The study of palliative therapy for intranasal tumors in dogs

（犬の鼻腔腫瘍における緩和療法に関する研究）

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Abstract

Intranasal tumors in dogs generally carry a poor prognosis, and radiation therapy is considered necessary for significantly increased survival time. Surgical resection alone does not improve survival time because complete removal of the tumor is usually impossible because of anatomic location and advanced disease at the time of diagnosis. For this reason, radiotherapy alone has been considered the standard treatment of choice.

An ideal head-immobilization method provides a high level of accuracy and reproducibility in the immobilization. Various head-immobilization methods have been published and are excellent in terms of accuracy; however, these methods are complicated to use and labor intensive. This study describes 2 new bite-block-type head-immobilization devices designed for greater stability and reduced vertical variation. The device designed in our previous study ("the bite-block-type head-immobilization device"; Device A) was modified by creating a groove on the upper surface of the horizontal plate (Device B) for a stable ventral–dorsal position or on the underside of the horizontal plate (Device C) for a stable dorsal–ventral position. These 3 devices were objectively compared with respect to setup time and accuracy of computed tomography (CT) images by 2 independent authors. Five healthy male beagles were used in this study. For each device, the setup time and variation in the coordinates were measured 5 times in each dog. The median setup time for Devices A, B, and C was 3.1, 1.6, and 2.3 min, respectively,

suggesting that the groove modifications were able to reduce setup time (in Device B, by at least 50%). Moreover, three-dimensional analysis of CT images revealed that the measurement variability of Device A (median, 1.3 mm) was significantly higher than that of Device C (median, 0.6 mm; $p < 0.001$). Collectively, our results showed that use of a bite-block-type head-immobilization device with a groove improves setup time and head-immobilization accuracy.

A fractionated radiation-therapy protocol is considered suitable for intranasal tumor in dogs. However, even with this protocol, median survival time is 12–15 months, and only 5–7 months in dogs with cribriform plate destruction. Further, dogs are treated daily from Monday to Friday with a fractionated protocol, which is demanding both for dogs and owners. The object of this study was to evaluate hypofractionated multiportal field and two-portion (rostral and caudal portions divided by the eyelid) radiation therapy in canine nasal tumor. Sixty-three dogs underwent multiportal hypofractionated radiation therapy. The radiation field was divided into rostral and caudal portions by the eyelid. Treatments were performed 4 times in 57 dogs. The median irradiation dose/fraction was 8 Gy (range, 5–10 Gy); the median total dose was 32 Gy (10–40 Gy). Improvement of clinical symptoms was achieved in 53 (84.1%) of 63 cases. Median survival time was 197 days (range, 2–1080 days). There was no significant difference between median survival time with and without destruction of the cribriform plate before radiotherapy (163 and 219 days, respectively). No other factors were related to survival according to a univariate analysis. All radiation side effects, except one, were Grade I according to the Veterinary

Radiation Therapy Oncology Group (VRTOG) classification. No dog required treatment for dermal side effects. One dog (1.6%) developed an oronasal fistula 1 year after completion of radiation therapy. This radiation protocol may be useful in reducing radiation side effects in dogs with cribriform plate destruction.

Despite recent advancement of radiation treatment machines, the median survival time of dogs with intranasal tumor typically does not exceed a year when treated with hypofractionated radiation therapy alone. In this retrospective study, we evaluated dogs treated with hypofractionated radiotherapy (28–32 Gy/4 fractions/4 weeks) followed by surgical resection. Subjects were staged according to the modified WHO staging protocol proposed by Adams *et al*. (1998), and the author further subclassified Stage III into substage a (no tumor extension in the orbit) and substage b (orbital involvement). Of 14 dogs, 3 were Stage I, 1 was Stage II, 2 were Stage IIIa, 3 were Stage IIIb, and 5 were Stage IV. Surgery was performed 1–2 months after the completion of radiation therapy in 6 dogs and immediately following the final radiation treatment in 8 dogs. Residual macroscopic tumor was still present after the surgical resection in 5 dogs. Tumor recurrence was noted in 9 dogs, including all the dogs with macroscopic residual tumors (median time to recurrence was 3 months). Observed late side effects were chronic rhinitis (n = 3) and nasocutaneous fistulae (n = 2). Univariate analysis indicated that tumor stage (I-IIIa; $p < 0.001$) and no recurrence ($p < 0.001$) had significant positive associations with survival time. In Stages I, II, and IIIa, the median disease-free interval was 18.5 months. The author concluded that this combination

therapy might lead to improved durations of local tumor control and survival in dogs with Stages I, II, and IIIa intranasal tumors.

The aim of combined radiotherapy and surgery is to resolve the disadvantages of the individual treatments; however, hypofractionated radiotherapy has a low total dose and the effect on tumor reduction is small. Hypofractionated radiotherapy followed by surgical resection was effective only in low-stage cases, and there was a high rate of complications. It was hypothesized that macroscopic resection with photodynamic therapy (PDT) would achieve comparable results and might decrease complications. Acridine orange (AO) has been used for PDT. In human medicine, it has been used locally after cytoreductive surgery. However, local administration of AO solution does not appear to result in penetration into deep tissues. Therefore, in invasive tumors, systemic administration of AO is useful. The purpose of this study was to evaluate the short-term safety of intravenous administration of AO (0.1 mg/kg) in dogs. Five beagles were used in this study. Initial evaluation (control) consisted of a physical examination, complete blood count (CBC), serum chemistry, and serum AO concentration. Clinical signs were observed every day for 1 month. CBC and serum chemistry were obtained 1, 3, 7, and 30 days after AO administration. Serum AO concentrations were measured 0, 7.5, 15, 30, 60, 90, 120, 150, 180, 240, and 300 min after 0.1 mg/kg AO was administered into the cephalic vein over 30 s. All dogs showed no clinical signs for 30 days. No photosensitivity was noted. All CBC and serum chemistry results were within normal limits. After intravenous injection of AO (0.1 mg/kg), serum AO level decreased rapidly

and was below the detection limit (5 ng/mL) 2 h after injection. These results show that intravenous administration of 0.1 mg/kg AO is safe on a short-term basis. Systemic administration of this drug should be limited to dogs with malignant tumors and a short life-span, because the long-term effects of systemic AO are unknown.

The author described a treatment protocol involving macroscopic tumor resection and intraoperative AO-PDT together with low-dose photon irradiation (5 Gy) of the tumor bed and, if the cribriform plate was damaged, high-dose electron irradiation of the cribriform plate. Four dogs were treated, including 1 dog that had previously received hypofractionated radiotherapy followed by surgical resection. In the latter dog, tumor recurrence was detected in the cranial part of the surgical site 1 month after completion of the therapy and in the caudal part of the surgical site 23 months after the completion of the first therapy. The tumor stage of all dogs in the study was I in 3 tumors and IV (cribriform plate destruction) in 2 tumors. All 5 tumors underwent AO-PDT and low-dose photon irradiation; in addition, the 2 tumors that had stage IV cancer received cribriform irradiation (15 or 25 Gy). Recurrence was detected in 2 of 4 dogs at 4 and 7 months after the therapy; 1 of 2 dogs underwent another treatment at 8 months, and the other dog was lost to follow-up at 13 months. One dog did not show recurrence after 7 months of therapy at the time of writing this case series. The other dog did not show recurrence after 33 months at the rostral tumor site, and after 11 months at the caudal tumor site, at the time of writing this case series. Median follow-up period was 11 months (range, 7–33 months), and side effects were mild

(subcutaneous emphysema, rhinitis, and nasal bleeding), except for one case where hypofractionated radiotherapy had already been performed. Thus, this combination treatment may increase survival time of dogs with macroscopically resectable intranasal tumors without severe side effects. Supplemental cribriform irradiation may control the tumors.

The author presents 5 important conclusions from the combined results of this study. First, improved stability and decreased vertical variation may be obtained with the two new bite-block-type head-immobilization devices. Second, hypofractionated multiportal field and two-portion (rostral and caudal portions divided by the eyelid) radiation therapy for canine intranasal tumors may reduce side effects and suit dogs with cribriform plate destruction. Third, hypofractionated radiotherapy followed by surgery may significantly improve survival time in dogs in early stage (Stages I, II, and III with subcutaneous involvement only) compared with hypofractionated radiotherapy alone; however, chronic infection and nasocutaneous fistulae are common complications. Fourth, systemic administration of AO (0.1 mg/kg) in dogs is safe on a short-term basis. Fifth, the combination of macroscopic tumor resection and intraoperative AO-PDT together with low-dose photon irradiation (5 Gy) of the tumor bed may increase survival time in dogs with macroscopically resectable intranasal tumor. Moreover, if the cribriform plate or nasal turbinate around the cribriform plate is destroyed, supplemental cribriform irradiation may control the local tumor. Thus, as a result of this study, the author developed a simplified positioner and proposed treatment options for dogs with intranasal tumors, which might be less

demanding on dogs and owners.

General introduction

Canine intranasal tumors are associated with a poor prognosis. Without treatment, survival time ranges from 0.9 to 5.5 months depending on the clinical stage at the time of diagnosis (MacEwen *et al*., 1977; Morris *et al*., 1994; Norris, 1979; Rassnick *et al*., 2006; Yoon *et al*., 2008). Surgical resection does not improve survival because it is extremely difficult to remove the tumor completely (MacEwen *et al*., 1977; Norris, 1979). For this reason, radiotherapy has been the treatment of choice for years (Henry *et al*., 1998; Turek and Lana, 2007). However, survival times did not exceed 20 months (Adams *et al*., 1998; Adams *et al*., 2005; Adams *et al*., 2009; Buchholz *et al*., 2009; Evans *et al*., 1989; Gieger *et al*., 2008; Hunley *et al*., 2010; Maruo *et al*., 2011; McEntee *et al*., 1991; Mellanby *et al*., 2002; Morris *et al*., 1994; Nadeau *et al*., 2004; Yoon *et al*., 2008), and most tumors recurred (Adams *et al*., 2005; Adams *et al*., 2009; Henry *et al*., 1998; LaDue *et al*., 1999; Lana *et al*., 2004; McEntee *et al*., 1991; Morris *et al*., 1994; Théon *et al*., 1993; Thrall *et al*., 1993b). Furthermore, radiotherapy may be accompanied by severe side effects (LaRue and Gillette, 2007) including vision loss and oronasal fistula (Adams *et al*., 1998; Adams *et al*., 2005; Gieger *et al*., 2008; Lana *et al*., 2004; Maruo *et al*., 2011; McEntee *et al*., 1991; Mellanby *et al*., 2002; Nadeau *et al*., 2004; Northrup *et al*., 2001; Roberts *et al*., 1987; Théon *et al*., 1993). Therefore, it is important to simplify the treatment by decreasing treatment frequency to reduce radiation side effects while maintaining treatment efficacy. Moreover, radiotherapy in dogs with intranasal tumor requires equipment to immobilize the head. A device has to be set up for each animal and is

time-consuming to construct. Thus, the author performed the following 5 studies to develop protocols to address these treatment problems and reduce side effects in dogs with intranasal tumor.

The first study was titled “Validation of new bite-block-type head-immobilization devices for radiotherapy in dogs.” An ideal head-immobilization method provides a high level of accuracy and reproducibility in the immobilization. Various head-immobilization methods for radiotherapy have been published and are excellent in terms of accuracy; however, these methods are complicated to use and labor intensive. This study describes 2 new bite-block-type head-immobilization devices designed for greater stability and decreased vertical variation. The device designed in our previous study (Device A) (Mori *et al*., 2009) was modified by creating a groove on the upper surface of the horizontal plate for a stable ventral–dorsal position (Device B) or on the underside of the horizontal plate (Device C) for a stable dorsal–ventral position. These 3 devices were compared with respect to setup time and accuracy of computed tomography (CT) images. The author then used these devices in the second study involving a radiotherapy protocol and in the third involving radiotherapy combined with surgery.

The second study was titled “Retrospective study of canine nasal tumors treated with hypofractionated radiotherapy.” In the fractionated protocol, dogs are treated daily from Monday to Friday, which is demanding for dogs and owners. The object of this study was to evaluate hypofractionated multiportal field and two-portion (rostral and caudal portions divided by the eyelid) radiation therapy for canine nasal tumors.

The title of the third study was “Combination therapy involving hypofractionated radiotherapy prior to surgical resection for canine intranasal tumors.” Despite recent advancement of radiation treatment machines, the median survival time of dogs with intranasal tumors typically does not exceed a year when treated with hypofractionated radiation therapy alone. The total dose of hypofractionated radiotherapy is lower than that of fractionated radiotherapy; however, controlling macroscopic tumors is generally difficult with low-total-dose radiotherapy (Zips, 2009). Here, the author hypothesized that a combination of hypofractionated radiotherapy prior to surgical resection for dogs with an intranasal tumor would control the local tumor. This study describes a protocol for hypofractionated radiotherapy prior to surgical resection of the tumor.

In humans, intra-lesional or partial tumor excision, followed by acridine-orange photodynamic therapy (AO-PDT) and low-dose photon irradiation (5 Gy) (Hashiguchi *et al*., 2002), has been used to treat musculoskeletal sarcomas (Kusuzaki *et al*., 2005). This approach reduces tumor recurrence rates without significant side effects (Kusuzaki *et al*., 2005; Matsubara *et al*., 2012). Therefore, the author sought to determine if this approach was suitable for the treatment of intranasal tumors in dogs. Moreover, intraoperative radiotherapy with an electron beam is effective in local control of tumors (Wolkov, 1998). The author therefore proposed that irradiation with a supplemental electron beam would be useful for tumor control in the case of tumor involvement of the cribriform plate. The

author investigated the toxicity of AOin the fourth study, and performed AO-PDT, low-dose photon irradiation, and cribriform irradiation in the fifth.

The fourth study was titled “Safety of intravenous administration of acridine orange in dogs.” AO selectively accumulates in tumor cells and is used for PDT and as a radiation sensitizer in humans (Hashiguchi *et al*., 2002; Kusuzaki *et al*., 2005). If AO is safe in dogs, it might be effective for dogs with tumors. Therefore, short-term safety of AO was determined in 5 healthy beagles.

The title of the fifth study was “Intraoperative PDT using AO and cribriform electron beam irradiation in canine intranasal tumors.” This study describes a protocol for treating intranasal tumors in 4 dogs. This protocol involved combined use of macroscopic resection and intraoperative AO-PDT, low-dose photon irradiation (5 Gy) of the macroscopically resected tumor bed in all cases, and (if the cribriform plate was damaged) high-dose electron irradiation of the cribriform plate. The results demonstrated that this combination therapy might increase survival time in dogs with macroscopically resectable intranasal tumors. Supplemental cribriform irradiation might control local tumors of the cribriform plate. This study was carried out with the hypothesis that macroscopic resection with PDT would achieve comparable results to hypofractionated radiotherapy prior to surgical resection (being effective in the early stages of the cancer), and may decrease complications including recurrence of the cancer at the cribriform plate.

Chapter 1: Validation of new bite-block-type head-immobilization devices for radiotherapy in dogs

Abstract

An ideal head-immobilization method provides a high level of accuracy and reproducibility in the immobilization. Various head-immobilization methods for radiotherapy have been published and are excellent in terms of accuracy; however, these methods are complicated to use and labor intensive. This study describes 2 new bite-block-type head-immobilization devices designed for greater stability and decreased vertical variation. The device designed in our previous study ("the bite-block-type head-immobilization device"; Device A) was modified by creating a groove on the upper surface of the horizontal plate (Device B) for a stable ventral–dorsal position or on the underside of the horizontal plate (Device C) for a stable dorsal–ventral position. These 3 devices were objectively compared with respect to setup time and accuracy of computed tomography (CT) images by 2 independent authors. Five healthy male beagles were used in this study. For each device, the setup time and variation in the coordinates were measured 5 times for each dog. The median setup time for Devices A, B, and C was 3.1, 1.6, and 2.3 min, respectively, showing that the groove modifications enabled reduced setup time (in Device B, by at least 50%). Moreover, three-dimensional analysis of the CT images revealed that the measurement variability of Device A (median, 1.3 mm) was significantly higher than that of Device C (median, 0.6 mm; $p < 0.001$). Collectively, our results show that use of a bite-block-type head-immobilization device

with a groove improves the setup time and head-immobilization accuracy.

Introduction

In recent years, radiotherapy has come to be widely used in the field of veterinary medicine (LaRue and Gillette, 2007). An ideal head-immobilization method provides a high level of accuracy and reproducibility in the immobilization, with the latter being particularly desirable for radiotherapy. To this end, several groups have published reports concerning various head-immobilization methods for radiotherapy (Bley *et al*., 2003; Green *et al*., 2003; Head 2002; Kent *et al*., 2009; Kippenes *et al*., 2000; Kippenes *et al*., 2003). Although these methods are excellent in terms of accuracy, they are complicated to use and labor intensive. To address these particular limitations, our group developed a bite-block-type head-immobilization device (hereafter referred to as "the head-immobilization device") in a previous study (Fig. 1) (Mori *et al*., 2009). The canine teeth of a feline or canine subject were placed on the immobilization device, which was able to maintain the head horizontally. However, because of the lack of fixation, arranging the canines in the same position in the long axis was not possible; further, movement to the right and left was able to occur. This lack of fixation allowed the possibility of variation in the lateral direction, and a significant amount of time was necessary to adjust the device. Moreover, because this immobilization method contacted only the tip of the canine teeth, variation in the vertical dimension remained possible.

This study describes 2 new bite-block-type head-immobilization devices designed for greater stability and reduced vertical variation. The device designed in our previous study (Device A) (Mori *et al*., 2009) was modified by creating a groove on the upper surface of the horizontal plate for a stable ventral–dorsal position (Device B) or on the underside of the horizontal plate (Device C) for a stable dorsal–ventral position. These 3 devices were compared with respect to setup time and accuracy of computed tomography (CT) images.

Materials and Methods

Animals and devices

Five healthy male beagles bred for research purposes were used in this study, with a mean age of 4.8 years (range, 2–6 years) and mean body weight of 10.0 kg (range, 6.0–12.0 kg). All procedures were performed in compliance with the guidelines of the Animal Research Committee of Azabu University. In this study, the author compared 3 rectangular-shaped bite-block-type head-immobilization devices. Device A was a previously reported bite-block-type head-immobilization device (Fig. 1) (Mori *et al*., 2009). Devices B and C were modified from Device A by the creation of a groove on the top and bottom sides, respectively, of the horizontal plate to hold the canine teeth. Dogs were placed in a dorsal–ventral position with Devices A and B and in a ventral–dorsal position with Device C.

Animal preparation

The dogs were maintained under general anesthesia during testing of each device. Atropine sulfate hydrate (0.025 mg/kg s.c.; Atropine Sulfate Injection, Mitsubishi Tanabe Pharma Co., Osaka, Japan) was administered 15 min before induction of anesthesia with propofol (6–8 mg/kg i.v.; Rapinovet, Takeda Schering-Plough Animal Health Corporation, Osaka, Japan). Each dog was positioned in lateral recumbency and intubated, after which anesthesia was maintained by isoflurane (Isoflurane for Animals, Mylan Inc., PA, USA). The tracheal tube was affixed to the lower jaw, while the upper jaw remained free, allowing for insertion of the horizontal plate of the head-immobilization device between the tracheal tube and the upper jaw. For Devices A and B (dorsal–ventral position), the tracheal tube system was passed through the head-immobilization device, and the canine teeth were inserted into the grooves on top of the horizontal plate (Fig. 1). The materials and dimensions of Device B were the same as those of Device A, with the exception of the size of the basement piece, which was long enough to allow use of the room lasers for adjustment of the long axis. The groove on the upper side of Device B was perpendicular to the long axis, and 3 mm in width and 3 mm in depth. The long axis of Device B was aligned with lasers. The dogs were placed in a dorsal–ventral position, and the tips of both canine teeth were placed in the groove.

For Device C (ventral–dorsal position), the nose of the dog was passed through the head-immobilization device, and the canine teeth were inserted into the groove on the underside of the

horizontal plate. The long axis of Device C was aligned with room lasers. A sponge (asterisk) was used to compress the muzzle from below to hold the tips of the canine teeth in the groove. The head was held in position with 3-point support (the tips of both canine teeth and the vertex of the head). The tracheal tube rested on top of the rectangular plate. The author measured setup time from lateral recumbency to completion of each position, and then conducted a CT scan. Five cycles of each setup and scan procedure were conducted (one cycle: Device A followed by Devices B and C) for each animal, and dogs were placed back in the lateral position after each scan.

The coordinates (x, y, z) were defined such that the x axis was the lateral direction, the y axis was the longitudinal direction, and the z axis was the vertical direction. The reference point of Device A was defined as the intersection point of the nasal apex, the philtrum, and the surface of the head-immobilization device. The long axis of the head was adjusted from the philtrum to the protuberantia occipitalis externa (Fig. 1, arrow) using the room lasers. For Devices B and C, the reference point of the y and z axes was drawn on the side of device, and the x axis was set between the first upper incisors using a ball-point pen. The long axis of the ball-point pen, which pointed between the first incisors, was aligned with the room lasers.

Computed tomography examination and analysis

Each animal underwent examination using a 4-slice helical CT scanner (Asteion, Toshiba,

Tokyo) with 4 × 1-mm slice thickness and 3.0 pitch. The scan parameters were as follows: 0.75-s rotation time, 120 kV, and 150 mA. CT images were reconstructed at 1-mm intervals using a head (reconstruction filter: FC21) algorithm. The head CT scans were conducted at a point 100 mm away from the reference point, and images were acquired 5 mm backward and forward from this reference point at 1-mm intervals (total, 1 cm). In the analysis of the slice 100 mm from the reference point, the same anatomical structure was compared between the first CT scan and the second to fifth scans for the same cross-section on the same screen. The author selected the ventral aspect of the bone at the nasopharynx, because it was almost parallel to the long axis (i.e., the position was almost the same in the scans back and forth) and was detected easily. For image comparison, 2 images (the first one left, the following one right) were loaded on the screen (Fig. 2). Initially, the image at 100 mm from the reference point and representing the first scan was shown on the left side of the screen. Afterwards, the image from the second scan was shown on the right side of the screen. The image on the right side was moved back and forth, and the author identified images in which the selected anatomical structure was in the same position. To assess the images, a window width of 300 and window level of 0 were used. The author measured the coordinates (x, y, z) of the first and second scans in which the structure was clearly defined. Consecutive scans through the fifth scan were displayed on the right side, and each coordinate was determined.

In each dog, the average coordinate was calculated, and the variation in the position of each coordinate was obtained by subtracting the average coordinate from each coordinate. The absolute value

of 5 dogs was used (total, 25 samples). The units for the x and z axes were pixels, while those for the y axis were millimeters (mm). For standardizing the units of measurement, 2 random points were selected, and the length between these points was measured in mm and pixels, allowing for the calculation of the number of millimeters per pixel (coefficient a). For obtaining the variation of X (mm) in the x axis, the author multiplied the absolute value of each x (pixels) by a. To obtain the variation of Z (mm) in the z axis, the author multiplied the absolute value of each z (pixels) by a. The variations of x, y, and z were analyzed, and a three-dimensional vector was calculated by $(X^2 + y^2 + Z^2)^{1/2}$.

Statistical analysis

For each device, the measurement of the setup time and the variation in the coordinates were measured 5 times for each dog (N = 25). The median and inter-quartile range (25%, 75%) of the setup time; the variation of X, y, Z; and the three-dimensional vector were calculated. The performance of these 3 devices was tested by analysis of variance, followed by the Mann–Whitney test with Bonferroni correction for multiple comparisons. Statistical significance was set at $p < 0.05$, and statistical analyses were conducted using a computer software program (JMP version 8.02, Institute Inc., NC).

Results

As shown in Figure 3, the median and inter-quartile range (25%, 75%) of the setup time for

Device A was 3.1 (2.6, 3.5) min and was significantly longer than that of Device B (upper groove; 1.6 [1.0, 2.0]; $p < 0.001$) and Device C (lower groove; 2.3 [1.9, 2.7]; $p < 0.001$). Furthermore, Device B was significantly more efficient than Device A ($p < 0.001$). These results showed that the groove modifications significantly reduced the setup time (in Device B, by at least 50%).

The accuracy of these 3 bite-block-type head-immobilization devices was compared on the basis of CT scan analysis of 5 repeated measurements in all 3 axes. Images analyzed along the *x* axis and *y* axis did not show significant difference (Figs. 4, 5). Thus, there was no significant difference between each device in lateral and longitudinal directions. However, the addition of a groove to the lower side of the horizontal plate improved the measurement accuracy in the *z* axis (0.5 [0.5, 1.1] mm for Device A vs. 0.2 [0.1, 0.3] mm for Device C; $p < 0.001$) (Fig. 6). Furthermore, the performance of Device C was significantly superior to that of Device B ($p = 0.002$). Thus, Device C was significantly more accurate in the vertical direction than Devices A and B. Three-dimensional analysis of the CT images confirmed that the measurement variability of Device A (1.3 [1.0, 2.3] mm) was significantly greater than that of Device C (0.6 [0.4, 0.9] mm; $p < 0.001$) (Fig. 7). Furthermore, the performance of Device B was significantly superior to that of Device A ($p = 0.017$).

Discussion

This study shows that a simple modification dramatically improves the performance of the

bite-block-type head-immobilization device in terms of setup time and accuracy. Our group previously reported that a bite-block-type head-immobilization device (Device A) reduced roll variation (Mori *et al*., 2009). The groove was able to hold both upper canine teeth, and this hold reduced yaw variation (Device B). Moreover, the groove created on the underside of the dorsolateral plate, with the dog positioned in the ventral–dorsal position, reduced pitch variation by 3-point support.

Radiation therapy requires an immobilization device, and a setup time is typically needed to fit the patient to the device. In this study, the setup time with these 3 bite-block-type head-immobilization devices was 1.6–3.1 min. The author also showed that groove modifications significantly reduced the setup time. The setup times for these 3 devices are considerable improvements compared with the 5–30 minutes required to create devices (using items such as inflatable pillows, headrests, thermoplastic masks, dental molds, and casting material) (Bley *et al*., 2003; Green *et al*., 2003; Kippenes *et al*., 2000). Hence, the results of the current study indicate that our head-immobilization device modified with a groove on the upper side of the horizontal plate is the most efficient among these various methods in terms of setup time.

Accurate and precise positioning of the patient is required in veterinary radiotherapy. A previous study examining an immobilization device consisting of a head holder and an inflatable pillow found that the device had a median reposition variation of 0.5–1.0 mm, with a standard deviation of 1.0–1.5 mm (Kippenes *et al*., 2000). Meanwhile, a different study reported that the mean displacement

value and standard deviation of the three-dimensional vector for a thermoplastic mask and a customized head support were 2.4 mm and 2.1 mm, respectively (Kent *et al*., 2009). In the current study, the variation of the three-dimensional vector for Device C was typically within 0.9 mm (i.e., the lower quartile) and was the lowest of these 3 tested devices. Thus, Device C is the most suitable for performing radiotherapy among these 3 tested devices.

Nonetheless, the devices in this study have several disadvantages. First, they cannot be used for dogs with canine teeth missing on one or both sides. In these dogs, dental moldings might be useful to support the maxilla. Second, these devices cannot be used for patients with maxillary tumors that extend beyond the canine teeth. In such cases, other devices such as an inflatable pillow might be useful. Third, if the tumor is located around the outside of the forehead or the vertex of the head, obtaining stability is difficult with Device C. Fourth, this study was conducted in a single breed of dog of similar head size and shape; in dogs with a large muzzle, or small breed dogs and cats, a larger or a smaller device than the ones in this study may be needed with Device C. Moreover, it may be difficult to use Device C in brachycephalic animals. Finally, another limitation of this study design is the small sample size and the absence of measurement in roll, pitch, or yaw, because these rotations might play a role in a patient that is fixed only by the canine teeth in Devices A and B.

The results of this study confirm that use of a bite-block-type head-immobilization device with a groove improves the setup time and accuracy of the head immobilization. These devices will be able to

reduce setup time and position variation in a clinical setting. Further clinical study is needed to test the efficacy of these devices by using coordinate differences and rotational shifts in various patients with tumors.

This study was published in *Vet Radiol Ultrasound* [in press].

Chapter 2: Retrospective study of canine nasal tumor treated with hypofractionated radiotherapy

Abstract

The object of this study was to evaluate hypofractionated multiportal field and two-portion (rostral and caudal portions divided by the eyelid) radiation therapy in canine nasal tumor. Sixty-three dogs underwent multiportal hypofractionated radiation therapy. The radiation field was divided into rostral and caudal portions by the eyelid. Treatments were performed 4 times each in 57 dogs. The median irradiation dose/fraction was 8 Gy (range, 5–10 Gy); the median total dose was 32 Gy (10–40 Gy). Improvement of clinical symptoms was achieved in 53 (84.1%) of 63 cases. Median survival time was 197 days (range, 2–1080 days). Median survival times with and without destruction of the cribriform plate before radiotherapy were 163 and 219 days, respectively. There was no significant difference between them. No other factors were related to survival according to a univariate analysis. All radiation side effects, except one, were Grade I according to the Veterinary Radiation Therapy Oncology Group (VRTOG) classification. No dog required treatment for dermal side effects. One dog (1.6%) developed an oronasal fistula 1 year after completion of radiation therapy. This radiation protocol may be useful in reducing radiation side effects in dogs with cribriform plate destruction.

Introduction

Tumors involving the nasal cavity and nearby sinuses are uncommon in dogs, and they account for approximately 1% of all reported canine tumors (Turek and Lana, 2007). Because nasal tumors metastasize rarely, late in the course of disease, therapy is directed at controlling localized disease (Moore and Ogilvie, 2006). Without treatment, the median survival time of dogs has been reported to be 1.5–4.1 months (Norris, 1979; Rassnick *et al*., 2006; Yoon *et al*., 2008). Surgery alone does not prolong survival time (MacEwen *et al*., 1977; Norris, 1979), and may deteriorate quality of life. In regard to chemotherapy, Langova *et al.* (2004) reported the effectiveness of alternating doses of doxorubicin and carboplatin in conjunction with oral piroxicam, although their sample size was small. Radiation therapy is the treatment of choice (Turek and Lana, 2007), and reported survival times have ranged from 7.4 to 47.7 months (Adams *et al*., 2009; Adams *et al*., 2005; Adams *et al*., 1998; Henry *et al*., 1998; LaDue *et al*., 1999; Lana *et al*., 2004; Lana *et al*., 1997; McEntee *et al*., 1991; Morris *et al*., 1994; Nadeau *et al*., 2004; Northrup *et al*., 2001; Roberts *et al*., 1987; Thrall *et al*., 1993ab; Yoon *et al*., 2008).

Various radiation treatment protocols in veterinary medicine have been reported. For increased total dose, 19 (LaDue *et al*., 1999) or 21 (Correa *et al*., 2003) fractions at 3 Gy/fraction over 1 month have been used. Because this method requires frequent anesthesia and is expensive, it is demanding on dogs and owners. Hypofractionated radiotherapy has been used to address these issues (Gieger *et al*., 2008; Mellanby *et al*., 2002).

Hypofractionated radiation therapy results in late side effects that are more severe than with

fractionated radiation therapy; blindness was reported following radiation treatment of dogs with nasal tumors (Gieger *et al*., 2008; Mellanby *et al*., 2002). To address this disadvantage, multiportal field and two-portion (rostral and caudal portions divided by the eyelid) radiotherapy (Fig. 8) has been used to deliver the radiation dose to the tumor and achieve a decreased dose to surrounding tissue, thus preventing severe side effects such as blindness and skin or bone necrosis, as previously reported in 18 cases (Shida *et al*., 2008).

The purpose of this study was to evaluate hypofractionated multiportal field and two-portion (rostral and caudal portions divided by the eyelid) radiation therapy in canine nasal tumor.

Materials and Methods

A total of 63 dogs with nasal tumors were treated with radiation therapy from November 2005 through February 2009 at Azabu University Veterinary Teaching Hospital. Dogs that underwent surgery were excluded.

Information obtained from the medical records or telephone interviews with the owner or referring veterinarian included breed, sex, age, body weight, clinical symptoms, duration of clinical symptoms from start to first visit, modified TNM classification, histopathology, radiation details (treatment number, portal number, irradiation dose/fraction, total dose, and total dose at the center of the eyeball on the irradiated side), response to treatment, post treatment computed tomography (CT) imaging

findings, survival time, cause of death, and radiation side effects.

The tumors were staged by clinical and CT findings using Adams's classification (a modification of the WHO system) (Adams *et al*., 1998). Clinical efficacy was assessed after irradiation, and response was assessed by CT 1 month after radiation therapy was completed. Survival time was calculated from the completion of radiation treatment to the time of death. The toxicity criteria of the Veterinary Radiation Therapy Oncology Group (VRTOG) (LaDue and Klein, 2001) were used to assess the radiation side effects.

For radiation therapy, a 4-MV linear accelerator (Mevatron; Toshiba, Tokyo, Japan) was used. Multiportal hypofractionated radiation therapy was performed once a week, with most of the dogs receiving 4 treatments. The radiation field was divided into rostral (Fig. 9, left) and caudal portions (Fig. 9, right) by the eyelid. The caudal portion was planned to avoid the orbit to reduce radiation side effects. The author planned to irradiate the gross tumor with more than 80% of the isocenter dose. Treatment was assessed by CT 1 month after completion of radiotherapy.

Survival analysis was performed using the Kaplan–Meier method, and the log-rank test was used to assess survival time and factors related to outcome, including age, body weight, duration of clinical signs from onset to first visit, presence or absence of facial deformity, and presence or absence of cribriform plate destruction. The level of significance was set at $p < 0.05$.

Results

The breeds commonly represented in this study included the Shiba Inu (n = 8), Golden Retriever (n = 7), Labrador Retriever (n = 7), Shetland sheepdog (n = 7), Pembroke Welsh Corgi (n = 5), Papillon (n = 3), Beagle (n = 3), Maltese (n = 3), and Miniature Dachshund (n = 2). Other breeds included the American Cocker Spaniel, Miniature Schnauzer, Toy Poodle, Siberian Husky, Border Collie, Alaskan Malamute (n = 1 each), and mixed breed (n = 11). There were 35 male and 28 female dogs; the mean age was 10 years (range, 4–15 years), and the median body weight was 13.0 kg (range, 3.0–47.0 kg). The clinical findings included epistaxis in 53 dogs (84.1%), sneezing in 43 dogs (68.3%), nasal discharge in 21 dogs (33.3%), facial deformity in 29 dogs (46%), exophthalmos in 10 dogs (15.9%), and neurological symptoms in 4 dogs (6.3%). The median duration of clinical signs from onset to first visit was 102 days (range, 8–1080 days).

One dog had Stage II disease; 17 dogs had Stage III disease, and 35 dogs had Stage IV disease. There were 5 dogs (7.9%) with regional lymph node metastases; none had lung metastases. The following tumor types were identified: adenocarcinoma (29, 46.0%), squamous cell carcinoma (10, 15.9%), osteosarcoma (6, 9.5%), undifferentiated carcinoma (3, 4.8%), transitional carcinoma (3, 4.8%), hemangiosarcoma (2, 3.2%), chondrosarcoma (2, 3.2%), and undifferentiated tumor (8, 12.7%).

Treatment was performed 4 times in 57 dogs; 3 times in 3 dogs; and 1, 2, and 5 times in 1 dog each. Portal number of the rostral portion in 58 dogs was rotational irradiation in 45 dogs, 4-portal

irradiation in 12 dogs, and single-portal irradiation in 1 dog. Portal number of the caudal portion was 3-portal irradiation in 55 dogs. Furthermore, 3 dogs had tumors that had progressed to the caudal part of the frontal sinus and were irradiated from the caudal part to the caudal portion by opposing portal irradiation. The median irradiation dose/fraction was 8 Gy (range, 5–10 Gy), and the median total dose was 32 Gy (10–40 Gy); the median total dose at the center of the eyeball on the irradiated side was 9.5 Gy (range, 0–35.5 Gy) for irradiation of the caudal portion in 55 of the dogs.

Improvement of clinical symptoms was achieved in 53 (84.1%) of the 63 cases. CT images at 1 month after completion of radiotherapy were available in 30 cases, and the result was complete regression in 3 cases (10.0%), partial regression in 16 cases (53.3%), stable disease in 10 cases (33.3%), and progressive disease in 1 case (3.3%). The median survival of all dogs was 197 days (range, 2–1080 days). The 1- and 2-year survival rates were 25.4% and 7.9%, respectively. Death was due to local disease in 37 dogs (90.2%) and disease other than nasal cancer in 4 dogs (9.8%).

Acute and late radiation side effects affected the skin and eyes. All except the late side effects were Grade I according to the VRTOG classification: hair loss, 15 dogs (23.8%); color change, 11 dogs (17.5%); and conjunctivitis, 13 dogs (20.6%). No treatment was needed for skin side effects. One year later, an oronasal fistula occurred after completion of radiation therapy in 1 dog (1.6%).

No factors were related to survival according to a univariate analysis. The dogs were divided into 2 groups by median duration of clinical signs from onset to first visit (102 days). There was no

significant difference between 102 days and <102 days, with median survival times of 296 and 197 days, respectively. There was no difference in median survival time between dogs with (n = 29, 150 days) and without (n = 34, 234 days) facial deformity. Similarly, there was no difference in median survival time between dogs with cribriform plate destruction before radiotherapy (n = 35, 163 days) and those without (n = 28, 219 days; Fig. 10). Furthermore, there were no significant differences by age, body weight, and radiation side effects.

Discussion

In this study, hypofractionated radiotherapy was effective for nasal tumors and resulted in mild acute radiation side effects. Improvement of clinical symptoms was achieved in 53 (84.1%) of 63 cases. CT images 1 month after completion of radiotherapy were available in 30 cases, and the result was complete regression in 3 cases (10.0%) and partial regression in 16 cases (53.3%). The median survival of all dogs was 197 days (range, 2–1080 days). All side effects, except for late ones, were Grade I according to the VRTOG classification, and no dogs required treatment for skin side effects.

Mellanby *et al.* (2002) conducted a retrospective study in 56 dogs treated for nasal tumor by megavoltage radiotherapy with a hypofractionated schedule consisting of 4 doses of 9 Gy given at intervals of 7 days. The median survival time in their study was 212 days. Gieger *et al*. (2008) reported data from 48 dogs with nasal carcinoma treated with hypofractionated radiation therapy because of the

extent of local or concurrent disease (40%) and the owners requesting palliative radiotherapy because of concerns about cost, potential for toxicity, or travel/hospitalization associated with definitive radiation therapy. The total radiation dose ranged from 16 to 40 Gy (median, 24 Gy), and the dose per fraction ranged from 4 to 10 Gy (median, 8 Gy). The median survival time in their study was 146 days. The median survival time of 197 days in this study does not differ greatly from that in either of these reports. However, a more fractionated protocol produces a better therapeutic gain than a hypofractionated protocol. Previous studies reported median survival times of 8.9–19.7 months with 10–19 fractions (Adams *et al*., 2009; Adams *et al*., 2005; LaDue *et al*., 1999; McEntee *et al*., 1991; Théon *et al*., 1993). In this study, the median survival time was shorter than that in previous studies because of the lower total irradiation dose, as is found with other hypofractionated protocols.

Advanced disease is believed to be associated with a poor prognosis. The reported prognostic factors are destruction of the cribriform plate (Adams *et al*., 2009), tumor type (Adams *et al*., 2009; Théon *et al*., 1993), tumor stage (Adams *et al*., 2009; Adams *et al*., 1998; Buchholz *et al*., 2009; Kondo *et al*., 2008; LaDue *et al*., 1999; Théon *et al*., 1993), age >10 years (LaDue *et al*., 1999), regional lymph node or pulmonary metastasis (Henry *et al*., 1998), resolution of clinical signs after treatment (Gieger *et al*., 2008; Northrup *et al*., 2001), tumor regression (Thrall *et al*., 1993b), facial deformity (Northrup *et al*., 2001), 3 or more treatments/week (Yoon *et al*., 2008), and a cumulative minimum tumor dose of at least 37 Gy (Yoon *et al*., 2008). In regard to the stage in particular, various stage classifications have been

reported, and lower stages resulted in longer survival than higher stages (Adams *et al*., 2009; Adams *et al*., 1998; Buchholz *et al*., 2009; Kondo *et al*., 2008; LaDue *et al*., 1999; Théon *et al*., 1993). However, this study and other hypofractionated protocols (Gieger *et al*., 2008; Mellanby *et al*., 2002) showed no significant difference between tumor stage and survival time; thus, it was thought that the hypofractionated protocol was inappropriate for early stages. However, a more fractionated protocol for dogs with destruction of the cribriform plate on CT images resulted in median survival times of 6.7 months (Adams *et al*., 2009) and 6.6 months (Kondo *et al*., 2008) (the tumors were classified together as Stage IV). The median survival times for Stages I, II, and III tumors were 23.1, 14.0, and 15.7 months (Adams *et al*., 2009), respectively, and that of the latter-stage III tumors was 15.1 months (Kondo *et al*., 2008). These survival times resembled our results for dogs with destruction of the cribriform plate. Thus, for dogs with destruction of the cribriform plate, the current protocol was useful, as it reduced radiation side effects and required less frequent treatments.

Local tumor control can be achieved with an adequate margin; generally, the larger the radiation field, the more serious the radiation side effects (Emami *et al*., 1991). Early side effects of radiation therapy are generally self-limiting, and recovery is rapid (LaRue and Gillette, 2007). Therefore, even if severe side effects are predicted, it is important to plan an adequate margin to control the tumor. Concerning the radiation side effects of fractionated protocols, McEntee *et al.* (1991) utilized an irradiation dose of 41.8–54 Gy and documented transient conjunctivitis (67%), oral mucositis (81%), and

significant ophthalmic complications (48%). Adams *et al*. (2005) utilized an irradiation dose of 42 Gy, and 15 out of 53 dogs lost sight in 1 (11 dogs) or both (4) eyes following radiotherapy. Roberts *et al*. (1987) reported ophthalmic complications following megavoltage irradiation of the nasal and paranasal cavities in dogs with a median total dose of 40 Gy (range, 36.8–50 Gy), and the ocular complications were classified as mild (17.2%) and severe (58.6%). On the other hand, with the use of hypofractionated protocols, Mellanby *et al*. (2002) reported that 57% of the dogs required treatment for acute side effects. No long-term side effects were observed, apart from in 1 dog that developed blindness as a result of keratitis. Gieger *et al*. (2008) reported that chronic ocular toxicities were reported in 13% of the dogs that had at least one eye irradiated. Chronic toxicities included conjunctivitis, corneal ulceration or perforation, loss of vision, keratoconjunctivities sicca, severe mucoid ocular discharge, cataract formation, blepharospasm, and uveitis (Gieger *et al*., 2008). The radiation side effects following nasal tumor treatment have included severe symptoms related to the orbit (Adams *et al*., 2005; Adams *et al*., 1998; Lana *et al*., 2004; McEntee *et al*., 1991; Nadeau *et al*., 2004; Northrup *et al*., 2001; Roberts *et al*., 1987; Théon *et al*., 1993). Serious radiation side effects and frequent treatment are of little benefit in dogs with high-stage tumors because the treatment for nasal tumors is palliation, not radical cure. Thus, a once-a-week, four-treatment hypofraction protocol was used to reduce frequent anesthesia, and multiportal fields and two portions (rostral and caudal portions divided by the eyelid) were planned to reduce exposure of the surrounding normal tissues and orbit to radiation. In our results, the median total

dose at the center of the eyeball was low, 9.5 Gy; therefore, the radiation side effects were thought to be mild.

The once-a-week multiportal field, two-portion protocol (rostral and caudal portions divided by the eyelid) with 4 hypofractions was useful to reduce the number of treatments, save cost, and reduce the burden on dogs and owners because the majority of cases experienced amelioration of clinical symptoms, and it was not necessary to treat radiation side effects in dogs with cribriform plate destruction.

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Chapter 3: Combination therapy involving hypofractionated radiotherapy followed by surgical resection for canine intranasal tumors

Abstract

Untreated canine intranasal tumors generally carry a poor prognosis. Despite recent advancement of radiation treatment machines, the median survival time of dogs with intranasal tumors typically does not exceed a year when treated with hypofractionated radiation therapy alone. In this retrospective study, the author evaluated dogs treated with hypofractionated radiotherapy (28–32 Gy/4 fractions/4 weeks) followed by surgical resection. Staging was carried out according to the modified WHO staging protocol proposed by Adams *et al*. (1998), and the author further subclassified stage III into substage a (no tumor extension in the orbit) and substage b (orbital involvement). Among 14 dogs, 3 were Stage I, 1 was Stage II, 2 were Stage IIIa, 3 were Stage IIIb, and 5 were Stage IV. Surgery was performed 1–2 months after the completion of radiation therapy in 6 dogs and immediately following the final radiation treatment in 8 dogs. Residual macroscopic tumor was present after the surgical resection in 5 dogs. Tumor recurrence was noted in 9 dogs, including all the dogs with macroscopic residual tumors (median time to recurrence was 3 months). Observed late side effects were chronic rhinitis (n = 3) and nasocutaneous fistulae (n = 2). Univariate analysis indicated that tumor stage (I–IIIa; $p < 0.001$) and no recurrence ($p < 0.001$) had significant positive associations with survival time. In Stages I, II, and IIIa, the

median disease-free interval was 18.5 months. The author concluded that this combination therapy may lead to improved local tumor control time and survival time in dogs with Stages I, II, and IIIa intranasal tumors.

Introduction

Dogs with intranasal tumors generally carry a poor prognosis, and radiation therapy is considered necessary for a significantly increased survival time (Turek and Lana, 2007). Surgical resection alone does not improve survival time because complete removal of the tumor is virtually impossible in all cases because of anatomic location and advanced disease at the time of diagnosis (MacEwen *et al*., 1977; Morris *et al*., 1994). For this reason, radiotherapy alone has been considered the standard treatment of choice (Henry *et al*., 1998; Turek and Lana, 2007), with reported median survival times ranging from 5 to 19.7 months (Adams *et al*., 1998; Adams *et al*., 2005; Adams *et al*., 2009; Buchholz *et al*., 2009; Evans *et al*., 1989; Gieger *et al*., 2008; Hunley *et al*., 2010; Maruo *et al*., 2011; McEntee *et al*., 1991; Mellanby *et al*., 2002; Morris *et al*., 1994; Nadeau *et al*., 2004; Yoon *et al*., 2008).

In general, fine fractionated protocols, which allow the administration of a higher total dose by using a smaller dose per fraction, are considered superior to hypofractionated protocols in terms of tumor control time. However, local tumor recurrence is still common, even with the use of fine fractionated radiation protocols with megavoltage radiation machines (Adams *et al*., 2005; Adams *et al*., 2009; Henry *et al*., 1998; LaDue *et al*., 1999; Lana *et al*., 2004; McEntee *et al*., 1991; Morris *et al*., 1994; Théon *et al*., 1993; Thrall *et al*., 1993ab). Therefore, various attempts have been made to achieve a longer local tumor control time, including surgery (Adams *et al*., 1987; Adams *et al*., 2005; LaDue *et al*., 1999; McEntee *et al*., 1991; Morris *et al*., 1994; Théon *et al*., 1993; Yoon *et al*., 2008), radiation sensitizers (Lana *et al*.,

1997; Lana *et al*., 2004; Nadeau *et al*., 2004), boost techniques (Gutiérrez *et al*., 2007; Thrall *et al*., 1993a), and accelerated radiotherapy (Adams *et al*., 1998; Adams *et al*., 2005). One study that evaluated the combination of accelerated radiotherapy followed by exenteration of the nasal cavity resulted in a median survival time of 47.7 months (Adams *et al*., 2005). However, severe late side effects including chronic rhinitis and vision loss were not uncommon in this study (Adams *et al*., 2005).

Because of lower cost and fewer sessions of general anesthesia, which is required for each radiation treatment, hypofractionated radiation therapy has been evaluated in multiple studies (Buchholz *et al*., 2009; Gieger *et al*., 2008; Maruo *et al*., 2011; Mellanby *et al*., 2002; Yoon *et al*., 2008). As mentioned before, the total dose administered with a hypofractionated radiotherapy protocol is lower than the dose administered in fine fractionated radiotherapy protocols. However, long-term control of the growth of macroscopic tumors is difficult with a lower total dose (Zips, 2009). The goal of this study was to evaluate our hypothesis that hypofractionated radiotherapy followed by surgical debulking of canine nasal tumors would improve local tumor control time.

Materials and Methods

The author treated 14 dogs with intranasal tumors by using the hypofractionated radiotherapy protocol followed by surgical resection from May 2009 through December 2011. Informed consent was obtained from the animals' owners to participate in this study. Information was obtained from either

medical records or telephone interviews with the owners or the referring veterinarians. The evaluated variables included breed, sex, age, body weight, clinical signs, duration of the clinical signs prior to presentation (months), tumor stage including the TNM (tumor, lymph node involvement, and distant metastasis), and histological type of the tumor. The collected data regarding the treatments included overall radiation treatment time, radiation dose per fraction, total dose, duration between the last radiation treatment and surgery, and other details regarding surgery (such as whether bone was removed or bone flaps were created, and whether surgery resulted in microscopic or macroscopic residual tumor). The collected data regarding the follow-ups included radiation acute/late side effects, surgical complications, local tumor recurrence, time from the completion of radiotherapy to the time of local tumor recurrence, and the duration of the follow-up period. The disease-free interval (DFI) was defined as the time from the completion of treatment to the time when recurrence was detected or suspected. The end point of this study was set as the time of recurrence, because other treatments (such as repeat surgery and photodynamic therapy) were performed upon recurrence. None of the dogs in this study were treated with either chemotherapy or nonsteroidal anti-inflammatory drugs.

The treatment schedule was as follows. Computed tomography (CT) was performed in all dogs prior to radiation therapy to determine the size, location, and extent of the tumor. In 6 dogs, CT was repeated 1 month after the completion of radiotherapy, and the residual tumor was resected, as described in the study by Adams *et al*. (2005). However, based on the study that the author performed previously,

only 10% of the dogs treated with hypofractionated radiation therapy alone achieved complete response (i.e., complete disappearance of the gross disease) (Maruo *et al*., 2011); hence, in the remaining 8 dogs, the author performed resection at the last session of the radiation therapy. CT scans were performed immediately before and immediately after surgery.

The author employed the modified WHO staging system (Owen, 1980), which was described by Adams *et al*. (1998; Table 1). In this study, the author further subclassified Stage III into substage a (no tumor extension in the orbit) and substage b (orbital involvement). Survival time was calculated from the completion of radiation treatment to the time of death. The toxicity criteria described by the Veterinary Radiation Therapy Oncology Group (VRTOG) were used to assess the radiation-related acute and late side effects (LaDue and Klein, 2001).

For radiotherapy, three-dimensional computer planning software (ARCS-III; Nihon Denshi Ohyo Co., Ltd., Tokyo, Japan) was used in 11 dogs. The gross tumor volume was irradiated with at least 80% of the prescribed dose of the isocenter dose in 1 dog (Maruo *et al*., 2011), and a single-portal field was used with a 1-cm margin around the gross target volume (GTV) in 11 dogs. A different radiation therapy software (XiO; Elekta Co., Ltd., Tokyo, Japan) was used in 3 dogs. A 4-MV photon beam generated by a linear accelerator (Mevatron; Toshiba, Tokyo, Japan) was used in 11 dogs, and a 6-MV photon beam generated by another linear accelerator (Primus, Toshiba) was used in 3 dogs. One-centimeter-thick commercially available bolus material was used for all dogs (MTCB 410 S; CIVCO

Medical Solutions, Kalona, IA, USA). Wedges (beam modifier) were not used. Heterogeneity correction was applied during the radiation therapy planning.

Prior to surgical debulking, the rostral and the caudal extents of the tumor were evaluated according to the distance from the rostral end of the nasal planum on CT images. The skin incision was extended 5 mm beyond the rostral and caudal extents of the tumor. A chisel was used to remove the bone and to expose the nasal cavity. When the removed bone was to be replaced, the bone was removed with the periosteum attached, then, after the surgical procedure, the periosteum was sutured to restore the bone in place. When the bone was to be removed permanently, the periosteum was separated from the bone, then, after the surgical procedure, the periosteum was sutured. When the tumor eroded through the bone, the bone and the associated periosteum were permanently removed. Tumor removal was performed using suction and curettage. The tumor and surrounding mucous membrane were removed until the bone was exposed. An intact bone flap was then returned or removed, and any visible tumor tissue invading the bone was removed. The bone flap with the periosteum attached was sutured using 4-0 absorbable material (Maxon; Covidien, Dublin, Ireland). The skin incision was closed with subcuticular sutures using 5-0 absorbable material and with simple interrupted sutures using 4-0 nonabsorbable material (Monosof, Covidien).

Survival analysis was performed using the Kaplan–Meier method, and the log-rank test was used to assess survival time and factors related to outcome, including age, body weight, duration of

clinical signs prior to presentation, tumor stage (I–IIIa vs. IIIb and IV), tumor type (carcinoma vs. sarcoma), timing of surgery relative to radiotherapy (during radiotherapy vs. post radiotherapy), macroscopic tumor remaining after surgery (yes vs. no), and recurrence. The Cox proportional hazards model was used for multivariate analysis. Statistical significance was set at $p < 0.05$, and statistical analyses were conducted using a computer software program (JMP version 8.0.2; JMP Institute Inc., NC, USA).

Results

The clinical information of the dogs is shown in Table 2. These patients included 4 neutered males, 2 intact males, 6 neutered females, and 2 intact females. The mean age was 12 years (range, 8–14 years), and the mean body weight was 18.3 kg (range, 6.7–36.7 kg). Clinical signs included epistaxis (n = 9), sneezing and/or nasal discharge (n = 7), and facial deformity (n = 8). The median duration of the clinical signs prior to presentation was 2 months (range, 1–6 months). Three dogs were classified as T1, 1 dog as T2, and the remaining 10 dogs as T3. Neither lymph node involvement nor distant metastasis were noted in any dogs at the time of staging. The tumors were at Stage I in 3 dogs, Stage II in 1 dog, Stage III in 5 dogs, and Stage IV in 5 dogs. There were 10 carcinomas (5 adenocarcinomas, 2 transitional carcinomas, 2 carcinomas, and 1 squamous cell carcinoma) and 4 sarcomas (2 osteosarcomas, 1 chondrosarcoma, and 1 fibrosarcoma).

Hypofractionated radiotherapy consisted of 4 weekly treatments using 7–8 Gy per fraction (median, 7 Gy) for a total period of 4 weeks. The total dose range was 28–32 Gy (median, 28 Gy). Surgical resection was performed after completion of hypofractionated radiotherapy in 6 dogs or immediately after the last session of hypofractionated radiotherapy in 8 dogs. The tissue dorsal to the nasal cavity was removed in 7 of 14 dogs that had tumor involving the subcutaneous tissue in this area to expose the nasal cavity. An intact bone flap was returned and sutured in 4 dogs, but removed in the remaining 3 dogs because of high morbidity (e.g., 2 of 4 returned and sutured dogs developed nasocutaneous fistulae). All macroscopic (visible) tumor was removed by surgery in 9 dogs (Table 3); however, in the remaining 5 dogs, macroscopic residual tumor was still present when the surgical procedure was complete.

All radiation side effects, acute and late combined, were classified as Grade I (mild) according to the VRTOG classification and were limited to the skin and the eyes. Observed acute effects included hair loss (n = 3) and mild conjunctivitis (n = 3). Late side effects that were noted included keratoconjunctivitis sicca (n = 1) and rhinitis (n = 7). Of the 7 animals that developed rhinitis, 3 developed chronic rhinitis, although the symptoms were alleviated in all dogs by the use of intermittent antibiotic drugs. One dog developed an abscess involving the frontal sinus due to bone infection on 2 separate occasions at the same location (13 and 20 months after completion of radiotherapy); this problem resolved following surgical debridement. Surgical complications were noted in 6 dogs, including

subcutaneous emphysema (n = 4) and infection of the bone flap (n = 2). Severe subcutaneous emphysema developed in 1 dog, which extended to the trunk, but this resolved within a month. Nasocutaneous fistulae developed in both the dogs with infection of the bone flap. Among the 5 dogs without a recurrence, 2 developed chronic rhinitis (one of these dogs developed a nasocutaneous fistula) and 1 developed an abscess. Table 3 summarizes the variables related to the treatments and outcomes.

Recurrence was detected in 4 dogs by CT scan, in 1 dog by radiography, in 4 dogs by development or progression of facial deformity, in 4 dogs by histopathological means, and in 1 dog by cytology. Among the 9 dogs with recurrence, 1 was Stage I, 3 were Stage IIIb, and the remaining 5 were Stage IV. Dogs with macroscopic residual lesions when the surgical procedure was complete developed recurrence. The median time to recurrence was 3 months (range, 1–5 months). The median DFI of 6 dogs with Stages I and II, and Stage IIIa with only subcutaneous involvement (i.e., dogs 1 and 2 in Table 3), was 18.5 months (range, 5–39 months).

Univariate analysis indicated that tumor type (carcinoma; $p = 0.065$), successful macroscopic tumor resection after surgery ($p = 0.052$), and timing of surgery ≥1 month after radiotherapy ($p = 0.110$) were potentially associated favorably (i.e., $p < 0.15$) with survival time, and tumor stage (I–IIIa; $p < 0.001$) and no recurrence ($p < 0.001$) were significantly associated favorably with survival time (Table 4). However, there was no significant factor in the multivariate analysis.

Discussion

The survival time of dogs with intranasal tumors treated with radiation therapy is typically less than 20 months (Adams *et al*., 1998; Adams *et al*., 2005; Adams *et al*., 2009; Buchholz *et al*., 2009; Evans *et al*., 1989; Gieger *et al*., 2008; Hunley *et al*., 2010; Maruo *et al*., 2011; McEntee *et al*., 1991; Mellanby *et al*., 2002; Morris *et al*., 1994; Nadeau *et al*., 2004; Yoon *et al*., 2008), and destruction of the cribriform plate has been shown to be a poor prognostic factor in some studies (Adams *et al*., 2009). However, in our previous study, involvement of the cribriform plate was not associated with treatment outcome in dogs treated with hypofractionated radiotherapy protocols. In fact, those dogs with intact cribriform plates survived 7.3 months, compared with 5.4 months in dogs with cribriform plate destruction (Maruo *et al*., 2011). The results of this study suggest that a combination of hypofractionated radiotherapy followed by surgical resection for dogs with an intranasal tumor may lead to longer tumor control, compared with hypofractionated radiation therapy alone (Gieger *et al*., 2008; Maruo *et al*., 2011; Mellanby *et al*., 2002), particularly if the tumors are in early stages (Stages I and II and Stage III with only subcutaneous involvement). Moreover, the median DFI was only 3 months for dogs that developed recurrence at the time of this writing, compared with 18.5 months for the dogs with early-stage tumors (Stages I, II, and III with only subcutaneous involvement). Hence, most dogs at Stage IIIb (orbital involvement) and Stage IV did not benefit from surgical debulking post radiotherapy. Moreover, the postoperative complication rate of this combination treatment was high, although the radiation side effects

were mild (Grade I). Further, a number of surgical complications were observed, including subcutaneous emphysema, nasocutaneous fistulae, and chronic rhinitis. It is possible, however, that radiation therapy led to the increased incidence and severity of the side effects associated with surgery, because higher surgical complication rates have been reported if surgery is performed in previously irradiated areas (Séguin *et al*., 2005).

Canine intranasal tumors are locally aggressive, and the median survival time of untreated dogs ranges from 0.9 to 5.5 months (MacEwen *et al*., 1977; Morris *et al*., 1994; Norris 1979; Rassnick *et al*., 2006; Yoon *et al*., 2008), compared with 5 to 19.7 months in dogs treated with radiation therapy (Adams *et al*., 1998; Adams *et al*., 2005; Adams *et al*., 2009; Buchholz *et al*., 2009; Evans *et al*., 1989; Gieger *et al*., 2008; Hunley *et al*., 2010; Maruo *et al*., 2011; McEntee *et al*., 1991; Mellanby *et al*., 2002; Morris *et al*., 1994; Nadeau *et al*., 2004; Yoon *et al*., 2008). Some groups have proposed therapies combining radiotherapy and surgery. One study evaluated dogs with intranasal tumors that underwent surgical resection followed by radiotherapy (41.8–54 Gy/10–12 fractions). The median survival time in this study was 15 months (McEntee *et al*., 1991). In another study (Adams *et al*., 2005), radiotherapy was followed by surgery, and the median survival time was 47.7 months. In the present study, the author found that hypofractionated radiotherapy followed by surgical debulking may extend the survival time of dogs with intranasal tumors in the early stages (Stages I and II and Stage III with only subcutaneous involvement); the study dogs showed a durable DFI (18.5 months). Consistent with Adams *et al*. (2005), the results of

this study suggest that better outcomes are achieved with radiotherapy before surgery than after surgery.

Adams *et al*. (2005) reported that 6 of 13 dogs developed local recurrence of intranasal neoplasia 3–48 months after treatment. In this study involving 14 dogs, recurrence was detected in 9 dogs; 3 of these dogs had Stage III with orbit invasion and 5 had Stage IV disease at the initial presentation. In the remaining 5 dogs, all visible tumor was removed, leaving no bulky disease. Of these 5 dogs, 2 were Stage I, 1 was Stage II, and 2 were Stage III with only subcutaneous involvement. Therefore, early recurrence is more likely in dogs with advanced stage. In our study, all recurrence occurred within 5 months after completion of radiotherapy. The period prior to recurrence was shorter than that observed in Adams *et al*. (2005) and may have resulted from the lower total dose of radiotherapy used in our study.

Complications of surgery and radiotherapy were classified into 2 categories: radiation-related side effects and surgical complications. Several studies have reported severe late radiation side effects in dogs with intranasal tumors treated with hypofractionated radiotherapy, including vision loss and oronasal fistulae (Gieger *et al*., 2008; Maruo *et al*., 2011; Mellanby *et al*., 2002). Adams *et al*. (2005) reported various complications in dogs treated with radiation therapy followed by surgery, including chronic rhinitis in 5 dogs, osteosarcoma of the maxilla 5 years after radiotherapy in 1 dog, and loss of vision within the radiation field in 3 dogs. In this study, surgical complications included subcutaneous emphysema in 4 dogs, infection of bone flaps in 2 dogs, and nasocutaneous fistulae in 2 dogs. At the time of writing, the author does not replace the incised bone after debulking nasal tumors, because 2 of the 4

dogs with replaced bone flaps developed infection followed by development of nasocutaneous fistulae. All radiation side effects were mild; however, the incidence of rhinitis was high. Removing the mucous membrane inside the nasal passage may predispose dogs to rhinitis. In dogs without local tumor recurrence, 2 of the 5 dogs suffered chronic rhinitis (nasocutaneous fistula in one of these dogs) and 1 dog developed an abscess. However, all of the radiation-related side effects were mild, which was likely related to the low total dose (28 Gy). These data demonstrate that the described combination treatment could lead to chronic infection and nasocutaneous fistulae.

The biological effective dose (BED) for the late responding tissue, using an alpha/beta ratio of 3, is 117.3 Gy (4 treatments using 8 Gy per fraction) and 93.3 Gy (4 treatments using 7 Gy per fraction). The late BED of the daily 10 treatment protocol (4.2 Gy per fraction; described by Adams *et al*. [2005]) is 100.8 Gy. Therefore, the risk of late side effects is comparable or potentially higher with the 4 weekly treatment protocol used in this study. This may be a concern, particularly in dogs with lower-stage tumors that may potentially live long term.

There are several limitations in this study. The sample size was small, and various histological tumor types were present. Further, the treatment protocol varied. The author is also aware that it is not always clear whether the tumor has recurred in the long-term survivors. There is also a chance that the dogs in this study that are still alive could potentially develop a radiation-induced tumor in the future.

Although hypofractionated radiation followed by surgery may significantly improve survival

time for dogs in early stages (Stages I, II, and III with subcutaneous involvement only) compared with hypofractionated radiation alone, chronic infection and nasocutaneous fistulae are common complications. The cost of the approach described herein is comparable or exceeds that associated with full-course radiation alone; therefore, the author recommends pursuing full-course radiation rather than taking the approach described in this study.

Chapter 4: Safety of intravenous administration of acridine orange in dogs

Abstract

Acridine orange (AO) has been used for photodynamic therapy. In human medicine, it has been used locally after cytoreductive surgery. However, local administration of AO solution does not appear to result in penetration into deep tissues. Therefore, in invasive tumors, systemic administration of AO is useful. The purpose of this study was to evaluate the short-term safety of intravenous administration of AO (0.1 mg/kg) in dogs.

Five beagles were used in this study. Initial evaluation (control) consisted of a physical examination, complete blood count (CBC), serum chemistry, and serum AO concentration. Clinical signs were observed every day for 1 month. CBC and serum chemistry were obtained 1, 3, 7, and 30 days after AO administration. Serum AO concentrations were measured 0, 7.5, 15, 30, 60, 90, 120, 150, 180, 240, and 300 min after 0.1 mg/kg AO was administered into the cephalic vein over 30 s.

All dogs showed no clinical signs for 30 days. No photosensitivity was noted. All CBC and serum chemistry results were within normal limits. After intravenous injection of AO (0.1 mg/kg), serum AO level decreased rapidly and was below the detection limit (5 ng/mL) 2 h after injection.

These results show that intravenous administration of 0.1 mg/kg AO is safe on a short-term basis. Systemic administration of this drug should be limited to dogs with malignant tumors and a short

life-span, because the long-term effects of systemic AO are unknown.

Introduction

Acridine orange (AO) was first extracted from coal tar as a weak basic dye over 100 years ago (Kusuzaki *et al*., 2007). The photosensitizing effect of AO is well established (Wainwright *et al*., 1997), and this drug is used in photodynamic therapy (PDT) (Coli *et al*., 2006; Kusuzaki *et al*., 2007; Kusuzaki *et al*., 2000ab; Tomson *et al*., 1974). The success of this treatment has been reported in mouse epithelial tumors (Tomson *et al*., 1974) and rat gastric tumors (Tatsuta *et al*., 1984).

Kusuzaki *et al*. (2005) advocated AO-PDT. In intra-lesional or partially marginal tumor excision, AO solution was administered locally, with removal of excess AO and washing out with saline. Then, the tumor was fluorovisualized by blue light excitation; the visualized tumor was treated surgically, and residual tumor was irradiated by xenon light to eradicate tumor cells. In an in vitro model, AO combined with low-dose X-ray irradiation of about 1 to 5 Gy had a strong cytocidal effect on cultured mouse osteosarcoma cells (radiodynamic therapy with AO, AO-RDT) (Hashiguchi *et al*., 2002). Kusuzaki *et al*. (2005) reported good local control of musculoskeletal sarcomas in humans. It appears that local administration of AO solution does not result in penetration into deep tissues. Therefore, in invasive tumors, systemic administration of AO is useful for AO-RDT. Satonaka *et al*. (2010) reported anti-tumor activity with intravenous (IV) administration of 1 mg/kg AO followed by illumination in a mouse osteosarcoma model. However, the toxicity of systemic AO has not been well studied.

Satonaka *et al*. (2006) reported that, based on the results of an acute toxicity study of AO, the

estimated LD_{50} of this substance following IV administration was 27.30 mg/kg in mice. However, the safety of AO in dogs has not been confirmed. The purpose of this study was to evaluate the short-term safety of IV administration of AO (0.1 mg/kg) in dogs.

Materials and Methods

Five male beagles were used in this study with an age of 5.4 ± 1.6 years (mean ± SD) and a body weight of 13.2 ± 1.6 kg. All procedures were performed in compliance with the guidelines of the Animal Research Committee of Azabu University.

Initial evaluation (control) consisted of a physical examination, complete blood count (CBC), serum chemistry, and serum AO concentration. Clinical signs were observed every day for 1 month. CBC and serum chemistry were obtained 1, 3, 7, and 30 days after AO administration. Serum AO concentrations were measured 0, 7.5, 15, 30, 60, 90, 120, 150, 180, 240, and 300 min after AO administration. The dogs were kept under fluorescent light with no light shielding.

AO (acridine orange hydrochloride solution, 10 mg/mL in H_2O; Sigma-Aldrich, St. Louis, MO, USA) at a dose of 0.1 mg/kg was administered into the cephalic vein over 30 s.

Determination of serum AO concentrations

Serum AO concentrations were determined by high-performance liquid chromatography with a

fluorescence detector (Em 492 nm, Ex 523 nm). Separation was achieved with a 3.0 mm × 75-mm column (Ascentis Express, Sigma-Aldrich) in which temperature was maintained at 40°C. The mobile phase composition was 0.025% phosphoric acid and 0.1% octanesulfonic acid in water as mobile phase A and 0.025% phosphoric acid and 0.1% octanesulfonic acid in 80% acetonitrile (20% water) as mobile phase B. A linear time gradient program [Time (min)/%B: 0/30, 3/42, 5/44, 5.01/100, 8/100, 8.01/30] at a flow rate of 1.0 mL/min was used. A mixture of serum sample (400 μL) and acetonitrile (800 μL) was centrifuged at 15,000 *g* for 10 min at 4°C. The supernatant of the mixture (5 μL) was injected and chromatographed under the above conditions. Each sample was measured in duplicate, and a standard curve was prepared in normal dog serum. The limit of quantification of the method was 5 ng/mL. The method was linear between 5 and 100 ng/mL. Inter- and intra-assay coefficients of variation were <10%.

Statistical analysis

The parameters of CBC and serum chemistry were compared using one-way analysis of variance for repeated measures and a posteriori testing with Dunnett's multiple comparison test. Differences were considered significant at $p < 0.05$.

Results

All dogs showed no clinical signs for 30 days. No photosensitivity occurred. Serum creatinine

was significantly different between control and 1 month after AO administration, but this variation was within the normal limit. Other results showed no significant differences between control and later time points, and all results were within normal limits (Table 5).

Time course changes in serum AO concentrations after IV administration of AO (0.1 mg/kg) are shown Fig. 11. The serum AO level decreased rapidly, and it was below the detection limit 2 h after injection.

Discussion

In this study, IV administration of AO at a dose of 0.1 mg/kg was considered safe on a short-term basis, because there were no clinical signs and CBC and serum chemistry values were within normal limits. The serum AO concentration reached a peak immediately after administration, and it was below the detection limit 2 h after injection.

Quinacrine hydrochloride, which is an acridine derivative, has been administered orally as an antiprotozoal drug, and its toxicities have been reported to date. In small animals, a yellow skin and urine color, gastrointestinal disturbances, abnormal behaviors, pruritus, and fever have been noted (Plumb, 2008). Quinacrine crosses the placenta and has been implicated in causing deformity in a human infant (Plumb, 2008). At high doses, it caused increased fetal death rates in rats (Plumb, 2008). Therefore, it was thought that AO, which is an acridine derivative, should not be administered to pregnant animals. IV

administration of AO did not show any side effects such as gastrointestinal disturbances, abnormal behaviors, or pruritus. However, because other toxicity remains unclear, this approach should be limited to dogs with a short life-span.

In general, PDT makes the skin and eyes sensitive to light for 6 weeks or longer after treatment (Ogilvie and Moore, 2006). Cutaneous photosensitization appears to be an uncommon problem in dogs and cats treated with PDT, which may reflect the limited use of Photofrin in these species, differences in photosensitizer distribution compared with humans, or differences in the skin and adnexa among species (Lucroy, 2007). Photosensitization with systemic AO has not been reported (Satonaka *et al*., 2010; Satonaka *et al*., 2006; Tomson *et al*., 1974). In this study, there was no evidence of photosensitivity. Thus, 0.1 mg/kg appears to be safe in dogs.

It was thought that the toxicity of this drug was mild. The International Agency for Research on Cancer (IARC) of the World Health Organization reported that this agent was considered not classifiable regarding carcinogenic effects (Class 3) (IARC, 1978). Some authors reported usage of AO solution at surgical sites in humans (Coli *et al*., 2006; Kusuzaki *et al*., 2005), and no toxicity was reported. With systemic administration, this drug was safe at an oral dose of 500 mg in humans, and the only side effect was mild gastrointestinal symptoms (nausea in 3 and vomiting in 1 of 35 patients) (Katou, 1970). It has been reported that the LD_{50} of IV AO was 27.3 mg/kg in mice (Satonaka *et al*., 2006), and that 1–10 mg/kg IV was safe in mice (Hashiguchi *et al*., 2002; Satonaka *et al*., 2010; Satonaka *et al*., 2011).

Satonaka *et al.* (2006) reported that AO at 0.1 mg/kg IV provided the best visual contrast on digital images. Thus, in this study, 0.1 mg/kg AO was administered IV, and no clinical signs were detected. The serum AO concentration decreased rapidly after the initial peak following injection and was below the limit of detection 2 h after the injection. Serum creatinine was increased 1 month after AO administration, but it was within the normal limit (0.76 ± 0.09 mg/dL). Thus, this was not thought to be clinically significant.

In this study, IV administration of 0.1 mg/kg AO was safe on a short-term basis. Systemic administration of this drug should be limited to dogs with malignant tumors and a short life-span, because the long-term effects of systemic AO are unknown.

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Chapter 5: Intraoperative photodynamic therapy using acridine orange and cribriform electron beam irradiation in canine intranasal tumors

Abstract

Canine intranasal tumors have a poor prognosis. The author describes a treatment protocol involving macroscopic tumor resection and intraoperative acridine orange photodynamic therapy (AO-PDT) together with low-dose photon irradiation (5 Gy) of the tumor bed and, with involvement of the cribriform plate, high-dose electron irradiation of the cribriform plate. Four dogs were treated including 1 dog that had already received hypofractionated radiotherapy followed by surgical resection. In the latter dog, tumor tissue was detected at the cranial part of the surgical site 1 month after completion of the therapy and at the caudal part of the surgical site 23 months after completion of the first therapy. Tumor stages of the 4 dogs included Stage I in 3 tumors and Stage IV (cribriform plate destruction) in 2 tumors. All 5 tumors underwent AO-PDT and low-dose photon irradiation, and cribriform irradiation (15 or 25 Gy) was performed in the 2 tumors that had Stage IV cancer. Recurrence was detected in 2 of 4 dogs at 4 and 7 months after therapy; 1 of these dogs underwent another treatment at 8 months, and the other dog was lost to follow-up at 13 months. One dog did not show recurrence after 7 months of therapy at the time of writing this case series. The dog that had received previous hypofractionated radiotherapy and surgery did not show recurrence after 33 months at the rostral part and after 11 months at the caudal

part of the tumor site at the time of writing this case series. Median follow-up period was 11 months (range, 7–33 months), and side effects were mild (subcutaneous emphysema, rhinitis, and nasal bleeding), except for the case in which hypofractionated radiotherapy had already been performed. Thus, this combination treatment may increase survival time of dogs with macroscopically resectable intranasal tumors without severe side effects. Supplemental cribriform irradiation may control the tumors.

Introduction

Canine intranasal tumors are associated with a poor prognosis. Without treatment, survival time ranges between 0.9 and 5.5 months depending on the clinical stage at the time of diagnosis (MacEwen *et al*., 1977; Morris *et al*., 1994; Norris 1979, Rassnick *et al*., 2006; Yoon *et al*., 2008). Surgical resection does not improve survival because it is extremely difficult to remove the tumor completely (MacEwen *et al*., 1977; Norris, 1979). For this reason, radiotherapy has been the treatment of choice for years (Henry *et al*., 1998; Turek and Lana, 2007). However, radiotherapy does not significantly improve prognosis in dogs with destruction of the cribriform plate, in which survival is only 5–7 months (Adams *et al*., 2009; Gieger *et al*., 2008; Kondo *et al*., 2008; Maruo *et al*., 2011). Furthermore, radiotherapy can be accompanied with severe side effects (LaRue and Gillette, 2007) including vision loss and oronasal fistula (Adams *et al*., 1998; Adams *et al*., 2005; Gieger *et al*., 2008; Lana *et al*., 2004; Maruo *et al*., 2011; McEntee *et al*., 1991; Mellanby *et al*., 2002; Nadeau *et al*., 2004; Northrup *et al*., 2001; Roberts *et al*., 1987; Théon *et al*., 1993).

Various forms of radiotherapy have been used to treat these tumors. Hypofractionated radiotherapy has been performed in dogs previously by using Co-60 (Yoon, 2008). Hypofractionated radiotherapy followed by surgical resection was performed in 14 dogs with intranasal tumors (Chapter 3). The aim of this combination was to prolong local tumor control more than that with hypofractionated radiotherapy alone. Local tumor control was achieved at Stages I and II and Stage III with only cutaneous

invasion by using this combination therapy. Thus, hypofractionated radiotherapy followed by surgical resection was effective only in early stages of the cancer. However, the frequency of complications was high.

Another form of radiotherapy involves the use of acridine orange (AO). AO was first extracted from coal tar as a weak basic dye over 100 years ago (Kusuzaki *et al*., 2007). AO has an absorption peak at 492 nm in 1 μM aqueous solution, and forms at least 2 complexes in solution with both DNA and RNA, with absorption maxima at 465 and 502 nm (Tatsuta *et al*., 1984). AO selectively accumulates in tumor cells and is used for photodynamic therapy (PDT) as it is a radiation sensitizer (Hashiguchi *et al*., 2002; Kusuzaki *et al*., 2005). AO accumulates to a greater extent in malignant musculoskeletal tumors than in benign tumors, normal muscle, and adipose tissue (Matsubara *et al*., 2006). In humans, intra-lesional or partial tumor excision, followed by AO-PDT and low-dose photon irradiation (5 Gy) (Hashiguchi *et al*., 2002), has been used to treat musculoskeletal sarcomas (Kusuzaki *et al*., 2005). This approach reduces tumor recurrence rates without significant side effects (Kusuzaki *et al*., 2005; Matsubara *et al*. 2012). Therefore, the author sought to determine if this approach was suitable for the treatment of intranasal tumors in dogs. Moreover, intraoperative radiotherapy using an electron beam is effective in controlling local tumors (Wolkov, 1998). The author previously reported that intraoperative radiotherapy (IORT) at the tumor bed is effective in prolonging local tumor control (Maruo *et al*., 2012b). The author therefore proposed that irradiation of damaged cribriform plate by supplemental electron beam would be useful to

control the tumor.

This study describes a protocol for treating intranasal tumors in dogs. This protocol involves combined use of macroscopic resection and intraoperative AO-PDT together with low-dose photon irradiation (5 Gy) of the macroscopically resected tumor bed in all cases and high-dose electron irradiation of the cribriform plate if the cribriform plate is damaged. The cases reported here demonstrate that this combination therapy may increase survival time in dogs with macroscopically resectable intranasal tumors. Supplemental cribriform irradiation may control local tumors of the cribriform plate. This study was carried out with the hypothesis that macroscopic resection with PDT would achieve comparable results (being effective in the early stages of the cancer) to hypofractionated radiotherapy followed by surgery, and may decrease complications including recurrence of the cancer at the cribriform plate.

Methods

Atropine sulfate hydrate (0.025 mg/kg s.c.; Atropin Sulfate Injection, Mitsubishi Tanabe Pharma Co., Osaka, Japan) and morphine hydrochloride hydrate (0.25 mg/kg s.c.; Morphine Hydrochloride, Shionogi & Co. Ltd., Osaka, Japan) were administered 15 min before induction of anesthesia; 0.1 mg/kg AO (acridine orange hydrochloride solution, stock solution, 10 mg/mL in H_2O; Sigma-Aldrich, St. Louis, MO, USA) was administered intravenously at the same time as anesthetic

induction. Systemic administration of AO as a radiation sensitizer was permitted by the Animal Research Committee of Azabu University, and the owners provided informed consent for the study. AO was sterilized by microfiltration with a membrane filter (25AS020AS; Advantex MFS, Inc., Dublin, CA, USA). Propofol (8 mg/kg i.v.; Rapinovet, Takeda Schering-Plough Animal Health Corporation, Osaka, Japan) was used for anesthetic induction and to aid intubation. After induction, anesthesia was maintained by isoflurane (Isoflurane for Animals, Mylan Inc., Osaka, Japan). Cefazolin (25 mg/kg i.v.; Cefamezin Alfa, Astellas Pharma Inc., Tokyo, Japan) was administered for preoperative microbial prophylaxis.

Prior to surgical debulking, the rostral and caudal margins of the tumor were identified on computed tomography (CT) images according to the distances from the rostral end of the nasal planum. The skin incision extended 5 mm beyond the rostral and caudal margins of the tumor. A chisel was used to remove the bone and expose the nasal cavity. The periosteum was separated from the bone and was sutured back to the bone after the procedure. If the tumor had metastasized into the bone, the bone and the associated periosteum were permanently removed. Tumor removal was performed using suction and curettage. The tumor and surrounding mucous membrane were removed until the bone was exposed. For residual tumors, AO-PDT was performed. The surgical site was packed with gauze soaked in AO solution (1 μg/mL AO in saline) for 5 minutes. Thereafter, xenon light (Xenon Nova 175; Karl Storz Endoscopy Japan K. K., Tokyo, Japan) was used to irradiate the area for 10 minutes (AO-PDT). Wavelength of the emitted light was 400–700 nm, and power density was 20.7 (mW/cm^2) at 10 cm from the light source.

This value was measured by a spectro-radiometer (USR-45DA-14; Ushio Inc., Tokyo, Japan). If destruction of the cribriform plate was evident, supplemental electron beam irradiation was performed at an intensity of 20 Gy (2-cm cone, 5–12 MeV; depending on the target depth, approximately 0.5 cm of gauze soaked in saline was used as a bolus) between AO-PDT and skin closure (Fig. 12).

After the skin was closed, irradiation was performed. For radiotherapy, 3D computer planning software (XiO; Elekta K. K., Tokyo, Japan) was used; the margin was set at 1 cm from the edge of the tumor, and 1 portal field was irradiated from above. A 6-MV linear accelerator (Primus; Toshiba Medical System, Tokyo, Japan) was used with a 1-cm bolus, and the tumor bed was irradiated with 5 Gy. After surgery, CT was performed. Fentanyl citrate (1–2 μg/kg/h; Fentanyl injection, Janssen Pharmaceutical K. K., Tokyo, Japan) was administered intravenously until the morning after surgery. The dogs were discharged the day after surgery and prescribed piroxicam (0.3 mg/kg p.o. s.i.d; Baxo, Toyama Chemical Co., Ltd., Tokyo, Japan) for analgesia for 1 week and cephalexin (20–30 mg/kg p.o. b.i.d.; Larixin, Toyama Chemical Co., Ltd.) for 1 week.

TNM classification was performed according to the guidelines of the World Health Organization (Owen, 1980), and the tumor was staged according to Adams *et al*. (1998). Radiation side effects were classified using the Veterinary Radiation Therapy Oncology Group (VRTOG) classification (LaDue and Klein, 2001).

Case 1

A 13-year-old male Shiba Inu weighing 10.9 kg was brought to Azabu University Veterinary Teaching Hospital with a 2-week history of nasal bleeding. Findings of physical examination, complete blood count (CBC), blood chemistry, and chest radiography were normal. Increased opacity of the right nasal cavity was noted on a ventrodorsal radiograph. A CT scan (Asteion; Toshiba Medical System, Tokyo, Japan) revealed a mass on the right side and slight destruction of the nasal turbinate around the cribriform (TAC) plate (Fig. 13 left, arrows). The bony tissues surrounding the nasal cavity and the cribriform plate were intact. The tumor was classified as T1N0M0 and Stage I. Following histologic examination of the tumor, adenocarcinoma was diagnosed.

This dog was operated on according to the method described above without cribriform irradiation, as this dog did not have cribriform plate destruction. The treatment procedure was performed 3 weeks after the initial diagnosis. The entire treatment procedure was performed in 180 min (Fig. 13 right).

The dog was discharged from our animal hospital on the day after treatment and did not re-attend for follow-up. The author conducted a telephonic follow-up interview with the referring veterinarian and the owner, who both reported no evidence of surgical complications or radiation side effects. Seven months after treatment, the dog presented to the referring veterinarian with nasal discharge and allergic dermatitis. A ventrodorsal radiograph showed that the caudal aspect of the affected side had

increased radiological density, suggesting recurrence around the cribriform plate. The dog was treated with prednisolone intermittently for allergic dermatitis; the nasal discharge subsequently disappeared. Thirteen months after surgery, the dog again presented to the referring veterinarian with diarrhea; thereafter, contact with the animal and owner was lost, and further follow-ups were not possible. At the last visit, the referring veterinarian did not note nasal symptoms or facial deformity. Acute and chronic radiation side effects were not detected, and the VRTOG classification was 0.

Case 2

A 12-year-old spayed female Beagle weighing 9.5 kg was brought into our department with a 2-week history of nasal discharge and facial deformity. Findings of CBC and serum chemistry were within normal limits. Destruction of the cribriform plate was observed on CT (Fig. 14). The tumor was classified as T3N0M0 and Stage IV. On histologic examination of the tumor, transitional carcinoma was diagnosed.

This dog was operated on according to the method described above. The dog was administered AO (0.1 mg/kg) intravenously before anesthesia. Electron beam irradiation of 12 MeV was selected (0 degrees, Fig. 12). The entire treatment procedure was performed in 150 minutes.

Subcutaneous emphysema from head to trunk persisted for 3 weeks after surgery, and chronic rhinitis was detected. However, photosensitivity did not occur. CT scans were obtained every month. Four

months after surgery, a mass was detected near the rostral surgical site, while destruction of the cribriform plate had progressed. On histological examination of the mass, transitional carcinoma was diagnosed and recurrence was confirmed. CT and MRI performed 7 months after surgery confirmed the presence of tumor at the rostral aspect (Fig. 15). Although the tumor had not advanced to the cribriform plate, fluid was detected, and progressive cribriform plate destruction was observed. The dog received further treatment 8 months after surgery because of invasion of the tumor into the skin. Acute and chronic radiation side effects were not detected, and the VRTOG classification was 0. There was no further follow-up after this, and the dog was euthanized 13 months after surgery because of a general deterioration in condition.

Case 3

A 12-year-old male Labrador Retriever weighing 31.0 kg was diagnosed with nasal transitional carcinoma. The tumor was classified as T3N0M0 and Stage III. The dog underwent radiotherapy once a week for 4 weeks and received a total radiation dose of 28 Gy. At the last session of radiotherapy, surgical resection of the tumor was performed and the bone flap was reattached. A nasocutaneous fistula was detected 1 month after surgery. The nasocutaneous fistula arose because of necrosis of the returned bone flap. On CT examination, tumor recurrence was observed at the rostral aspect of the nasal cavity (premolar level 1–2), and the tumor was classified as T1N0M0 and Stage I.

The animal was operated on according to the method described above without cribriform irradiation, because the tumor did not induce destruction of the cribriform plate. The entire treatment procedure was performed in 90 minutes. No recurrence of the tumor was identified for 33 months after the procedure and at the time of writing this case series.

At 22 months after AO-PDT, a mass was observed below the nasocutaneous fistula at the cribriform plate, which was located at the caudal border of the first surgery. The tumor extended 3 cm in diameter around the cribriform plate, and slight lysis of the cribriform plate was detected on CT (Fig. 16). The tumor was classified as T3N1M0 and Stage IV, and diagnosed as nasal transitional carcinoma. At this time, the dog had developed keratoconjunctivitis sicca (KCS, VRTOG Grade 1), cataract (VRTOG Grade 2), chronic rhinitis, and nasocutaneous fistula.

As this animal showed signs of cribriform destruction, irradiation was performed at a 75-degree angle from the vertical (5 MeV, 10 Gy). The irradiation dose was reduced as the site had already been irradiated. The entire treatment procedure was performed in 120 min.

Clinical symptoms had not become worse 11 months after the second AO therapy. The dog is still alive 11 months after the second AO therapy and at the time of writing this case series.

Case 4

A 13-year-old female Labrador Retriever weighing 25.0 kg was diagnosed with nasal

adenocarcinoma. The tumor was classified as T1N0M0 and Stage I (Fig. 17).

The animal was operated on according to the method described above without cribriform irradiation, because the tumor did not induce destruction of the cribriform plate. The entire treatment procedure was performed in 120 min.

Side effects of this treatment were transient rhinitis and nasal bleeding. These signs resolved within 1 month. The tumor has not recurred for 7 months and at the time of writing this case series.

Discussion

In general, PDT has been used as a single treatment modality for the treatment of canine intranasal tumors (Lucroy *et al*., 2003; Osaki *et al*., 2009). This case report describes 3 additions to the traditional PDT approach: macroscopic tumor resection; intraoperative AO-PDT; low-dose (5 Gy) irradiation of the tumor bed and, if the cribriform plate is damaged, high-dose electron irradiation of the cribriform plate. This unique combination resulted in survival times that were comparable with those of other treatment modalities; this is remarkable considering that 2 patients were diagnosed with Stage IV tumors. These findings may represent an efficacy comparable with that of radiation monotherapy and considerable improvements in safety.

Several drugs, including pyropheophorbide-a-hexyl ether (Lucroy *et al*., 2003) and benzoporphyrin derivative monoacid ring-A (Osaki *et al*., 2009), have been used for PDT therapy in cases

of canine intranasal tumor. Lucroy *et al*. reported that clinical signs were controlled for a variable time, although long-term responses were comparable with those of radiation therapy in 2 of 3 animals (Lucroy *et al*., 2003). Osaki *et al*. (2009) reported that the 1-year survival rate of 7 dogs with intranasal tumor was 57%; however, 50% of the dogs developed nasocutaneous or oronasal fistula. In this report, 2 dogs survived for 8 and 13 months, while another dog survived without recurrence of the tumor for 33 months at the rostral part and 11 months at the caudal part of the original tumor site. This result was comparable to those of other reports (Lucroy *et al*., 2003; Osaki *et al*., 2009). Thus, intraoperative AO-PDT may be useful to treat dogs with intranasal tumor.

In this report, the tumor bed was irradiated with low-dose radiation (5 Gy), while high-dose (total 15 and 25 Gy) radiation was only used for the cribriform plate. The animal described in Case 3 developed KCS and cataracts, but these may have been side effects of prior hypofractionated radiotherapy, as this protocol involved irradiation of only 5 Gy to the eyes. Gradual bone lysis at the cribriform plate was also observed in 1 dog, but other severe side effects were not detected. Chronic rhinitis was detected in 2 cases, Case 2 and 3. The animal in Case 3 had already undergone prior hypofractionated radiotherapy, and the animal in Case 2 had undergone extensive resection and cribriform irradiation. However, the animals in Case 1 and Case 4 developed only transient rhinitis as a side effect. These findings indicate that the use of AO-PDT and low-dose photon irradiation may not be associated with significant side effects.

Cutaneous photosensitization appears to be an uncommon problem in dogs and cats treated with PDT; this may reflect several factors: the limited use of Photofrin in these species, differences in photosensitizer distribution compared with humans, or differences in the skin and adnexa among species (Lucroy, 2007). Photosensitization with systemic AO has not been reported in mice (Satonaka *et al*., 2010; Satonaka *et al*., 2006; Tomson *et al*., 1974). This drug was found to be safe at an oral dose of 500 mg in humans, and the only side effects reported were mild gastrointestinal symptoms (3 cases of nausea and 1 case of vomiting out of 35 patients enrolled), and photosensitivity was not described (Katou, 1970). Some studies reported usage of AO solution at surgical sites in humans, and no side effects were reported (Coli *et al*., 2006; Kusuzaki *et al*., 2005). The author previously confirmed the safety of systemic AO (0.1 mg/kg) in dogs (Maruo *et al*., 2012a). All dogs showed no clinical signs for 30 days, and no photosensitivity was reported. All blood parameters such as CBC and serum chemistry were within normal limits. In this study, there was no evidence of AO side effects including photosensitivity. It is thought that chronic rhinitis observed in Cases 2 and 3 resulted from cribriform irradiation and hypofractionated radiotherapy, respectively. Thus, local and systemic administration of AO (0.1 mg/kg) appears to be safe in dogs.

Similar studies on treatments of epithelial tumors in mice and implanted gastric tumors in rats used argon lasers (Tatsuta *et al*., 1984; Tomson *et al*., 1974). Kusuzaki *et al*. performed AO-PDT with an interference filter (466.5 nm) for selection of the blue beam from a xenon lamp (Kusuzaki *et al*., 2005).

Ueda *et al*. reported that strong unfiltered light from a xenon lamp is more effective and feasible than weak filtered blue light for effective AO-PDT in clinical practice (Ueda *et al*., 2005). Thus, the author used an unfiltered xenon lamp during our procedure.

Destruction of the cribriform plate is associated with a poor outcome in cases of canine intranasal tumor (Adams *et al*., 2009). In cases where the cribriform plate was destroyed, median survival time was only 5–7 months after radiotherapy (Adams *et al*., 2009; Gieger *et al*., 2008; Kondo *et al*., 2008; Maruo *et al*., 2011). In Cases 2 and 3 involving the caudal part of the nasal cavity, recurrence was predicted around the cribriform plate. However, recurrence around the cribriform plate was not detected during a 7-month follow-up period in either dog. In Case 1, the cribriform plate was intact, but destruction was detected at the TAC. Tumors located at the TAC may be difficult to remove completely; this likely accounted for recurrence of the tumor in this location in this case. In dogs with intranasal tumors, supplemental electron beam irradiation of the cribriform plate may delay tumor recurrence or control the local tumor.

Several limitations were identified during the course of the study. The main limitation is that there was a small sample size, and the study involved animals with intranasal tumors at various stages. The animal described in Case 3 had already undergone radiotherapy and surgery. IORT is usually applied at approximately 25 Gy at the cribriform plate (Hoekstra *et al*., 1989), and the protocol already uses radiation at a dose of 5 Gy. Thus, a cribriform irradiation dose of 20 Gy was chosen for the second round

of surgery, because the animal had already received a radiation dose of 28 Gy/4 fractions. The dog was then given a dose of 15 Gy. The animal in this case also showed symptoms of rhinitis and other side effects, but it was difficult to distinguish between side effects due to radiation or AO-PDT. In this study, the author performed surgery and AO-PDT for dogs with macroscopically resectable intranasal tumor, as the effects of AO and xenon light irradiation are only superficial. The author believes that local administration of AO does not result in uniform distribution of AO in residual tumor mass at the TAC, and therefore the author administered AO systemically in Case 2. However, the efficacy of systemic administration was not determined in this study.

In summary, this case report describes an efficient combination therapy for the treatment of canine intranasal tumor. The combination of macroscopic tumor resection and intraoperative AO-PDT, and low-dose photon irradiation (5 Gy) of the tumor bed, may increase survival time for dogs with macroscopically resectable intranasal tumor. In animals with tumor-induced damage to the cribriform plate or TAC, supplemental cribriform irradiation may be useful in controlling the tumor.

Conclusion

The author revealed five important findings with the results of this study. First, the 2 new bite-block-type head-immobilization devices yield greater stability and reduced vertical variation. Second, hypofractionated multiportal field and two-portion (rostral and caudal portions divided by the eyelid) radiation therapy in canine intranasal tumor reduces side effects and is suitable in dogs with cribriform plate destruction. Third, hypofractionated radiotherapy followed by surgery may significantly improve survival time in dogs in early stages (Stages I, II, and III with subcutaneous involvement only) compared with hypofractionated radiotherapy alone, but chronic infection and nasocutaneous fistulae are common. Fourth, systemic administration of AO (0.1 mg/kg) in dogs is safe on a short-term basis. Fifth, the combination of macroscopic tumor resection and intraoperative AO-PDT together with low-dose photon irradiation (5 Gy) of the tumor bed may increase survival time in dogs with macroscopically resectable intranasal tumors. Moreover, if the cribriform plate or nasal turbinate around the cribriform plate is destroyed, supplemental cribriform irradiation may control the local tumor. Thus, as a result of this study, the author developed a simplified positioner and reduced the treatment burden for dogs and owners.

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Table 1. Clinical staging method of this study (Adams *et al*. [1998])

Stage and substage	Description
I	Confined to one nasal passage, paranasal sinus or frontal sinus, with no bone involvement beyond turbinates
II	Any bony involvement (beyond turbinates), but with no evidence of orbit/subcutaneous/submucosal mass
III	Orbit involved or subcutaneous or submucosal mass
IIIa	Tumor extension not in the orbit
IIIb	Orbital involvement
IV	Tumor causing lysis of the cribriform plate or extension into nasopharynx

Table 2. Characteristics of the dogs

No.	Breed	Clinical signs	Duration of clinical signs prior to presentation (months)	TNM classification (Owen, 1980)	Tumor stage (Adams *et al*., 1998)	Tumor type
1	Shetland sheepdog	Ep, SD, FD	1	T3N0M0	IIIa	CA
2	Pembroke Welsh Corgi	FD	6	T3N0M0	IIIa	AC
3	Labrador Retriever	SD, FD	2	T3N0M0	IV	CSA
4	Shiba Inu	Ep, FD	2	T3N0M0	IV	FSA
5	German Shepherd	Ep, SD	2	T1N0M0	I	AC
6	Pembroke Welsh Corgi	Ep, FD	1	T3N0M0	IIIb	OSA
7	Miniature Dachshund	Ep, FD	2	T3N0M0	IV	AC
8	Labrador Retriever	Ep	2	T3N0M0	IV	TC
9	Labrador Retriever	FD	1	T3N0M0	IIIb	OSA
10	Pomeranian	SD	1	T3N0M0	IIIb	TC
11	Golden Retriever	Ep	1	T1N0M0	I	CA
12	Labrador Retriever	Ep, SD	2	T1N0M0	I	AC
13	Pembroke Welsh Corgi	Ep, SD, FD	2	T3N0M0	IV	AC
14	Shih Tzu	SD	1	T2N0M0	II	SCC

Ep, epistaxis; SD, sneezing and nasal discharge; FD, facial deformity

AC, adenocarcinoma; CA, carcinoma; SCC, squamous cell carcinoma; TC, transitional carcinoma;

OSA, osteosarcoma; CSA, chondrosarcoma; FSA, fibrosarcoma

Table 3. Summary of treatment methods and outcome

No.	Total dose / fractions	Timing of surgery relative to RT	Bone flap	Surgical complications	Rhinitis	Macroscopic tumor remaining after surgery?	Recurrence	DFI (months)
1	32 Gy/4	1.5 months post RT	Removed	ND	ND	No	-	>26
2	28 Gy/4	2 months post RT	Removed	ND	Abscess (frontal sinus)	No	-	39
3	28 Gy/4	1.5 months post RT	Removed	ND	Rhinitis	Yes	+	2
4	28 Gy/4	1 month post RT	Removed	Subcutaneous emphysema	ND	Yes	+	3
5	28 Gy/4	1.5 months post RT	Returned	ND	Chronic rhinitis	No	-	16
6	28 Gy/4	Last RT (4th)	Removed	Subcutaneous emphysema	ND	Yes	+	3
7	28 Gy/4	Last RT (4th)	Removed	Subcutaneous emphysema	ND	No	+	2
8	28 Gy/4	Last RT (4th)	Returned	Bone necrosis, fistulae	Rhinitis	No	+	1
9	28 Gy/4	Last RT (4th)	Removed	ND	ND	Yes	+	2
10	28 Gy/4	Last RT (4th)	Returned	ND	Rhinitis	Yes	+	3
11	28 Gy/4	Last RT (4th)	Returned	Bone necrosis, fistulae	Chronic rhinitis	No	-	>21
12	28 Gy/4	Last RT (4th)	Removed	ND	ND	No	+	5
13	28 Gy/4	Last RT (4th)	Removed	Subcutaneous emphysema	Rhinitis	No	+	3
14	28 Gy/4	1 month post RT	Removed	ND	Chronic rhinitis	No	-	>12

ND, not detected; DFI, disease-free interval

Table 4. Results of univariate analysis

Variables	*P* value
Sex	0.369
Body weight (<15 kg vs. ≥15 kg)	0.285
TNM classification	0.290
Stage (I–IIIa vs. IIIb and IV)	<0.001
Tumor type (carcinoma vs. sarcoma)	0.065
Macroscopic tumor remaining after surgery	0.052
Recurrence	<0.001
Duration of clinical signs prior to presentation (1 month vs. ≥2 months)	0.204
Timing of surgery relative to RT (during RT vs. ≥1 month post RT)	0.110

Table 5. CBC and serum chemistry results over time

	Reference range	Control	After 1 day	After 3 days	After 7 days	After 1 month
Total WBC ($\times 10^3/\mu L$)		10.8 ± 2.3	13.0 ± 3.8	12.5 ± 1.6	13.0 ± 3.2	13.8 ± 5.0
RBC ($\times 10^6/\mu L$)		6.62 ± 0.58	6.13 ± 0.94	6.78 ± 0.35	6.60 ± 0.31	6.61 ± 0.54
Hemoglobin (g/dL)		15.7 ± 0.7	14.7 ± 2.3	15.9 ± 0.6	15.6 ± 0.8	15.4 ± 1.5
PCV (%)		45.4 ± 2.5	42.1 ± 6.2	46.2 ± 1.2	44.9 ± 1.6	45.0 ± 4.0
MCV (fL)		68.7 ± 3.5	68.7 ± 3.4	68.2 ± 3.2	68.0 ± 3.1	67.2 ± 1.6
MCH (pg)		23.8 ± 1.5	24.1 ± 1.7	23.5 ± 1.5	23.7 ± 1.6	23.0 ± 0.5
MCHC (%)		34.7 ± 0.9	35.0 ± 0.9	34.4 ± 0.7	34.8 ± 1.0	34.2 ± 0.3
Platelets ($\times 10^3/\mu L$)		327 ± 121	358 ± 188	379 ± 947	373 ± 98	257 ± 29
Total protein (g/dL)	5.2–8.2	6.6 ± 0.6	6.3 ± 0.5	6.6 ± 0.6	6.4 ± 0.5	6.4 ± 0.5
Albumin (g/dL)	2.7–3.8	3.0 ± 0.4	2.9 ± 0.4	3.0 ± 0.4	2.9 ± 0.3	2.9 ± 0.3
A/G		0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.3
Aspartate aminotransferase (IU/L)	0–50	30.2 ± 4.6	30.6 ± 9.4	31.4 ± 7.5	29.2 ± 6.6	30.2 ± 5.3
Alanine aminotransferase (IU/L)	10–100	46.2 ± 16.4	45.0 ± 14.1	51.8 ± 18.7	46.4 ± 15.2	37.2 ± 17.0
Alkaline phosphatase (IU/L)	23–212	79.2 ± 33.7	80.8 ± 30.2	74.8 ± 27.0	69.8 ± 19.6	71.0 ± 12.6
γ-glutamyltransferase (IU/L)	0–7	4.0 ± 1.0	4.2 ± 0.8	4.8 ± 1.3	4.6 ± 0.5	4.8 ± 0.8
Amylase (IU/L)	500–1500	793 ± 177	748 ± 146	790 ± 154	779 ± 68	936 ± 67
Lipase (IU/L)	200–1800	267 ± 120	283 ± 197	275 ± 79	249 ± 79	426 ± 183
Urea nitrogen (mg/dL)	7–27	11.8 ± 1.1	10.0 ± 1.6	14.2 ± 4.7	13.8 ± 5.8	17.0 ± 5.6
Creatinine (mg/dL)	0.5–1.8	0.60 ± 0.07	0.62 ± 0.08	0.70 ± 0.12	0.62 ± 0.08	0.76 ± 0.09*
Total cholesterol (mg/dL)	110–320	146.0 ± 15.3	134.8 ± 8.5	141.0 ± 11.8	137.6 ± 8.8	138.2 ± 12.0
Triglycerides (mg/dL)	10–100	53.4 ± 46.7	21.4 ± 5.5	75.0 ± 41.2	64.8 ± 30.9	38.6 ± 14.7
Na^+ (mEq/L)	134–153	148.4 ± 2.9	147.4 ± 3.2	147.0 ± 2.7	147.8 ± 1.8	147.2 ± 2.4
Cl^- (mEq/L)	105–118	111 ± 2	112 ± 2	110 ± 4	111 ± 3	114 ± 2
K^+ (mEq/L)	3.4–4.6	3.9 ± 0.4	4.0 ± 0.3	3.9 ± 0.1	4.1 ± 0.3	4.2 ± 0.1
Calcium (mg/dL)	7.9–12.0	10.4 ± 0.5	10.2 ± 0.4	10.2 ± 0.3	10.1 ± 0.4	10.1 ± 0.5
Inorganic phosphorus (mg/dL)	2.5–6.8	4.0 ± 0.5	4.4 ± 0.8	4.1 ± 0.6	3.9 ± 0.6	3.8 ± 0.3
Glucose (mg/dL)	77–125	93.8 ± 3.9	103.0 ± 8.0	90.4 ± 15.2	90.8 ± 16.6	86.4 ± 3.3

Data are expressed as mean ± SD of 5 dogs. *$p < 0.05$ vs. Control with one-way analysis of variance for repeated measures and a posteriori testing with Dunnett's multiple comparison test. WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; A/G, albumin/globulin.

Figure legends

Fig. 1. Use of the bite-block-type head-immobilization devices. Device A was constructed from acrylate resin (1 cm in thickness), and was rectangular shaped and 13 cm in length, 5 cm in width, and 7 cm in height. The dogs were placed in the dorsal–ventral position, and the canine teeth were placed on the head of the immobilization device. In Device B, a groove (arrow) was created perpendicular to the long axis and the tips of both canine teeth were placed in the groove. In Device C, the groove was created on the underside of the dorsolateral plate (arrow indicates groove). The size was the same as that of Device B. The dogs were placed in the ventral–dorsal position. To maintain the tips of the canine teeth in the groove, a sponge (asterisk) was used to compress the muzzle from below.

Fig. 2. For image comparison, 2 images were loaded on the screen. The left image was inserted first followed by the right image. Compared with the left image slice, the upper right image was 1 mm back (caudal), the middle right image was almost the same, and the lower right image was 1 mm forward (cranial). Arrows indicate significantly different parts between images.

Fig. 3. A comparison of the setup time for Devices A, B, and C. The data are expressed as the variability of 5 setup times (min) for 5 dogs with Device A, Device B (upper groove), and Device C (lower groove). The boxes show the range of 25–75%; the whiskers, the range of 5–95%; and the small dots, the means. The horizontal lines show the median values.

Fig. 4. Comparison of computed tomography scan variability on the *x* axis for the 3 bite-block-type head-immobilization devices in the study. Data are expressed as the variability of 5 measurements (mm) conducted in 5 dogs with Device A, Device B (upper groove), and Device C (lower groove). The boxes show the range of 25–75%; the whiskers, the range of 5–95%; and the small dots, the means. The horizontal lines show the median values. NS: not significant.

Fig. 5. Comparison of computed tomography scan variability on the *y* axis for the 3 bite-block-type

head-immobilization devices in the study. Data are expressed as the variability of 5 measurements (mm) conducted in 5 dogs with Device A, Device B (upper groove), and Device C (lower groove). The boxes show the range of 25–75%; the whiskers, the range of 5–95%; and the small dots, the means. The horizontal lines show the median values. NS: not significant.

Fig. 6. Comparison of computed tomography scan variability on the *z* axis for the 3 bite-block-type head-immobilization devices in the study. Data are expressed as the variability of 5 measurements (mm) conducted in 5 dogs with Device A, Device B (upper groove), and Device C (lower groove). The boxes show the range of 25–75%; the whiskers, the range of 5–95%; and the small dots, the means. The horizontal lines show the median values. NS: not significant.

Fig. 7. Comparison of computed tomography scan variability of the three-dimensional vectors of the 3 bite block-type head-immobilization devices in the study. Data are expressed as the variability of 5 measurements (mm) conducted in 5 dogs with Device A, Device B (upper groove), and Device C (lower groove). The boxes show the range of 25–75%; the whiskers, the range of 5–95%; and the small dots, the means. The horizontal lines show the median values. NS: not significant.

Fig. 8. To reduce the dose of the eyeballs, the irradiation field was divided into 2 portions (rostral and caudal portions) by the eyelid.

Fig. 9. Radiotherapy planning of the posterior portion by the eyelid. To reduce the radiation dose in the eye and other surrounding tissues, radiation was divided into 3 portal fields, and the tumor was irradiated with at least 80% of the isocenter dose (left and right: rostral and caudal portions, respectively). The median total dose at the center of the eyeball on the irradiated side was 9.5 Gy (range, 0–35.5 Gy) for irradiation of the caudal portion in 55 of the dogs.

Fig. 10. Kaplan–Meier survival curves with or without cribriform plate destruction. There was no difference in median survival time between dogs with cribriform plate destruction before radiotherapy (gray line; n = 35, 163 days) and those without (black line; n = 28, 219 days).

Fig. 11. Time course changes in serum concentration of acridine orange (AO) after intravenous administration of AO (0.1 mg/kg). Data are expressed as mean ± SD of 5 dogs. The serum AO level decreased rapidly and was below the detection limit (5 ng/mL) 2 h after injection.

Fig. 12. Isodose curves from the dorsal plane in Case 2. The tumor bed was irradiated over 5 Gy (aqua line), and the cribriform plate was irradiated over 20 Gy (green line).

Fig. 13. CT findings in Case 1 before surgery (left; 3 weeks before surgery) and after surgery (right). Tumor filled the right side of the nasal cavity (A), and slight destruction of the nasal turbinate around the cribriform plate was detected (B, arrows). Macroscopic resection of the tumor was possible. CT images immediately after surgery (C and D).

Fig. 14. CT findings in Case 2. Destruction of the cribriform plate was detected.

Fig. 15. CT and MRI (T2-weighted) findings 7 months after surgery in Case 2. The tumor recurred between the rostral and middle aspects, and fluid was detected around the cribriform plate (A). Progressive cribriform plate destruction was observed (B).

Fig. 16. CT findings 22 months after AO therapy in Case 3. Slight lysis of the cribriform plate was detected.

Fig. 17. CT findings in Case 4. Tumor was located only in the nasal passage.

Figure 1

Figure 2

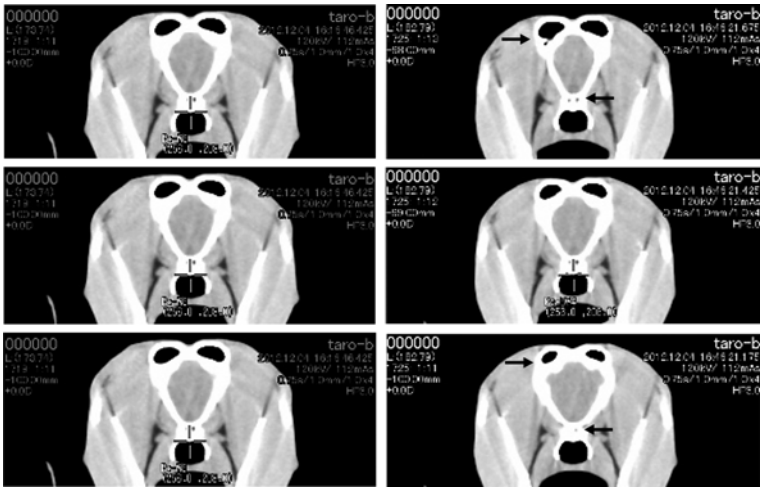
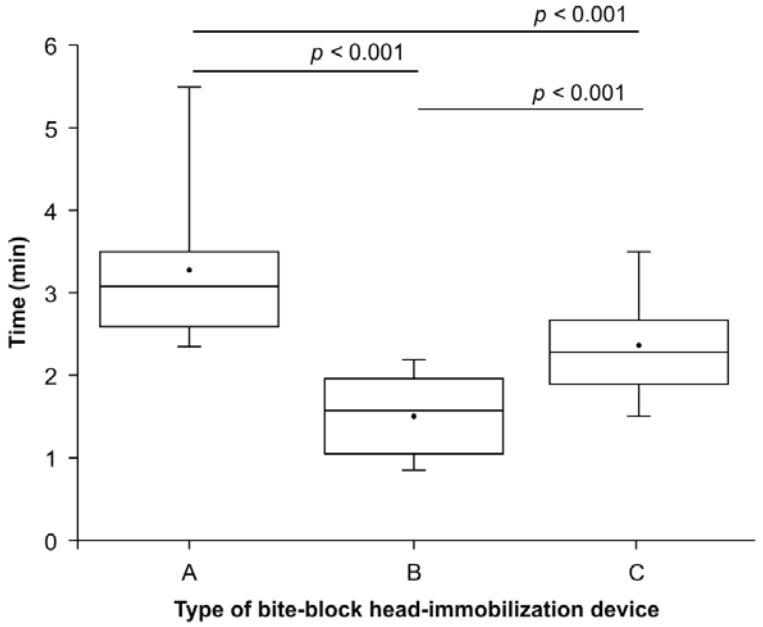


Figure 3



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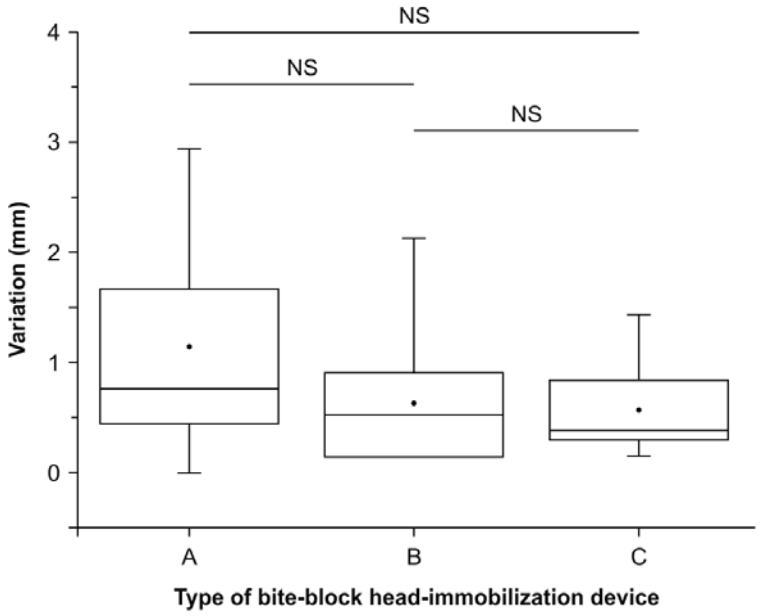

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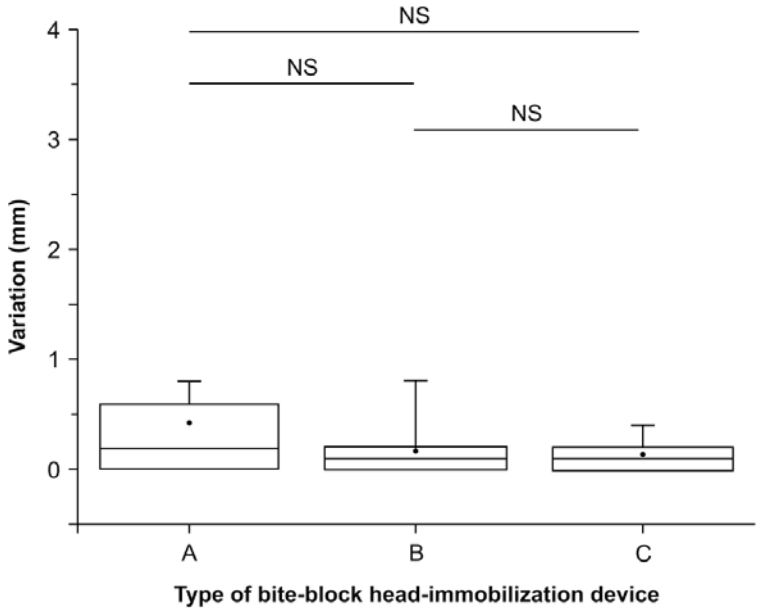

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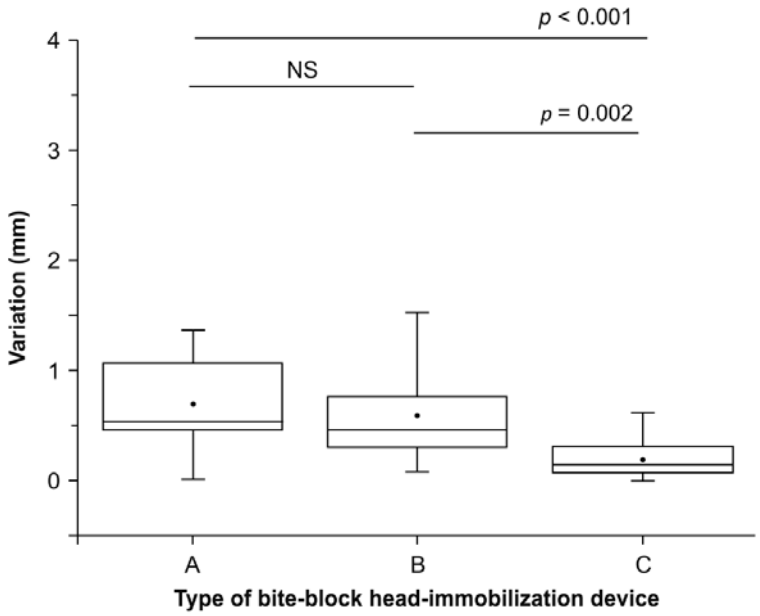

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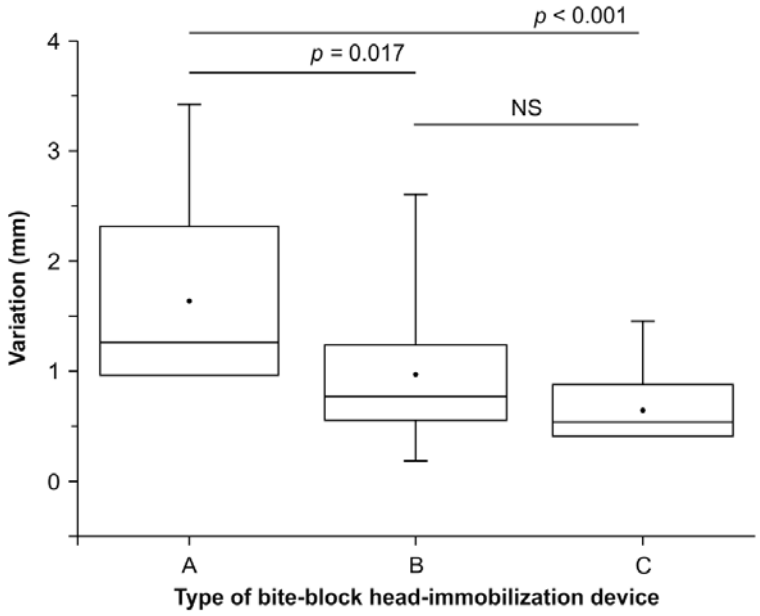

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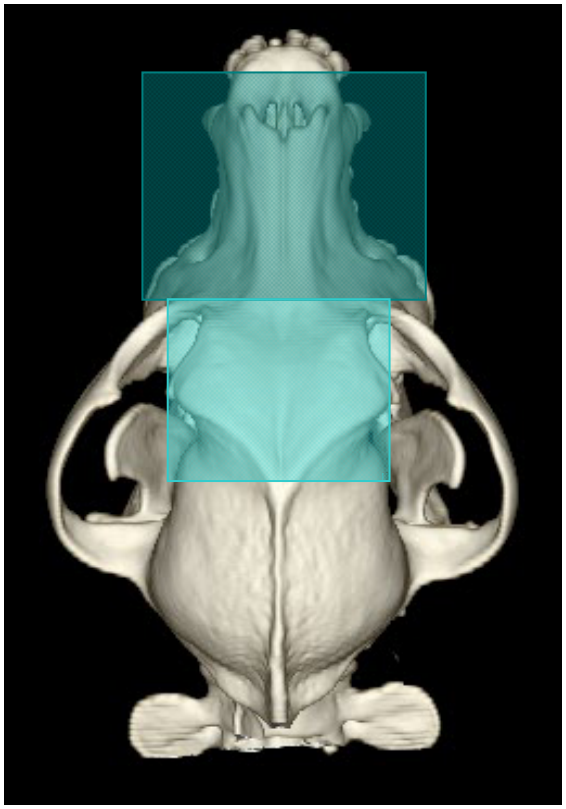

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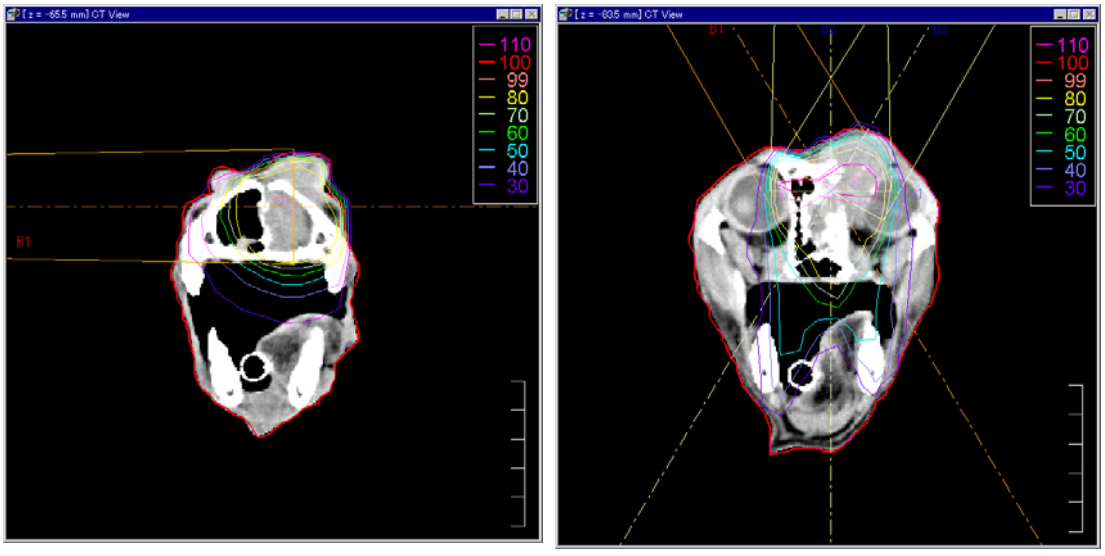

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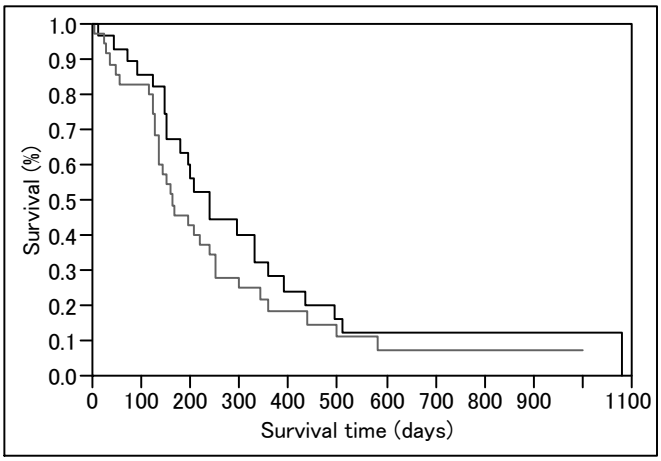

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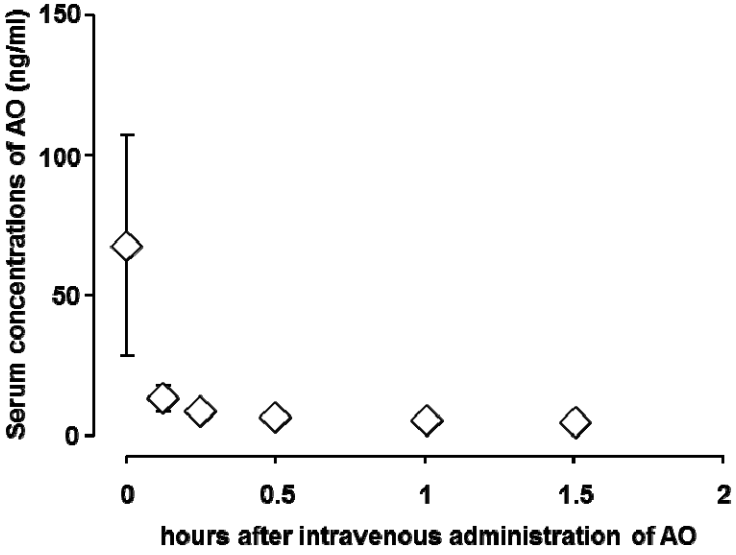

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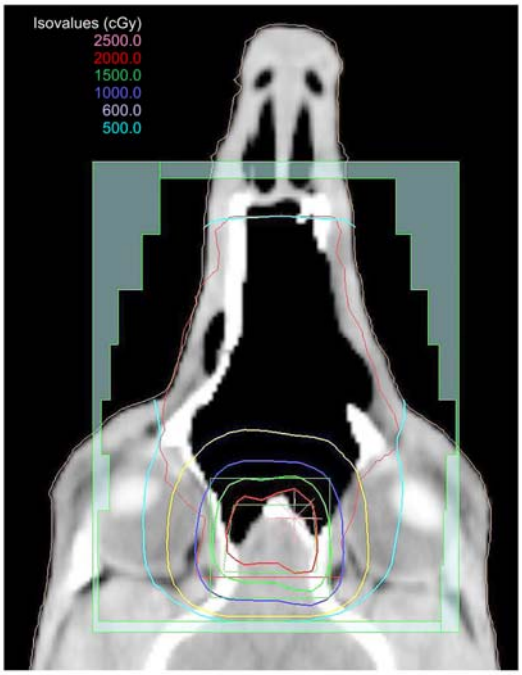

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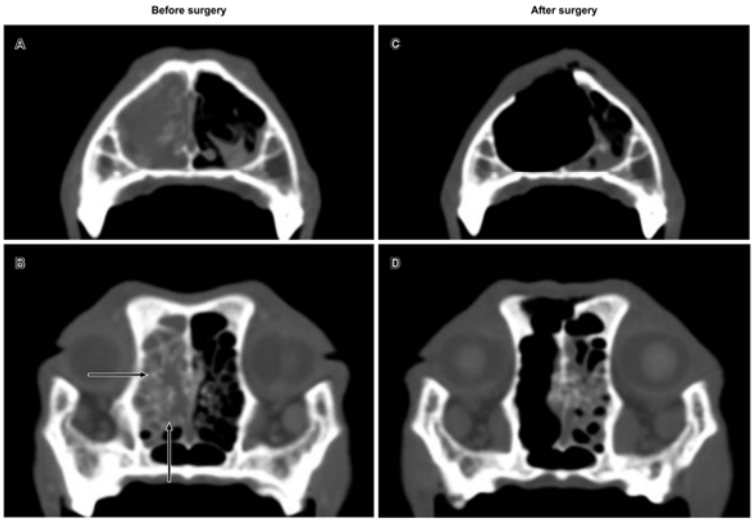

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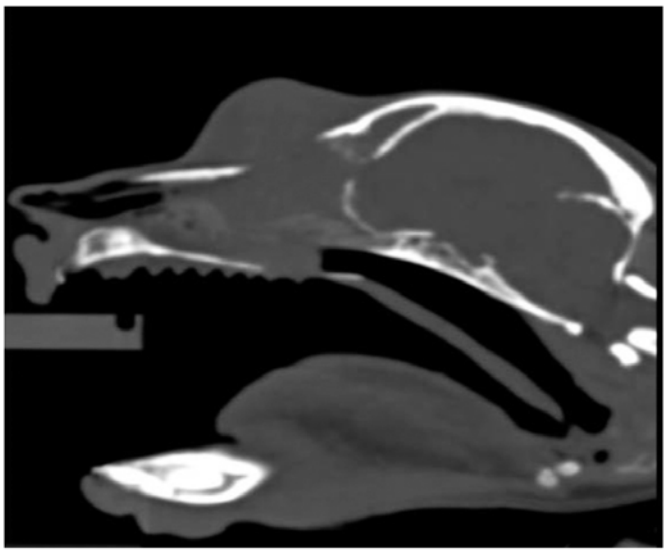

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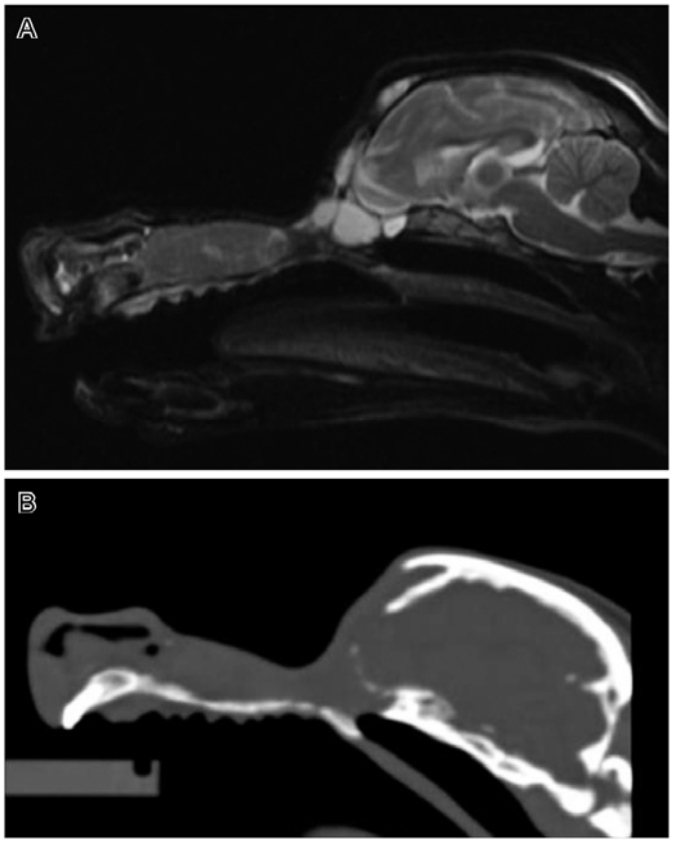

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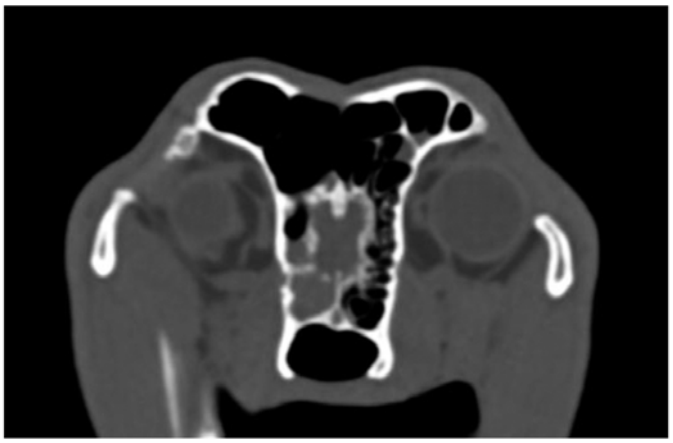

Figure 17