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Sulfoquinovosyl Acyl Panediol (SQAP) as a Radiation Sensitizer for Dogs with Tumors: A Pilot Study

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Abstract: Sulfoquinovosylacylpanediol (SQAP) was discovered to be a radiation sensitizer in 1997. However, the safety of SQAP and radiotherapy to treat naturally occurring tumors in dogs remains unknown. The purpose of this study was to evaluate the safety of SQAP and hypofractionated radiotherapy in healthy beagle dogs, and dogs with tumors. Healthy beagle dogs were administered SQAP at doses of 0, 1, 2, 4, and 8 mg/kg for 5 consecutive days on the first week, while radiotherapy was performed once weekly for 4 weeks. Five dogs with soft tissue sarcoma, thyroid carcinoma, undifferentiated carcinoma, hemangiosarcoma, and liposarcoma were also enrolled in this prospective study. SQAP at 4 mg/kg was administered to the dogs with tumors. The adverse events of SQAP included angialgia in most cases. The adverse events of SQAP and radiotherapy were mild. The systemic administration of SQAP and concomitant radiotherapy appears to be safe for short-term use.
Keywords: Dog, Radiation sensitizer, Radiotherapy, Sulfoquinovosyl monoacylglyceride, Tumor.

Introduction

Sulfoquinovosyl monoacylglyceride (SQMG), the lead compound of chemically synthesized sulfoquinovosylacylpanediol (SQAP), was first isolated from the small intestine of sea urchins as a result of natural products research[1)]. This drug has shown antiangiogenic effects[2)]; moreover, anti-tumor effects have been reported in cultured tumor cells and in implanted solid tumors in mice[3-5)]. Furthermore, SQMG had a radiation sensitization effect on human cancer cells implanted into nude mice[4,6)]. Several studies in which mice[3,5)] were administered 1 to 20 mg/kg of SQMG or SQAP have been reported but, to our knowledge, no toxicity study of SQAP has been performed in dogs with cancer receiving radiation therapy. Therefore, this study performed safety tests using healthy dogs administered the same treatment as the actual treatment protocol and also treated dogs with cancer combined with radiation therapy and SQAP.

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Materials and Methods

The SQAP used in this study was chemically synthesized by NARD Chemicals Ltd. (Osaka, Japan) and was of good laboratory practice (GLP) grade.

Preclinical study

In our previous study, toxicity tests were entrusted to Ina Research Inc. (Ina, Japan) to obtain a fair evaluation of the safety of SQAP in dogs. In the toxicity tests, 8, 16, and 32 mg/kg of SQAP were intravenously administered daily to

3 healthy beagle dogs of each sex for 2 weeks for research purposes. The toxicity tests included complete blood count (CBC), blood chemistry, urinary analysis, and histopathology. Differences between experimental groups and the control were analyzed with Dunnett's test.

Acute radiation toxicity test

In this study, 5 healthy beagle dogs were used in this preliminary study for research purposes. As such, a preliminary study was approved by our animal research committee (approval number 081224−1) and in compliance with the guidelines of the Animal Research Committee of Azabu University. A safety test on healthy dogs administering the same treatment as the actual treatment protocol was performed using doses ranging from 1 to 8 mg/kg. We administered SQAP (0, 1, 2, 4, and 8 mg/kg) for 5 consecutive days to each dog and also administered irradiation of the muzzle to healthy young beagles. The SQAP was diluted in 100 mL saline and was administered by drip infusion for 1 hour through the cephalic vein. A linear accelerator (Mevatron, Toshiba, Tokyo) was used to administer the radiation therapy. The collected data included clinical symptoms (vigorous, appetite, and local and systemic adverse events), body weight, and radiation side effects.

Clinical cases

Five dogs with naturally-occurring tumors were presented to Azabu University Veterinary Teaching Hospital between February 2009 and August 2009. This study was approved by the steering committee of our veterinary teaching hospital. All owners agreed and signed to enroll their dogs in this prospective study (Cases 1−5). Data were collected from medical records, including breed, sex, age, body weight, adverse events, and survival times. The responses to treatment, as well as adverse events from radiotherapy and from drug administration, were classified based on guidelines from the Response Evaluation Criteria in Solid Tumors (RECIST)[7], the Veterinary Radiation Treatment Oncology Group (VRTOG)[8], and the Veterinary Co-operative Oncology Group–Common Terminology Criteria for Adverse Events (VCOG-CTCAE)[9], (Table 1–3).

Treatment protocol

In actual clinical trials, because the dogs are suffering from cancer, a test dose of 4 mg/kg was set as half of the safe dose for healthy animals. SQAP (4 mg/kg) was administered daily as a 2-h intravenous infusion for 5 consecutive days in each dog with a tumor(s).

Hypofractionated radiotherapy was performed using a 4 MV linear accelerator (Mevatron, Toshiba, Tokyo) on the 4th day of SQAP administration, and once weekly for 4 sessions (every Thursday). Treatment plan software (ARCS 3, Japan Electronic Applications Co., Ltd., Toyama, Japan) was used to calculate the irradiation dose. The prescribed dose was calculated at the isocenter of each legion. The planning target volume was set at 5 mm outside of the gross tumor volume and rotational irradiation was used for 4 of the dogs (Cases 2−5), while parallel-opposed irradiation was used for the other dog (Case 1).

Table 1 Response evaluation criteria in solid tumors (RECIST) classifications

	Definition
Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD):	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of 1 or more new lesions is also considered progression).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Table 2 Toxicity criteria of the Veterinary Radiation Therapy Oncology Group (VRTOG)

VRTOG Acute radiation morbidity scoring scheme

	Grade			
	0	1	2	3
Skin	No change over baseline	Erythema, dry desquamation, alopecia/epilation	Patchy moist desquamation without edema	Confluent moist desquamation with edema and/or ulceration, necrosis, hemorrhage
Eye	No change over baseline	Mild conjunctivitis and/or scleral injection	KCS requiring artificial tears, moderate conjunctivitis or iritis necessitating therapy	Severe keratitis with corneal ulceration and/or loss of vision, glaucoma

KCS: keratoconjunctivitis sicca

Table 3 VCOG-CTCAE classifications of adverse events

	Grade				
Adverse Event	1	2	3	4	5
Administration site conditions					
Injection site reaction	Tenderness with or without associated signs (e.g. warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis, severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Blood/bone marrow					
Packed cell volume (dog)	30% to <LLN	20 to <30%	15 to <20%	<15%	Death
Neutrophilia		50,000−100,000/μL	>100,000/μL	Clinical manifestations of leucostasis; urgent intervention indicated	−
Constitutional clinical signs					
Weight loss	<10% from baseline; intervention not indicated	10−15% from baseline; nutritional dietary modification indicated	>15% of baseline	−	Death
Metabolic/laboratory					
Alkaline phosphatase (dog)	>ULN to 2.5×ULN	>2.5−5.0×ULN, transient	>5.0−20×ULN	>20×ULN	−
Alanine aminotransferase (dog)	>ULN to 1.5×ULN	>1.5−4.0×ULN, transient	>4.0−10×ULN	>10×ULN	−

LLN: lower limit normal, ULN: upper limit normal.

Results

Preclinical study

The toxicity tests for SQAP revealed no abnormalities in any of the examined items at a dose of 8 mg/kg. The dogs administered 16 and 32 mg/kg showed decreased red blood cell (RBC) counts in CBC and hemolysis in urine analysis (Table 4 and 5). In histopathology, all groups showed blood clots, perivascular inflammation, edema, and fibrous response around administered vein. As a result, the safe dose of SQAP was determined to be 8 mg/kg.

Acute radiation toxicity test

The clinical symptoms of vigorous and appetite did not decrease after SQAP administration. Systemic adverse events were not detected after administration. Extravasation occurred in 2 dogs (4 and 8 mg/kg), who licked the area around the extravasated area for several days. The mean body weight before the study was 8.9 ± 0.4 kg and was 9.5 ± 0.6 and 10.7 ± 0.6 kg 1 day (day 6) and 29 days after the

Table 4 Red blood cell count on female (left) and male (right)

	Pre ($\times10^6$/μL)	2 wks later ($\times10^6$/μL)
Control	7.24 ± 0.55	7.35 ± 0.59
8 mg/kg	7.46 ± 0.62	6.81 ± 0.49
16 gm/kg	7.98 ± 0.50	7.05 ± 0.33
32 mg/kg*	7.47 ± 0.03	6.03 ± 0.26

	Pre ($\times10^6$/μL)	2 wks later ($\times10^6$/μL)
Control	7.08 ± 0.66	6.89 ± 0.64
8 mg/kg	71.7 ± 0.46	6.45 ± 0.34
16 gm/kg	7.19 ± 0.12	6.58 ± 0.13
32 mg/kg*	7.43 ± 0.31	5.72 ± 0.05

* $p<0.05$

Table 5 Urine occult blood on female (left) and male (right)

	Pre	2 wks later
Control	–/–/–	–/–/–
8 mg/kg	–/–/–	–/–/–
16 gm/kg	–/–/–	–/+++/+++
32 mg/kg	–/–/–	+++/+/+++

	Pre	2 wks later
Control	–/–/–	+/–/–
8 mg/kg	–/–/–	+/–/+++
16 gm/kg	–/–/–	++/–/–
32 mg/kg	–/–/–	+++/+++/+++

Table 6 Acute toxicity results in beagle dogs

		No. 1	No. 2	No. 3	No. 4	No. 5
Control	Eye	0	0	0	0	0
	Skin	0	0	0	0	0
Last RT	Eye	2	2	2	1	1
	Skin	1	1	1	0	1

No. 1: control, No. 2–5 are 1, 2, 4, and 8 mg/kg of SQAP, respectively, 5 consecutive days.
RT: radiotherapy.

completion of SQAP administration, respectively.

The acute radiation adverse events are shown in Table 6. An increased dose of SQAP did not induce severe acute radiation side effects compared to those of radiotherapy alone.

Clinical cases

Summaries of the case characteristics and treatment responses are shown in Tables 7, and 8, respectively.

Table 7 Case characteristics

Cases	Breed	Age	Sex	Body weight (kg)	Location	Diagnosis
No. 1	Shih Tzu	12	Female	5.9	Axillary cavity	Soft tissue sarcoma
No. 2	Beagle	11	Spayed female	13.4	Ventral neck	Bilateral thyroid carcinoma
No. 3	French Bulldog	2	Male	13.6	Muzzle	Undifferentiated sarcoma
No. 4	Shih Tzu	9	Spayed female	5.6	Left ventral neck	Hemangiosarcoma
No. 5	Shetland Sheepdog	13	Male	17.8	Ventral neck	Liposarcoma

Table 8 Treatments and outcomes

	Total dose / fraction	Response*	SQAP adverse events (VCOG-CTCAE grade)	Acute radiation adverse events (VRTOG grade)	Late radiation adverse events (VRTOG grade)	Cause of death	Survival time (months)
No. 1	32 Gy/4 times	SD	Pain at injection site (2)	Alopecia (1)	ND	Local tumor	13
No. 2	32 Gy/4 times	SD	Pain at injection site (2)	ND	ND	Local tumor	36
No. 3	28 Gy/4 times	SD	Pain at injection site (2)	Mild conjunctivitis (1)	-	Metastatic disease	3
No. 4	24 Gy/4 times	PR	Pain at injection site (2)	ND	-	Metastatic disease	2
No. 5	32 Gy/4 times	SD	ND	ND	-	Metastatic disease	3

VCOG-CTCAE: Veterinary Co-operative Oncology Group-Common Terminology Criteria for Adverse Events, VRTOG: Veterinary Radiation Treatment Oncology Group, SD: stable disease, ND: not detected, PR: partial response.
* Response was evaluated 1 month after completion of radiotherapy (No. 2 was evaluated at the time of last radiotherapy).

Case 1

A 12-year-old female Shih Tzu weighing 5.9 kg presented with a 12-cm mass around the left axillary cavity. The lesion was diagnosed as a soft tissue sarcoma and no metastasis was detected by computed tomography (CT). A CBC and the blood chemistry parameters fell within the reference ranges. During the administration of SQAP, the dog licked the skin around the indwelling catheter, suggesting the presence of mild vascular pain (angialgia). The VCOG-CTCAE grade of the pain at the injection site reaction was 2. The dog was irradiated with 32 Gy once weekly for 4 weeks using parallel-opposed irradiation. After the last round of radiation therapy, CBC and chemistry parameters remained within the reference ranges and the dog's body weight had slightly decreased to 5.5 kg (grade 1). At 1 month after treatment completion, the tumor size had not changed; thus, the RECIST classification was stable disease (SD). In a contrast-enhanced CT scan of the tumor at 1 month after radiotherapy, a decrease in blood vessels was observed as compared to the number seen before radiotherapy. Before and a month after the mean CT value of the mass were 40 and 31.5 Hounsfield Unit, respectively. The tumor gradually increased in size, reaching 17 cm, 11 months after treatment. A mild grade 1 skin adverse event, alopecia, was noted. No other adverse events were observed. The dog died 13 months after radiotherapy. No chronic adverse events from the radiation were reported by the owner in a telephone interview.

Case 2

An 11-year-old female beagle weighing 13.4 kg presented with a 12-cm mass in her ventral neck region. The lesion was diagnosed as a bilateral thyroid carcinoma that had invaded the surrounding normal tissues. A CT scan showed no metastatic lesions. A CBC was normal, and grade 3 abnormal blood chemistry parameters were noted for alanine aminotransferase (ALT) (458 IU/L; reference range: 18−71) and alkaline phosphatase (ALP) (1,562 IU/L; reference range: 36−259). During the administration of SQAP, the dog showed mild, VCOG-CTCAE grade 2 angialgia. The dog was irradiated with 32 Gy. Following treatment completion, the abnormal blood chemistry parameters had slightly decreased (grade 2 for ALT [160 IU/L] and ALP [1211 IU/L]). The body weight did not change. No response to treatment was detected; thus, the response was categorized as SD by RECIST classification. Neither acute or chronic adverse events from the radiation nor any other adverse events were noted. At 1 month after radiotherapy, the tumor size had decreased compared to that at the time of referral to our hospital. Subsequently, 5 cycles of carboplatin (200 mg/m^2) were administered. The dog survived for 3 years after irradiation; however, she died due to dysphagia and respiratory insufficiency.

Case 3

A 2-year-old male French Bulldog weighing 13.6 kg presented with an undifferentiated sarcoma in his muzzle area. The tumor measured 6 cm and had invaded the surrounding normal tissues, including the skin and right eyelid. A CT scan showed that the tumor had not metastasized to the lungs and the CBC and blood chemistry values were within the reference ranges. During the administration of SQAP, the dog showed mild, VCOG-CTCAE grade 2, angialgia. The dog was irradiated with 28 Gy. After the last round of radiation therapy, grade 2 neutrophilia (12,450 / μL; reference range: 3,000−11,500) was detected and body weight had not changed. No treatment response was noted; thus, the RECIST classification was SD. Mild conjunctivitis was noted as an acute adverse event of the radiation, with a VRTOG classification of 1. No other adverse events were noted. At 2 months after the radiotherapy, multiple lung metastases of 1 to 2 cm were detected by CT. The dog died owing to metastasis 3 months after radiotherapy.

Case 4

A spayed 9-year-old female Shih Tzu weighing 5.6 kg presented with a left cervical mass. The 16-cm tumor was determined to be a hemangiosarcoma that spanned from the base of the mandible to the inlet of the thorax as shown by a CT scan. A CT scan also showed that the tumor had metastasized to the lungs. A CBC revealed decreased packed cell volume (PCV) (grade 1; 32.8%; reference range: 37−55). During the administration of SQAP, the dog showed mild angialgia, with a VCOG-CTCAE grade of 2. The dog

was irradiated with 24 Gy. After the last round of radiation therapy, the PCV was 35.9% (grade 1), and the other CBC and chemistry parameters were within the reference ranges. Her body weight had slightly decreased to 5.1 kg (grade 1). The tumor size had decreased from 16 cm to 9 cm (30%) at the completion of radiotherapy for a RECIST classification of partial response (PR). Neither acute adverse events from the radiation nor any other adverse events were noted. One month after the completion of radiotherapy, the dog died due to metastasis.

Case 5

A 12-year-old male Shetland Sheepdog weighing 17.5 kg presented with a liposarcoma on his ventral thorax. The tumor's maximum diameter was 13 cm and it was affixed to the surrounding normal tissues. Masses were also detected in the liver, spleen, and kidney. A CBC was normal and ALT (177 IU/L; reference range; 18−71), ALP (738 IU/L; reference range; 36−259), total cholesterol (TChol) (376 mg/dL; reference range; 107−304), and triglyceride (TG) (123 mg/dL; reference range; 17−102) values were elevated. The dog was irradiated with 32 Gy. After the last round of radiation therapy, the abnormal blood chemistry parameters had not changed (ALT [115 IU/L], ALP [677 IU/L], TChol [288 mg/dL], and TG [69 mg/dL]. Moreover, his body weight had not changed. The tumor had not responded; thus, the RECIST classification was SD. Neither acute adverse events from the radiation nor any other adverse events were noted. At 2 months following radiotherapy, the number of masses detected in the liver, spleen, and kidney had increased. This dog died due to metastasis 3 months after completing radiotherapy.

Discussion

This study had 3 important findings. First, angialgia was an adverse event of SQAP administration; however, it was a self-limited problem. Second, systemic adverse events were not detected after 8mg/kg or less SQAP administration in beagle dogs and clinical cases; therefore, the systemic administration of SQAP appeared to be safe for short-term use. Third, SQAP and concomitant radiotherapy also appeared to be safe. These findings are discussed in more detail below.

Mild angialgia (VCOG-CTCAE grade 2 of injection site reaction) appeared as an adverse event from SQAP administration in 2 of 5 beagle dogs, and 3 of the 5 clinical cases. Dogs licked the area around the extravasated area for several days, and it was deemed to be a self-limiting occurrence. Since no skin ulcers developed and only mild pain was observed during the administration of SQAP in the present study, the administration of SQAP at 4 mg/kg appeared not to adversely affect the skin around the injection site.

CBC and blood chemistry parameters did not change consistently, except anemia (32 mg/kg SQAP) in the preclinical study. All of 32 mg/kg dogs, and a half of 16 mg/kg dogs were shown urine occult blood, therefor hemolysis would be the cause of anemia in the adverse events. Three dogs had abnormal CBC and blood chemistry test values prior to treatment and none of these values deteriorated after treatment except for neutrophilia (grade 1) in 1 dog. Moreover, body weight decrease (grade 1) occurred in 2 dogs. Consistent systemic adverse events were not detected in the acute radiation toxicity test, and clinical cases. The above-mentioned reasons indicate that the systemic administration of SQAP 4 mg/kg appears to be safe for short-term use.

SQAP and concomitant radiotherapy appeared to be safe for use in all the study dogs. In the beagle study, there was no tendency for severe side effect with dose escalation. All acute radiation side effects of radiation alone in our clinical study of intranasal tumors were grade 1 [11]. All cases in this pilot study also grade 1. SQAP did not aggravate acute radiation side effects in this study. Therefore, the combination of SQAP (4 mg/kg) and radiotherapy appears to be safe for short-term use.

SQAP is believed to suppresses angiogenesis; therefore, tumors likely undergo necrosis due to hypoxia[4,5]. Moreover, the mechanism of the radiation sensitization effect of SQAP may involve the re-oxygenation of tumor cells[11]. In this study, 3 dogs died early (within 3 months following treatment) due to metastases; therefore, we were unable to evaluate the efficacy of the combination of SQAP and irradiation in these dogs. The dog with the soft tissue sarcoma survived for 13 months and the dog with the thyroid tumor survived for 3 years. The survival time of palliative radiation

therapy is 3−4 months[12]. While the survival times in the present study are better than those previously reported[12], the efficacy of the combination of SQAP (4 mg/kg) and radiotherapy could not be evaluated in this pilot study.

Our study had several limitations. The late adverse events of radiation could not be assessed due to the short survival times of 3 of the dogs and only 1 dog survived for 3 years. In addition, the chronic adverse events of SQAP could not be assessed. Finally, it was difficult to evaluate the efficacy of this combination treatment because of the differences in tumor types, tumor stages, and irradiation doses.

The results of this pilot study suggest that SQAP 4mg/kg is well-tolerated and support further investigation to evaluate its efficacy in the treatment of dogs with tumors.

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