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ラットにおけるα_1-酸性糖蛋白（α_1AG）の動態に関する研究

Variations of α_1-acid glycoprotein (α_1 ・ AG) in healthy rats

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要　約　異なる炎症刺激を与えて急性炎症を惹起させた9週齢のSprague - Dawley系雄性ラットにおいて，α_1 - 酸性糖蛋白（α_1 ・ AG），インターロイキン（IL）1，6およびCytokine-induced neutrophil chemoattractant-1（CINC-1）の血清中濃度の推移を検討した。α_1 ・ AGは，炎症刺激後48時間に最高値を示した。そのα_1 ・ AG値は，テレピン油（0.4 ml/head）投与例では投与前値の33.7倍で，インドメタシン（20 mg/kg）投与例では投与前値の15.5倍であった。CINC-1は，テレピン油，インドメタシンおよび黄色ブドウ球菌投与後に増加し，投与12時間後に最高値を示した。しかし，IL-6は，テレピン油投与時のみに増加（最高値27.4倍）し，インドメタシンおよび黄色ブドウ球菌投与時には増加を示さなかった。テレピン油投与例でIL-1に変化は認められなかった。

1. Purpose

The aim of this study was to be investigated serum levels of α_1 ・ AG during artificially induced acute inflammation in rats caused by various methods. Furthermore, the relationships between α_1 ・ AG, interleukin (IL)-1, IL-6 and cytokine-induced neutrophil chemoattractant-1 (CINC-1) were investigated.

2. Materials and Methods

Organisms

Pseudomonas aeruginosa (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) were provided by Dr. Katsunori Furuhata. *S. aureus* was isolated from human and *P. aeruginosa* was isolated from natural water. *S. aureus* was suspended at 1×10^8 CFU/ml in sterilized saline. *P. aeruginaosa* was suspended at 1×10^9 CFU/ml in sterilized saline.

Chemical agents

Turpentine oil (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan) and indomethacin (ICN Biomedicals, Inc., Ohio, USA) were used. Indomethacin was suspended in saline with a few drops of Tween 80 (ICN Biomedicals, Inc., Ohio, USA) at a concentration of 2.5 mg/ml for 10 mg/kg or 5 mg/ml for 20 mg/kg. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) that has been reported to induce gastrointestinal lesions after subcutaneous administration[1]. Indomethacin was used as an inducer of gastrointestinal lesions.

Rats and treatment

Thirty-five nine-week-old male Sprague-Dawley (SD) rats (Charles River Japan, Inc., Kanagawa, Japan) were

used in this experiment. Turpentine oil was intramuscularly injected at 0.4 ml. Indomethacin was subcutaneously administrated at 10 mg/kg or 20 mg/kg in a volume of 4 ml/kg body weight. *P. aeruginosa* was inoculated by the oral route at 2 ml/kg body weight. *S. aureus* was inoculated subcutaneously at 0.1 ml/head. Blood was collected from the venae cervicalis superficialis under ether anesthesia before treatment and at 6, 12, 18, 24, 30, 48, 72, 96 and 144 h after injection of turpentine oil, before treatment and at 12, 18, 24, 48, 72 and 96 h after indomethacin administration or inoculation with *P. aeruginosa* or *S. aureus*. Sera were obtained after centrifugation at 2,200 g for 15 min. Sera were stored at -80 ℃ until analysis.

Quantification of α_1 · AG, IL-1, IL-6 and CINC-1

Serum levels of α_1 · AG were measured by single radial immunodiffusion (SRID), as directed by the diagnostic kit manufacturer (Institute for Metabolic Ecosystem Co., Ltd., Miyagi, Japan). Serum levels of α_1 · AG were measured at pretreatment and at 6, 12, 18, 24, 30, 48 and 72 h after injection of turpentine, pretreatment and 24, 48, 72 and 96 h after administration of indomethacin or inoculation with *P. aeruginosa* or *S. aureus*.

Serum levels of IL-1, IL-6 and CINC-1 were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits. ELISA kits for IL-1 and IL-6 were obtained from BioSource International, Inc. (California, U.S.A.) and the ELISA kit for CINC-1, developed by the Institute for Cyto Signal Co., Ltd, was obtained from Panapharm Laboratories Co., Ltd. (Kumamoto, Japan). Serum levels of IL-1, Il-6 and CINC-1 were measured at pretreatment and at 6, 12, 18, 24, 30 and 48 h after injection of turpentine oil. IL-6 and CINC-1 were measured at pretreatment and at 12, 18, 24 and 48 h after administration of indomethacin or inoculation with *P. aeruginosa* or *S. aureus*.

All experiments were approved by Animal Research Committee of Azabu University and were conduced in accordance with the Guidelines for Animal Experimentation (Japanese Association for Laboratory Animal Science, JALAS, 1987).

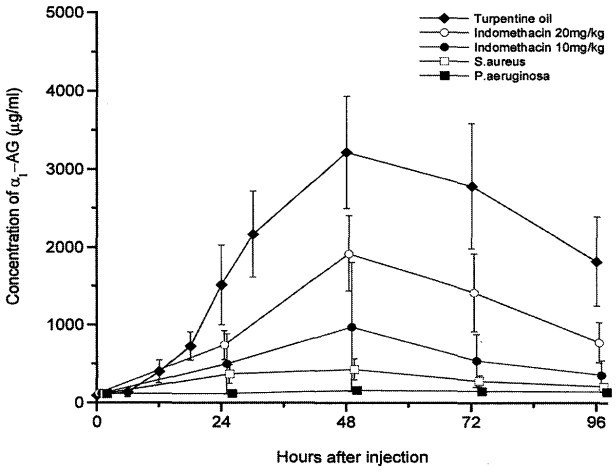

Fig. 1 Changes in serum α_1-acid glycoprotein (α_1 · AG) concentrations in rats after injection of turpentine oil, indomethacin (10, 20 mg/kg) or inoculation of *Staphylococcus aureus* (*S. aureus*) or *Pseudomonas aeruginosa* (*P. aeruginosa*). Each point represents mean ± SD of 6 or 12 rats.

3. Results and discussion

Changes in serum levels of α_1 · AG after injection of turpentine oil, indomethacin, inoculation of *S. aureus* or *P. aeruginosa*. are shown in Fig. 1. Serum levels ranged from 2328.8 to 4841.6 μg/ml (mean, 3218.2 ± 721.2 μg/ml), 33.7-fold higher than the pretreatment mean value (95.6 μg/ml), at 48 h after injection of turpentine oil, after which time levels gradually decreased. Serum levels of α_1 · AG increased after administration and peak levels were observed at 48 h after administration. Mean peak levels were 977.4 ± 830.0 μg/ml at 10 mg/kg of indomethacin, 1920.5 ± 482.9 μg/ml at 20 mg/kg, respectively. These levels were 7.9- at 10 mg/kg and 15.5-fold at 20 mg/kg higher than pretreatment values, respectively. Serum levels of α_1 · AG increased after inoculation and reached peak levels at 48 h after inoculation with *S. aureus*, with the mean peak level being 430.1 ± 136.4 μg/ml. Serum levels of α_1 · AG scarcely increased after inoculation with *P. aeruginosa*. Mean peak levels were 160.4 ± 45.7 μg/ml at 48 h after inoculation, 1.4-fold higher than the pretreatment mean value (118.7 μg/ml).

Changes in serum levels of IL-6 and CINC-1 after injection of turpentine oil, indomethacin, inoculation of *S.*

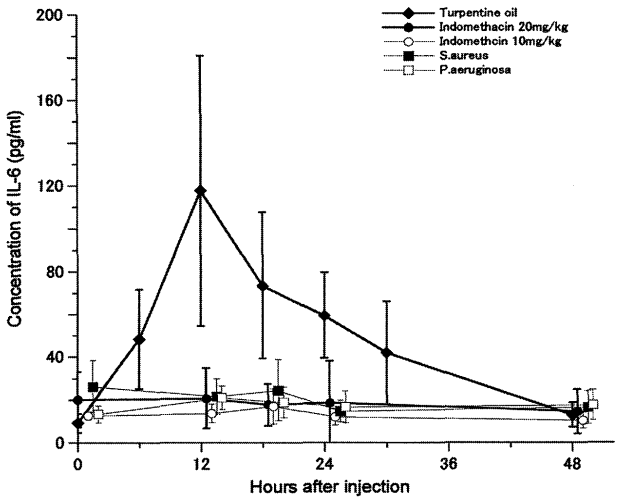

Fig. 2 Changes in serum interleukin-6 (IL-6) concentrations in rats after injection of turpentine oil, indomethacin (10, 20 mg/kg) or inoculation of *Staphylococcus aureus* (*S. aureus*) or *Pseudomonas aeruginosa* (*P. aeruginosa*). Each point represents mean ± SD of 6 or 12 rats.

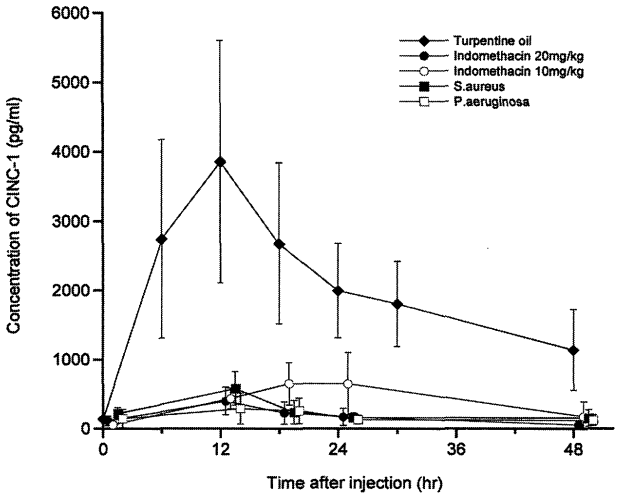

Fig. 3 Changes in serum cytokine-induced neutrophil chemoattractant-1 (CINC-1) concentrations in rats after injection of turpentine oil, indomethacin (10, 20 mg/kg) or inoculation of *Staphylococcus aureus* (*S. aureus*) or *Pseudomonas aeruginosa* (*P. aeruginosa*). Each point represents mean ± SD of 6 or 12 rats.

aureus or *P. aeruginosa* are shown in Figs. 2 and 3, respectively. Peak IL-6 levels ranged from 17.5 to 223.1 pg/ml (mean, 117.6 ± 63.1 pg/ml), which was 12.9-fold higher than the pretreatment mean value (9.1 pg/ml). Peak CINC-1 levels ranged from 784.7 to 6694.0 pg/ml (mean, 3858.1 ± 1750.3 pg/ml), which was 27.4-fold higher than the pretreatment mean value (140.8 pg/ml). Serum levels of IL-1 did not change. Changes in serum levels of IL-6 and CINC-1 after inoculation with *P. aeruginosa* or *S. aureus* are shown in Figs. 6 and 9, respectively. IL-6 levels did not change after administration of indomethacin and inoculation with *S. aureus* or *P. aeruginosa*. On the other hand, serum levels of CINC-1 increased after administration of indomethacin. Mean peak levels at 10 mg/kg of indomethacin, 398.1 ± 198.6 pg/ml, were observed at 12 h after administration, while peak levels at 20 mg/kg of indomethacin, 651.2 ± 303.6 pg/ml, were observed at 18 h after administration. In the case of inoculation with *S. aureus*, mean peak CINC-1 levels were 581.0 ± 240.8 pg/ml at 12 h after inoculation. Serum levels of CINC-1 increased slightly in the case of inoculation of *P. aeruginosa*. Mean peak levels of CINC-1 were observed at 12 h after inoculation (291.4 ± 223.2 pg/ml).

Serum levels of α_1 · AG increased and peak levels were observed at 48 h after injection of turpentine oil, which is similar to the timing observed with α_2 · MG [2]. Peak levels were 35.7-fold higher than pretreatment for α_1 · AG, while α_2 · MG increased 50-290-fold over pretreatment levels. Thus, the rate of increase for α_1 · AG was smaller than that for α_2 · MG. Serum levels of IL-6 and CINC-1 increased, and peak levels were observed at 12 h after injection, which was in agreement with the timing reported by Jinbo *et al.* [2]. However, the peak levels of IL-6 in this study were smaller than the levels reported by Jinbo *et al.* [2]. IL-1 levels did not change in this study, and this is also in agreement with a previous reports [2]. This study confirmed that α_1 · AG levels increase during various types of inflammation in rats. Thus, α_1 · AG is a useful inflammatory marker in rats infected with microorganisms, exhibiting gastrointestinal problems, or with inflamed local lesions. Serum levels of IL-6 and CINC-1 increased prior to α_1 · AG, and thus these cytokines may also be used as inflammation markers. However, different kinetics were observed for IL-6 and CINC-1 depending on the method of induced inflammation. Caution is therefore are required if these cytokines are used as surrogate inflammation markers. Further studies are needed to clarify the cytokine-regulated production of α_1 · AG in rats.

4. Summary

Serum levels of α_1 · AG, IL-1, IL-6 and CINC-1 were estimated in twenty-five 9-week-old male SD rats following various types of acute inflammatory stimulation. Serum levels of α_1 · AG and IL-6, CINC-1 were measured by SRID and ELISA, respectively, using commercial kits. α_1 · AG increased after inflammatory stimulation and peak levels were observed after 48 h. Serum levels of α_1 · AG also increased after inoculation with *S. aureus*, but were scarcely increased after inoculation with *P. aeruginosa*. However, only CINC-1 increased after injection of indomethacin (20 mg/kg); IL-6 levels did not change. This phenomenon was also observed in rats inoculated with *S. aureus*. IL-6 increased only after injection of turpentine oil and did not change after injection of indomethacin or inoculation with *S. aureus*. IL-1 did not change after injection of turpentine oil. α_1 · AG is useful as an inflammatory marker in rats

5. References

1) Takeuchi, K., Ueki, S. and Okabe, S. *Dig. Dis. Sci.*, 31, 1114-1122, 1986.
2) Jinbo, T., Sakamoto, T. and Yamamoto, S. *Lab. Anim.*, 36, 153-157, 2002.