Alteration of Radioprotective Effects of Heat-killed Lactobacillus casei in X-irradiated C3H/He Mouse Related to Blood Level of Proinflammatory Cytokines by Corticoids

Izumi TANAKA, Mika TANAKA, Akiko SATOH, Ayako KUREMATSU, Akiko ISHIWATA, Keiko SUZUKI and Hiroshi ISHIHARA *

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It is well known that a pre-administration of proinflammatory cytokines alters hematopoietic progenitor cells to promote an increase resistance against radiation and increases the survival rate in mice irradiated with lethal doses of radiation. Inflammation stimulators, such as some bacterial constituents, are also reported to have similar radioprotective action. We found that pre-administration of heat-killed Lactobacillus casei (HLC) to mice increases the level of interleukin (IL)-1 beta in circulation as well as the survival rate following lethal dose of radiation. Since HLC stimulates early immune responses, effects by drugs to modify inflammation were studied. The increase of both blood IL-1 beta levels and survival rates by HLC were simultaneously accelerated by coadministration of mineralocorticoid and inhibited by glucocorticoids or corticotropin. Neither parameter was modified by non-steroidal anti-inflammatory or anti-rheumatoid drugs. This suggests that both expected radioprotective action and unexpected systemic action, realized as an increase in plasma cytokines, by inflammation-related radioprotectors can be controlled by the coadministration of drugs at least in C3H/He mice, based on consideration of their pharmacological properties.

INTRODUCTION

Various compounds are recognized as radioprotectors because of their ability to increase the survival rates of experimental animals exposed to ionizing radiation at doses that diminish the hematopoietic cells. Among the radioprotectors, dead cells of microorganisms such as Lactobacillus casei 1) and Saccharomyces 2) are classified as immunomodulators, 3) and enhances survival rates of animals. In the case of C3H/He mouse models, hematopoietic damages are the major cause to death after 7 to 8 Gy of whole-body irradiation. 1,2,4) After the administration of such substances, the animals show complicated response, presumably as a result of crosstalk between the injection site and the systemic regulatory tissue mediated by cytokines and hormones. To reveal the mechanism of these radioprotectors, it is necessary to perform detailed studies focused on the spatial and temporal response following the injection of these substances.

Inflammation seems to contribute to the survival of lethally irradiated mice, since microorganism-derived radioprotectors are typical stimulators of inflammation. Radioprotective effect by the injection of proinflammatory cytokine interleukin (IL)-1 to animals 18 hours before irradiation has been reported by Neta et al. 5–7) Based on their studies, the proinflammatory cytokines not only IL-1, but also tumor necrosis factor alpha 8) and stem-cell factor, 9) contribute to endogenous protective mechanisms against hematopoietic damage. Although these cytokines have drastic systemic effects on organisms, the undesirable actions are expected to be reduced by simultaneous usage of related cytokines with minimal injection doses 6,9) or use of nonapeptide derivatives based on IL-1. 10,11)

The radioprotective effect of the proinflammatory cytokines is considered to enhance resistance of hematopoietic cells against radiation, probably as a result of the population change in cell cycles in the hematopoietic precursor cells 6,12) and/or of the activation of cellular scavenging activity against superoxides by the induction of superoxide dismutase. 13) Simultaneously, the proinflammatory cytokines stimulate neuroendocrine regulation systems such as the hypothalamic-pituitary-adrenal (HPA) axis (reviewed in 14).
The activation of the HPA-axis by radiation is known to increase serum levels of glucocorticoid\(^{15}\) that is endogenous anti-inflammatory agent. The glucocorticoids aggravate hematopoietic damage after radiation\(^{16}\) via enhancement of apoptosis in lymphoid cells.\(^{17}\) Thus, it is predicted that the modifications of the HPA-axis using various drugs interfere with radioprotective effect of proinflammatory cytokines in animal experimental models.

We reconfirmed the effectiveness of pre-administration of heat-killed *Lactobacillus casei* (HLC) using supralethally irradiated mice and found an elevation of blood IL-1 beta levels by HLC. The IL-1 beta levels and the survival rates were simultaneously modulated by the coadministration of HLC with various drugs that pharmacologically modify inflammation. The pharmacological effects of these drugs are discussed in this paper.

**MATERIALS AND METHODS**

**Animals**

Male C3H/He inbred mice of 6 to 7 week age were obtained from Japan SLC Co. (Hamamatsu, Japan) based on our previous study.\(^{13}\) Upon receipt, the mice were housed 5 per cage and acclimatized for 2 weeks at a room temperature of 23°C, humidity of 55%, with a 12 h light cycle from 7:00 to 19:00 at 400 to 800 lx (10 to 50 lx in the cage) to simulate their daytime. When a mouse with physical injury to the body or tail was found, no mice from the same cage were used for any experiment to avoid adverse effects on inflammation by the injury. For the experiments to determine survival rate following supralethal dose of radiation, mice were grouped 10 per treatment group. The data for each treatment were obtained as the average of at least 3 experiments using different production lots of mice. For total blood collection, mice were anesthetized with ether, dissected to expose trachea, fluid and debris were wiped to minimize lymph contamination, then blood was taken from the carotid artery. For nighttime blood collection, the mice were kept under darkness and operated under red-LED lamp of less than 0.2 lx until anesthetized. After collection, blood was centrifuged, filtered, divided to 0.22 ml aliquots of plasma and frozen at −80°C. All the animals were handled and treated in accordance with the Guideline for Experimental Animals of our Institute.

**Irradiation with rotation**

Mice were set in a cylindrical irradiation container (dia. 23 cm, height 4 cm, mouse area inner radius 3 to 11 cm, 12 radially separated chambers per container, made of acrylic resin of 5 mm-thickness) and the container was put onto the concentric irradiation area of an X-ray generator (PANTAK HF-320, Shimadzu, Kyoto, Japan) at a focus-sample distance of 800 mm (0.49 to 0.51 Gy/min). The concentric irradiation area was determined based on admeasured distribution data of X-rays from the generator, and the ionization chamber for measurement of absorbed doses was calibrated against an international standard by the Department of Technical Support and Development of our Institute. To avoid the effect of circadian rhythm, 10 mice (one mouse each from 10 different groups, each comprising 5 mice) was put into the irradiation container, exposed, and returned to the same group for each running. In all the experiments, less than 50 mice per day were irradiated between 09:00 and 10:30. Since the average irradiation time was 09:45, times for preadministration of HLC and drugs and for blood collection were scheduled at equal intervals.

**HLC, drugs and reagents**

*Lactobacillus casei* subsp. *casei* (JCM-1134) from Japan Collection of Microorganisms (JCM) of RIKEN BioResource Center (BRC, Saitama, Japan) was cultured in MRS broth containing 10.0 g polypeptone, 10.0 g beef extract, 5.0 g yeast extract, 20.0 g glucose, 1.0 g Tween 80, 2.0 g K\(_2\)HPO\(_4\), 5.0 g sodium acetate, 2.0 g diammmonium citrate, 0.2 g MgSO\(_4\)-7H\(_2\)O, and 0.05 g MnSO\(_4\)-5H\(_2\)O per liter (pH 6.5), at 37°C. *Bacillus subtilis* subsp. *subtilis* (JCM-1465, JCM, BRC, Saitama, Japan) was cultured in nutrient broth of 10 g polypeptone, 10 g beef extract, and 5.0 g NaCl per liter (pH 7.0), at 30°C. Bacterial cells during late exponential phase were harvested, washed with distilled water, boiled at 100°C for 30 min, and lyophilized. The lyophilized bacteria were reconstituted with 1% polyethylene glycol (PEG) 400 in physiological salt solution (PSS) and sonicated, then subcutaneously injected into the mice at 30 mg/kg. We chose drugs that contained ignorable levels of impurity or additives based on the respective datasheet. Drugs for injection used in the study were as follows: dexamethasone sodium phosphate (Decadron\(^\text{®}\) phosphate injection, Banyu Pharmaceutical Co., Ltd.), triamcinolone acetonide (Kenacort®-A intramuscular, Bristol-Myers Suquibb Company), methylprednisolone sodium succinate (Solu-medrol® 40, Pfizer Japan Inc.), ACTH (tetracosactide acetate with 1.5 mg ZnCl\(_2\)/mL, Cortrosyn® Z, Daiichi Sankyo Co., Ltd.), indomethacin sodium (Indacin® IV 1mg, Banya Pharmaceutical Co., Ltd.), and methotrexate (Methotrexato® parenteral 5 mg, Wyeth Pharmaceuticals). Water soluble reagent of D(−)-penicillamine (Superior grade, Wako Pure Chemical Indust. Ltd.) was also used. Water-insoluble reagents, acetylsalicylic acid (Superior grade, Wako Pure Chemical Indust. Ltd.) and aldosterone (Cell biology grade, Wako Pure Chemical Indust. Ltd.), were dissolved at high concentration with dimethylsulfoxide and diluted with water for injection. Doses of the drugs to inject into mice were determined based on maximum daily doses per kg body weight for humans, which were recommended by the manufacturers or in clinical reports as follows: dexamethasone (0.4 mg/kg), triamcinolone (1.5 mg/kg), methylprednisolone (40 mg/kg), tetracsoactide (0.01 mg/kg), acetylsalicylic acid (90 mg/kg).
indomethacin (0.5 mg/kg), aldosterone (0.008 mg/kg), methotrexate (0.2 mg/kg), and penicillamine (20 mg/kg). All the injections were administered subcutaneously in the area between the scapulas of mice.

**Measurement of plasma cytokine**

At least 9 mice from more than 3 different production lots were used in each treatment group. Using blood samples from each mouse, cytokine levels were measured in duplicate using Immunoassay Kits for mouse IL-1 beta, mouse TNF-alpha, mouse IL-6, and mouse IL-1 alpha (Biosource International Inc., California, USA).

**Statistics**

The p values were obtained by logrank tests of survival curve by Kaplan-Meier’s method to estimate significance on survival rate. To examine significance of cytokine levels in circulation, the p values were calculated from multiple comparisons by Dunnett’s test following confirmation of homoscedasticity by Levene’s test.

**RESULTS**

We used whole-body irradiation to C3H/He mice at a supralethal X-ray dose of 8.0 Gy at Day 0 to explicitly show protective effect on the survival rate, based on the data for LD50/28 of X-rays at 7.5 Gy in the mice obtained in the preliminary experiments (data not shown). Without injection of any microbial product, no mouse was surviving on Day 17 and the average survival time was 11.2 days (n = 70), as shown in Fig. 1. Administration of heat-killed *Bacillus subtilis* 24 h before irradiation showed radioprotective effects, but lower survival rate of 13% (n = 30) on Day 28. In contrast, very high survival rates of 70% (n = 30), 80% (n = 60), and 73% (n = 30) were obtained by a single pre-administration of HLC at 16 h, 24 h or 48 h before the X-irradiation, respectively. These surviving mice frequently possessed abscess of 0.5 to 1 mm diameter at the injection site on Day 28. Body weights of survivors at Day 28 were higher than the initial weights. On the other hand, mice injected with HLC within 15 min after the irradiation showed a lower survival rate of 27% (n = 30). Their body weights after irradiation were lower than the initial levels, and no abscesses were found by dissection on Day 28. Although the levels of life-saving effect of heat-killed *Lactobacillus casei* before and after the 7.5 Gy gamma-ray irradiation were similar, the survival curves between pre-administration and post-administration of HLC showed significant difference at supralethal dose at 8.0 Gy of X-rays in this study, suggesting that their mechanisms of survival are different. Since the precedent administration provided a very high survival rate, we focused on the effect of HLC in this study.

**Increase of proinflammatory cytokines in circulation by HLC in mouse**

To study the effects of HLC, levels of proinflammatory cytokines in the plasma after administration of HLC were measured by ELISA. Plasma IL-1 beta, which was below the detectable levels (< 15.6 pg/ml-plasma) before HLC-injection, was markedly increased from 8 to 24 h, reaching a peak at 16 h, and decreased to undetectable levels after 48 h (Fig. 2). Increase in plasma IL-6 levels by the HLC was also observed, but the difference was not evident as compared with IL-1 beta. In the case of the administration of *Bacillus subtilis*, which resulted in a lower survival rate as shown in Fig. 1, plasma IL-1 beta was increased with a similar profile. However, the IL-1 beta levels were lower (Fig. 2) and IL-6 in plasma did not reach the detectable level (< 4.9 pg/ml). We could not detect plasma TNF-alpha (less than 19.5 pg/ml) or IL-1 alpha (less than 15.6 pg/ml) after administration of HLC.

**Effects of inflammation modifiers on the blood IL-1 beta levels and the HLC-induced survival rate following irradiation**

Various drugs are clinically used to alleviate or exacerbate inflammation. Each drug was coadministered with HLC to mice 24 h before x-irradiation at 8.0 Gy and the effects on the survival were compared (Fig. 3a). To estimate the level of inflammation before irradiation, IL-1 beta level in the cir-
Calculation 16 h after injection of HLC with the drug were also measured (Fig. 3b). Co-administration of HLC with dexamethasone, triamcinolone, or methylprednisolone, which are anti-inflammatory synthetic glucocorticoids, markedly reduced induction levels of plasma IL-1 beta induced by single dose of HLC (Fig. 3b). Similarly, the induction was inhibited by ACTH, which stimulates secretion of endogenous glucocorticoid from the adrenal gland. When the mice were irradiated 24 h after the co-administration of both HLC and any of the glucocorticoids or ACTH, survival rates were significantly reduced (Fig. 3a).

Mineralocorticoid showed opposite profiles in both blood IL-1 beta levels and survival rate after HLC injection as compared with glucocorticoid. Co-injection of aldosterone with HLC significantly elevated the levels of IL-1 beta in the circulation (Fig. 3b) and the survival rates following irradiation (Fig. 3a). Non-steroidal anti-inflammatory drugs (NSAIDs), indomethacin and acetylsalicylic acid, as well as non-steroidal anti-rheumatoid drugs (NSARDs), methotrexate and penicillamine, did not significantly alter either increase in IL-1 beta levels or survival rate by HLC (Fig. 3). When any of these drugs were injected without HLC, plasma IL-1 beta levels were not increased and no mice survived after the irradiation (data not shown).

**Fig. 2.** Plasma levels of proinflammatory cytokines following administration of HLC. Plasma was prepared at indicated times after injection of HLC (white and black bars) or *Bacillus subtilis* (hatched bars). Levels of IL-1 beta (black and hatched bars) and IL-6 (white bars) were measured using ELISA. Range below the detection limit is shadowed. Means and S.E.M.s among more than 9 mice from 3 different production lots are shown.

**Fig. 3.** Effect of co-administration of inflammation modifiers with HLC on survival and plasma IL-1 beta levels. a: Mice were injected without (n = 70, open triangles, numbered as 0) or with HLC plus saline (n = 60, closed circles, 1), dexamethasone (n = 30, open squares, 2), triamcinolone (n = 30, open triangles, 3), methylprednisolone (n = 30, closed triangles, 4), ACTH (n = 30, closed squares, 5), acetylsalicylic acid (n = 30, closed triangles, 6), indomethacin (n = 30, open squares, 7), aldosterone (n = 50, open circles, 8), methotrexate (n = 30, closed squares, 9), or penicillamine (n = 30, open circles, 10). Twenty four hours after the injection, mice were X-irradiated at 8.0 Gy. Averages of survival rate in all the experiment are shown. P-values determined by logrank test are shown. b: Plasma was prepared 16 h after co-administration of HLC with saline (1), dexamethasone (2), triamcinolone (3), methylprednisolone (4), ACTH (5), acetylsalicylic acid (6), indomethacin (7), aldosterone (8), methotrexate, (9) or penicillamine (10) to measure IL-1 beta levels by ELISA. Means and S.E.M.s of 9 mice from 3 different production lots are shown. Statistically significant data of the p value less than 0.05 are indicated with their p-values determined by Dunnett’s multiple comparison test.
DISCUSSION

In this study, we confirmed high life-saving effects against supralethal dose of radiation in mice (Fig. 1) induced by HLC, which resembles LC9018 in structure.1,4 Since the pre-administration of HLC resulted in remarkable effects on the survival rate as compared with the post-injection, we focused on the effects of the pre-treatment of HLC on biological parameters. Injection of HLC into C3H/He mice causes simultaneous increase in IL-1 beta and IL-6 in the circulation (Fig. 2). Radioprotective effect of proinflammatory cytokines is well established by leading studies by Neta et al., which revealed that the effect of IL-1β is enhanced by coadministration with other related cytokines such as tumor necrosis factor alpha,8 stem-cell factor,9 or IL-12.5 Therefore it is suggested that the remarkable radioprotection of HLC when administrated before irradiation is mediated by the simultaneous secretion of proinflammatory cytokines IL-1 beta and IL-6. Among various effects of IL-1,8 effects on hematopoietic cells that may lead the resistance against radiation damage probably contribute to increase in survival rate in mouse.6,12

Levels of plasma IL-1 beta were modified by coadministration of various drugs with HLC (Fig. 3b). Treatments to reduce or augment the IL-1 beta level resulted in diminishment or increase of survival rate, respectively, when mice were supralethally irradiated (Fig. 3a). The results support that the endogenous IL-1 beta contributes to mouse on the survival.6

It is presumed that various inflammation-related responses including a secretion of IL-1 beta after injection of HLC would occur. Since glucocorticoids, strong anti-inflammatory drugs, inhibited the secretion of plasma IL-1 beta (Fig. 3b), it is speculated that the production of IL-1 beta was modified by the steroids at secretion level9,20 or at the transcriptional level mediated by the activated glucocorticoid receptor.20,21 Similar inhibitions on plasma IL-1 beta level and survival rates were found by coinjection of ACTH that stimulates HPA-axis to secrete endogenous glucocorticoid. Since the plasma level of glucocorticoid has reported to change immediately after giving physical and psychological stresses to animals,22 systemic parameters in the studies of radioprotectors may be varied by the fluctuation of HPA-axis including experimental conditions, such as breeding and handling.

Inflammation is known to be ameliorated by NSARDs and NSAIDs that reduce metabolites of cyclooxygenase and hydroperoxidase23 by the inhibition of these enzymes.24 These drugs showed no significant alteration on the plasma IL-1 beta levels and survival by HLC (Fig. 3), although NSAIDs25,26 and NSARDs27,28 have potential to induce apoptotic cellular death as glucocorticoids16,17 Therefore, the results show that a part of inflammation by HLC can be alleviated by these drugs without suppression on survival rate at least in the experimental animal model.

Aldosterone enhanced the HLC-induced increase in the survival rate and plasma IL-1 beta level (Fig. 3). The result suggests that inflammation by HLC may be partially enhanced by exogenously introduced aldosterone, because of aldosterone receptor30,31 is included in the pathway induced by angiotensin II. Alternatively, aldosterone may prevent actions of endogenous glucocorticoid by heterodimerization of glucocorticoid and mineralocorticoid receptors.30,31

In this study, we showed the effects of HLC and typical drugs that modify inflammation pharmacologically. Although HLC *per se* is hardly applicable to humans, the results show that coadministration of appropriate drugs can control both expected and another effects of radioprotectors. For example, NSAIDs and NSARDs can be applicable for amelioration of inflammation without reduction of IL-1 beta production. Although a limited number of mineralocorticoid derivative is available currently in the market, modification of the chemical structure of mineralocorticoid is expected to develop the drug that enhance IL-1 beta production. Based on this strategy, new combinations of drugs and new design of chemicals as radioprotective treatment can be developed in future.

REFERENCES

1. Tsuneoka, K., Ishihara, H., Dimchev, A. B., Nomoto, K., Yokokura, T. and Shikita, M. (2004) Timing in administration of a heat-killed *Lactobacillus casei* preparation for radioprotection in mice. J. Radiat. Res. 35: 147–156.
2. Anzai, K., Ikota, N., Ueno, M., Nyui, M. and Kagiya, T. V. (2008) Heat-treated mineral-yeast as a potent post-irradiation radioprotector. J. Radiat. Res. 49: 425–430.
3. Nair, C. K., Parida, D. K. and Nomura, T. (2001) Antioxidant activity of *Lactobacillus casei* treatment in radioprotection. Exp. Mol. Pathol. 70: 156–169.
4. Neta, R. (1997) Radioprotective effects of *Lactobacillus casei* on radiation injury: suggested mechanisms of action. Environ. Health Perspect. 105 Suppl. 6: 1463–1465.
5. Neta, R., Douchez, S. and Oppenheim, J. J. (1986) Interleukin 1 is a radioprotector. J. Immunol. 136: 2483–2485.
6. Neta, R., Oppenheim, J. J., Schreiber, R. D., Chizzonite, R., Ledney, G. D. and MacVittie, T. J. (1991) Role of cytokines (interleukin 1, tumor necrosis factor, and transforming growth factor beta) in natural and lipopolysaccharide-enhanced radioresistance. J. Exp. Med. 173: 1177–1182.
7. Neta, R., Oppenheim, J. J., Wang, J. M., Snapper, C. M., Moorman, M. A. and Dubois, C. M. (1994) Synergy of IL-1 and stem cell factor in radioprotection of mice is associated with IL-1 up-regulation of mRNA and protein expression for c-kit on bone marrow cells. J. Immunol. 153: 1536–1543.
10. Bajpai, K., Singh, V. K., Sharan, R., Yadav, V. S., Haq, W., Mathur, K. B. and Agarwal, S. S. (1998) Immunomodulating activity of analogs of noninflammatory fragment 163-171 of human interleukin-1beta. Immunopharmacology 38: 237–245.

11. Singh, V. K., Srinivasan, V., Seed, T. M., Jackson, W. E., Miner, V. E. and Sree Kumar, K. (2005) Radioprotection by N-palmitoylated nonapeptide of human interleukin-1beta. Peptides 26: 413–418.

12. Neta, R., Keller, J. R., Ali, N., Blanchette, F. and Dubois, C. M. (1996) Contrasting mechanisms of the myeloprotective effects of interleukin-1 against ionizing radiation and cytotoxic 5-fluorouracil. Radiat. Res. 145: 624–631.

13. Wong, G. H. and Goeddel, D. V. (1988) Induction of manganese superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science 242: 941–944.

14. Tumblin, A. V. and Rivier, C. L. (1999) Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol. Rev. 79: 1–71.

15. Lebaron-Jacobs, L., Wysocki, J. and Griffiths, N. M. (2004) Differential quantitative and temporal changes in the response of the hypothalamus-pituitary-adrenal axis in rats after localized or total-body irradiation. Radiat Res. 161: 712–722.

16. Nam, S. Y. and Chung, H. Y. (2005) The suppression of radiation-induced NF-kappaB activity by dexamethasone correlates with increased cell death in vivo. Biochem. Biophys. Res. Commun. 336: 603–608.

17. Hahn, P. J., Lai, Z. W., Nevaldine, B., Schiff, N., Fiore, N. C., and Goeddel, D. V. (1988) Induction of manganese superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science 242: 941–944.

18. Dinarello, C. A. (1996) Biologic basis for interleukin-1 in disease. Blood 87: 2095–2147.

19. Dinarello, C. A. (1998) Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. Ann. N. Y. Acad. Sci. 856: 1–11.

20. Barnes, P. J. (1998) Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin. Sci. (Lond). 94: 557–572.

21. Clark, A. R., Martins, J. R. and Tchen, C. R. (2008) The role of dual specificity phosphatases in biological responses to glucocorticoids. J. Biol. Chem. 283: 25765–25769.

22. Veličković, N., Djordjević, A., Matić, G. and Horvata, A. (2008) Radiation-Induced Hyposuppression of the Hypothalamic-Pituitary-Adrenal Axis is Associated with Alterations of Hippocampal Corticosteroid Receptor Expression. Radiat. Res. 169: 397–407.

23. Smith, W. L. (1989) The eicosanoids and their biochemical mechanisms of action. Biochem. J. 259: 315–324.

24. Kontogiorgis, C. A. and Hadjipavlou-Litina, D. J. (2002) Non steroidal anti-inflammatory and anti-allergy agents. Curr. Med. Chem. 9: 89–98.

25. Hanif, R., Pittats, A., Feng, Y., Koutsos, M. I., Qiao, L., Staiano-Coico, L., Shiff, S. I. and Rigas, B. (1996) Effects of non-steroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-induced pathway. Biochem. Pharmacol. 52: 237–245.

26. Rahman, M. A., Dhar, D. K., Masunaga, R., Yamanoi, A., Hohno, H. and Nagasue, N. (2000) Sulindac and exisulind exhibit a significant antiproliferative effect and induce apoptosis in human hepatocellular carcinoma cell lines. Cancer Res. 60: 2085–2089.

27. Genestier, L., Paillot, R., Fournel, S., Ferraro, C., Miossec, P. and Revillard, J. P. (1998) Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. J. Clin. Invest. 102: 322–328.

28. Okazaki, H., Sato, H., Kanimura, T., Hirata, D., Iwamoto, M., Yoshio, T., Mimori, A., Masuyama, J. I., Kano, S. and Minota, S. (2000) In vitro and in vivo inhibition of activation induced T cell apoptosis by bucillamine. J. Rheumatol. 27: 1358–1364.

29. Viengchareun, S., Le Menuet, D., Martinerie, L., Munier, M., Pascaud-Le Tallec, L. and Lombès, M. (2007) The mineralocorticoid receptor: insights into its molecular and (patho) physiological biology. Nucl. Recept. Signal. 5: e012.

30. Liu, W., Wang, J., Sauter, N. K. and Pearce, D. (1995) Steroid receptor heterodimerization demonstrated in vitro and in vivo. Proc. Natl. Acad. Sci. U. S. A. 92: 12480–12484.

31. Savory, J. G., Préfontaine, G. G., Lamprecht, C., Liao, M., Walther, R. F., Lefebvre, Y. A. and Haché, R. J. (2001) Glucocorticoid receptor homodimers and glucocorticoid-mineralocorticoid receptor heterodimers form in the cytoplasm through alternative dimerization interfaces. Mol. Cell. Biol. 21: 781–793.

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