Association of immunological disorders in lethal side effect of NSAIDs on β-glucan-administered mice

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Received 11 September 2000; received in revised form 6 November 2000; accepted 14 November 2000

First published online 2 March 2001

Abstract

(1→3)-β-D-Glucan (β-glucan) is a biological response modifier that regulates host immune response. We have found that the combination of a β-glucan and a non-steroidal anti-inflammatory drug (NSAID), indomethacin (IND), induced lethal toxicity in mice [Yoshioka et al. (1998) FEMS Immunol. Med. Microbiol., 21, 171–179]. This study was undertaken to analyze the mechanism of the lethal side effect. Combination of a β-glucan and IND increased the number of leukocytes, especially macrophages and neutrophils, in various organs and these cells were activated. The activated state of these cells was supported by the enhanced production of interferon-γ in the presence of IND in vitro culture of the peritoneal exudate cells. Intestinal bacterial flora was translocated into the peritoneal cavity in these mice to cause peritonitis. Comparing the toxicity of various NSAIDs, nabumetone, a partially cyclooxygenase-2-selective NSAID with weaker toxicity to the gastrointestinal tract, did not exhibit a lethal side effect. These facts strongly suggested that gastrointestinal damage by NSAIDs was more severe in β-glucan-administered mice, resulting in peritonitis by enteric bacteria and leading to death. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: β-Glucan; Indomethacin; Lethal toxicity; Non-steroidal anti-inflammatory drug; Nabumetone

1. Introduction

Indomethacin (IND) is a non-steroidal anti-inflammatory drug (NSAID) that suppresses prostaglandin (PG) synthesis by inhibiting cyclooxygenase (COX). NSAIDs and aspirin are widely used for the treatment of pain, fever and inflammatory diseases, particularly in patients with rheumatoid arthritis, osteoarthritis, cancer and gout. Recently they are also applicable to Alzheimer’s disease, which is also categorized as a chronic inflammatory disease, to significantly slow the decline in cognitive function. It is worth remembering that NSAIDs are excellent analgesics and anti-inflammatories, and bring huge benefits to many people. However, NSAIDs are associated with a number of adverse side effects, and the most important one is the mucosal damage on the gastrointestinal tract. NSAIDs and aspirin cause gastric erosions that can become ulcers. The ulcers may bleed, and indeed some people may die of a bleeding ulcer caused by NSAIDs [1–7]. It has been shown that the rate of NSAID-related gastrointestinal deaths is higher than that of cervical cancer, asthma or malignant melanoma. In addition, Reye’s syndrome and influenza-associated encephalitis-encephalopathy, a rare but serious illness affecting children and teenagers, has been associated with aspirin use [8].

The other important side effect is, so called, aspirin-sensitive asthma (ASA, also called aspirin-intolerant asthma). ASA can be induced not only by acidic analgesics (including acetylsalicylic acid), which effectively inhibit COX, but also by cross-reactivity with paraben, and other chemical additives [9–13]. This distinct clinical syndrome is characterized by a typical sequence of symptoms, intense eosinophilic inflammation of nasal and bronchial tissues, combined with an overproduction of cysteinyl-leukotrienes (cys-LTs). After aspirin challenge, cys-LTs are released into nasal and bronchial secretions.

In order to reduce the side effects mentioned above, various new materials have been developed. Extensive studies are required both from basic as well as clinical points of view to regain the usefulness of NSAIDs. COX catalyzes the conversion of arachidonic acid to PGs and
thromboxane. Two forms of COX have been identified, COX-1, which is constitutively expressed in most tissues and organs, and the inducible enzyme, COX-2, which has been localized primarily to inflammatory cells and tissues. The side effects of NSAIDs are suggested to be mediated by inhibiting COX-1 [14–18].

(a→3)-β-D-Glucans (β-glucans) are found in various species such as fungi, yeast, algae, bacteria and higher plants, and are biological response modifiers (BRMs) that regulate host immune response [19–22]. Some β-glucans, including lentinan and sonifilan (schizophyllan, SPG), are used clinically as antitumor agents in Japan [23–25]. Many β-glucans have immunopharmacological activity, although the activity varies in each glucan tested. The differences are at least partially dependent on structural parameters, such as solubility, molecular mass (Mₘ), degree of branching (DB) and conformation. The mechanism of β-glucan-mediated BRM activity and antitumor activity has not been fully elucidated due to the diversity of the activity as shown above and thus molecular mechanisms, such as the receptor and the signaling pathways, are insufficiently characterized. Our laboratory has been analyzing the molecular mechanism of β-glucan-mediated immunopharmacological activity from various points of view [26–32]. In a previous study, we analyzed the contribution of the acute inflammatory response on β-glucan-mediated antitumor activity. β-Glucans, especially watersoluble glucans, do not usually trigger serious side effects, but we found that the mortality of IND-administered mice was significantly elevated by treatment with SPG [33]. A similar effect was observed with other β-glucans including, SSG (a β-glucan from Sclerotinia sclerotiorum IFO 9395), grifolan, zymosan A and zymocel. Interferon (IFN)-γ, interleukin (IL)-6 and colony stimulating factor (CSF) concentrations in sera of IND/SSG (α→1,3)-glucan treatment induces lethality in mice by mal-adjusting the cytokine network. However, this was a quite surprising and completely unexpected result. Thus, in this study, to generalize the phenomena and to examine the mechanisms of lethal side effect of IND in β-glucan-administered mice, additional parameters were collected by in vivo and in vitro experimental systems. In this study we used SSG, one of the well-characterized immunomodulating β-glucans we have been studying, such as host-mediated antitumor activity, antimicrobial activity, adjuvant activity, enhancement of hematopoiesis, enhancement of cytokine synthesis and so on [26–28,31–34].

2. Materials and methods

2.1. Animals

Mice between 5 and 8 weeks of age were purchased from Japan SLC, Shizuoka, Japan. Mice were maintained under specific pathogen-free conditions. Experiments were performed on male ICR mice unless otherwise stated.

2.2. Reagents

SSG [34] was prepared as described previously. IND, aspirin and glucose-B test were purchased from Wako Pure Chemical Co. IND was suspended with 2% polyoxyethylene (20) sorbitan monooleate in 0.5% sodium carboxymethyl cellulose. The water-soluble form of IND was purchased from Banyu Pharmaceutical Co. Nabumetone and diclofenac were purchased from Sigma. Fetal calf serum (FCS) was from Atlanta Biologicals. Blood agar (sheep) was purchased from Nissui Seiyaku Co. Peroxidase activity was measured by TMB substrate systems (KPL Inc., MD, USA).

2.3. Enzyme-linked immunosorbent assay (ELISA)

A 96-well plate (Sumitomo Bakelite Co., Tokyo) was coated with rat anti-mouse IFN-γ monoclonal antibody (mAb) (Pharmingen, San Diego, CA, USA) in 0.1 M NaHCO₃ (pH 8.2) by incubation at 4°C overnight. The plate was washed with phosphate-buffered saline containing 0.05% Tween 20 (PBST; Wako Pure Chemical Co.) and blocked with 0.5% bovine serum albumin (Sigma) at 37°C for 40 min. After washing, the plate was incubated with recombinant mouse IFN-γ (Pharmingen, San Diego, CA, USA) or 50 µl of samples at 37°C for 40 min. Then, the plate was washed with PBST and treated with biotinylated rat anti-IFN-γ mAb (Pharmingen, San Diego, CA, USA). The plate was then treated with peroxidase-conjugated streptavidin (Zymed Laboratories) and developed with a TMB substrate system (KPL Inc., MD, USA). Color development was stopped with 1 N phosphoric acid and the optical density at 450 nm was measured.

2.4. Cell preparation

Mononuclear cells (MNC) were harvested from various mouse tissues. Hepatic lymphocytes were isolated by Percoll density gradient. Briefly, after sacrificing mice, the liver was removed, pressed through a 200-gauge stainless steel mesh and then suspended in Hanks’ balanced salt solution containing 2% heat-inactivated FCS. MNC were isolated from parenchymal hepatocytes and the nuclei of hepatocytes by the Percoll discontinuous gradient method using 30, 44 and 70% Percoll. Lymphocytes were obtained by pressing the spleen through a 200-gauge steel mesh; erythrocytes in the spleen were disrupted by ACK-lysing buffer (8.29 g l⁻¹ NH₄Cl, 1 g l⁻¹ KHCO₃, 37.2 mg l⁻¹ EDTA/Na). Peritoneal-exuded cells (PEC) were collected from the peritoneal cavity by washing twice with Hanks’ balanced salt solution. In vitro cell culture was performed in RPMI-1640 medium (Sigma) containing 10% heat-inactivated FCS in a 5% CO₂ incubator at 37°C.
2.5. Flow cytometric analysis

The surface phenotype of cells was analyzed using mAbs in conjunction with a two-color immunofluorescence test. The mAbs used here included fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated reagents of anti-CD3 (145-2C11), anti-IL-2R\(\alpha\) (TM-b1), anti-NK1.1 (PK136), anti-CD4 (RM4-5), anti-CD8 (53-6.7), anti-Mac-1 (M1/70) and anti-Gr-1 (RB6-8C5) mAbs (Pharmingen, San Diego, CA, USA). The fluorescent-positive cells were analyzed by FACS Calibur (Becton Dickinson). Dead cells were excluded according to their forward scatter and side-scatter gating. Intracellular IFN-\(\gamma\) was stained using an IC screen® intracellular staining kit for mouse IFN-\(\gamma\) (Biosource International, Camarillo, CA, USA), according to the standard protocol.

2.6. Statistical analysis

Statistical significance was analyzed by Student’s \(t\)-test.

3. Results

3.1. Effect of strains and NSAIDs on lethal toxicity induced in \(\beta\)-glucan-administered mice

In order to generalize the lethal side effect mediated by IND in \(\beta\)-glucan-administered mice, various strains and various NSAIDs were tested for their lethality. Table 1 summarizes the effect of strains on the lethality mediated by IND in \(\beta\)-glucan-administered mice. SSG (250 \(\mu\)g mouse\(^{-1}\)) was administered to these mice on days \(-5\), \(-3\) and \(-1\), IND was per orally (p.o.) administered to these mice daily from day 0, and lethality was monitored. Eleven strains of mice were tested for the side effect. The dose of IND was slightly varied depending on the strains tested, because of the direct lethal toxicity. The strains include, C5 deficiency (DBA/2, AKR/N), phospholipase A2 deficiency (C57BL/6), T-cell deficiency (BALB/c nu/nu, KSN), mast cell deficiency (WBB6F1), low responder to bacterial lipopolysaccharide (C3H/HeJ), and low natural killer activity (A/J) [35-42]. However, of quite an interest, the effect was almost independent of the genetic background and all of the strains tested showed enhanced lethality by this administration protocol.

Table 2 summarizes the effect of various NSAIDs on the lethality. Aspirin, diclofenac and sulindac showed a similar effect with IND, but nabumetone did not show such toxicity. It is well known that NSAIDs often show toxicity on the gastrointestinal tract, and nabumetone is known to show less side effects on the tract [43,44]. These facts strongly suggested the significant contribution of the gastrointestinal tract on the lethality.

From the data shown in Tables 1 and 2, the lethal side effect of NSAIDs in \(\beta\)-glucan-administered mice was a generally considerable phenomenon.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Life span (days, mean ± S.D.(^{\ast}))</th>
<th>Number of mice (dead/total on day 14)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND (mg kg(^{-1}))</td>
<td>SSG ((\mu)g mouse(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICR</td>
<td>5</td>
<td>12 ± 4.47</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.8 ± 1.79**</td>
<td>5/5</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>5</td>
<td>14 ± 2.0</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.8 ± 3.70</td>
<td>3/5</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>5</td>
<td>13 ± 2.24</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.8 ± 2.28**</td>
<td>5/5</td>
</tr>
<tr>
<td>AKR/N</td>
<td>5</td>
<td>13.6 ± 0.89</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.6 ± 0.55**</td>
<td>5/5</td>
</tr>
<tr>
<td>BALB/c</td>
<td>5</td>
<td>14 ± 2.0</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.2 ± 4.32*</td>
<td>4/5</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>5</td>
<td>14 ± 2.0</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.6 ± 4.93**</td>
<td>4/5</td>
</tr>
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<td>DBA/2</td>
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<td></td>
<td>5</td>
<td>3.2 ± 0.45*</td>
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</tr>
<tr>
<td>A/J</td>
<td>5</td>
<td>12.4 ± 2.07</td>
<td>4/5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6 ± 4.18*</td>
<td>5/5</td>
</tr>
<tr>
<td>BALB/c nu/nu</td>
<td>3</td>
<td>13 ± 2.17</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.6 ± 1.82**</td>
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<td>KSN</td>
<td>5</td>
<td>7.2 ± 2.28</td>
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</tr>
<tr>
<td></td>
<td>5</td>
<td>3.6 ± 0.55*</td>
<td>5/5</td>
</tr>
<tr>
<td>WB6F1</td>
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</tr>
<tr>
<td></td>
<td>5</td>
<td>4.8 ± 2.17**</td>
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</tr>
</tbody>
</table>

SSG was i.p. administered on days \(-5\), \(-3\) and \(-1\) before IND administration on day 0. The significance was evaluated by Student’s \(t\)-test against the corresponding IND-administered group. \(^{\ast}P < 0.05\), \(^{\ast\ast}P < 0.01\).

\(^{\ast}\)Mortality was monitored from the day of IND administration.
3.2. Physiological parameters of IND/SSG-administered mice

To clarify the mechanism of IND/β-glucan-induced death, some physiological parameters influenced by IND/β-glucan administration were examined. SSG was intraperitoneally (i.p.) administered three times, every other day from day 35. IND was singly p.o. administered on day 0. Body temperature, serum glucose and cytokine concentrations were monitored from day 0 to day 2. The mean body temperature of the IND/SSG group is shown in Fig. 2. It decreased from 24 h after IND administration. The serum glucose level of the IND/SSG group exhibited the same tendency (Fig. 1). In contrast, serum IFN-γ concentration gradually and significantly increased after IND/SSG administration (Fig. 3).

3.3. Increased numbers of granulocytes and macrophages in various tissues of treated mice

Cells were collected from the liver, spleen and peritoneal cavity of mice which were administered SSG and/or IND. The total cell number was counted with a hemocytometer. These cells were stained with mAbs and analyzed with a two-color immunofluorescence test. Lymphocyte number and population were analyzed by staining with CD3/B220, CD3/IL-2Rβ and CD4/CD8 (Fig. 4). The ratio of these cell populations did not significantly change under...
three experimental conditions. On the other hand, the number of granulocytes (Mac-1⁺Gr-1⁺) and macrophages (Mac-1⁺Gr-1⁻) did significantly increase and were calculated by FACS Calibur. These cells increased in any tissues of SSG/IND-treated mice (Figs. 5–7). Markedly, granulocytes were increased in PEC.

3.4. Activation of leukocytes in IND/SSG-administered mice assessed in vivo and in vitro

It is well known that neutrophils, included in granulocytes, have a numerous amount of myeloperoxidase in the cell. To evaluate the granulocytosis more precisely, peroxidase activity was compared. Spleen cells were collected by the method as described above. Cells were lysed by freezing and thawing, and the peroxidase activity of these cell lysates was measured. As shown in Fig. 8, the IND/SSG group showed the highest peroxidase activity. These facts well correlated with granulocytosis.

In order to examine the activation of leukocytes more precisely, peritoneal exudate cells were stimulated with IND in vitro and IFN-γ productivity was compared. As the first experiment, a whole-cell preparation of PEC was used and various concentrations of IND were added and cultured. As shown in Fig. 9, the concentration of IFN-γ increased dose-dependently in PEC collected from SSG-administered mice. To clarify the cells producing IFN-γ, PEC were separated into adherent and non-adherent cell preparations and IFN-γ production was compared in response to IND. As shown in Fig. 9, removal of the non-adherent cells significantly reduced the productivity of IFN-γ. In addition, cultured cells were fixed, permeabilized, stained with PE-labeled anti-IFN-γ, and analyzed by FACS. As shown in Fig. 10, the population of lymphocytes prepared from SSG-administered mouse produced a higher concentration of IFN-γ. These facts strongly suggested that leukocytes were increased as well as activated by IND/SSG treatment.

3.5. Bacterial translocation induced by IND/SSG treatment

As shown in Table 2, nabumetone, a lower toxic compound for gastrointestinal tract, did not show any lethal side effect in this experimental protocol. Damage in the intestine might induce lethal toxicity, thus, bacterial numbers were compared in these mice. As shown in Table 3, IND/SSG-administered mice contained a significantly higher number of bacteria in the peritoneal cavity. These facts strongly suggested that severe damage and increased permeability of the gastrointestinal tract induced ulcers and peritonitis in IND/β-glucan-administered mice.

4. Discussion

β-Glucan is a BRM that regulates host immune response [19–25,31,32]. We have found that the combination of a β-glucan and NSAIDs induced lethal toxicity in mice [33]. Combination of a β-glucan and IND increased the number of leukocytes, especially macrophages and neutrophils, in various organs and these cells were activated. The activated state of these cells was supported by the in vitro culture of the PEC of β-glucan-administered mice, which produced a higher concentration of IFN-γ in the presence of IND (Fig. 9). Intestinal bacterial flora were translocated into the peritoneal cavity in these mice to cause peritonitis. Comparing the toxicity of various NSAIDs, nabumetone, a partially COX-2-selective NSAID with weaker toxicity in neutrophils, in various organs and these cells were activated. The activated state of these cells was supported by the in vitro culture of the PEC of β-glucan-administered mice, which produced a higher concentration of IFN-γ in the presence of IND (Fig. 9). Intestinal bacterial flora were translocated into the peritoneal cavity in these mice to cause peritonitis. Comparing the toxicity of various NSAIDs, nabumetone, a partially COX-2-selective NSAID with weaker toxicity in the gastrointestinal tract, did not show the lethal side effect. These facts strongly suggested that gastrointestinal damage by NSAID was more severe in β-glucan-administered mice, resulting in peritonitis by intestinal bacterial flora and leading to death.

We have long been working on immunopharmacological parameters induced by β-glucans. It is generally accepted that β-glucans show a broad spectrum of immunopharmacological activity. We have shown that β-glucan is an IFN-γ inducer and this property is strongly related to the nitrogen oxide synthesis of macrophages in vivo, and enhanced production of tumor necrosis factor (TNF)-α in response to lipopolysaccharide. The lethal toxicity of IND/β-glucan-administered mice might be related to the above properties. IFN-γ is known to be an inducer of COX-2, an inducible type of COX responsible to the inflammatory responses. We did not directly examine the synthesis of COX-2 under these experimental conditions, an increased concentration of IFN-γ strongly suggested the production of the enzyme in β-glucan-administered mice. It is well characterized that the arachidonate cascade, producing

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Fig. 3. IFN-γ production in sera of SSG/IND-administered mice. Sera were prepared 6–24 h after IND administration. Concentration of IFN-γ was measured by ELISA. The significance was evaluated by Student’s t-test against Nil. *P < 0.05, **P < 0.01.
Fig. 4. Lymphocyte phenotypes in various tissues. Cells were collected from the liver, spleen and peritoneal cavity of mice which were administered SSG/IND. The surface phenotypes of these cells were analyzed by a two-color immunofluorescence test using CD4 and CD8, CD3 and IL-2Rβ, or TCRαβ and TCRγδ. The fluorescence was analyzed by flow cytometry. (A) shows plots of cells collected from the mouse 48 h after IND administration. (B) shows the parentage of each phenotype at 6, 24 and 48 h after IND administration. ● Nil; ■ SSG; △ IND; × S+I.
Fig. 4 (Continued).

**A**

- **Nil**
- **SSG**
- **IND**
- **S+I**

**B**

<table>
<thead>
<tr>
<th>(%)</th>
<th>CD3^+IL-2R β^- cell</th>
<th>TCR α β^+ cell</th>
<th>CD3^int IL-2R β^+ cell</th>
<th>CD4^+ cell</th>
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<td>24hr</td>
<td>48hr</td>
<td>6hr</td>
<td>24hr</td>
<td>48hr</td>
</tr>
</tbody>
</table>

- ◆ - Nil
- ■ - SSG
- △ - IND
- × - S+I

Fig. 4 (Continued).
Fig. 4 (Continued).

A

<table>
<thead>
<tr>
<th>Group</th>
<th>PEC</th>
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<tbody>
<tr>
<td>Nil</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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</tr>
<tr>
<td>IND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S+I</td>
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</tr>
</tbody>
</table>

B

- CD3^+IL-2Rβ^- cell
- TCRαβ^+ cell
- CD3^+IL-2Rβ^- cell
- CD4^- cell

---

- ● Nil
- ■ SSG
- △ IND
- × S+I

Fig. 4 (Continued).
various bioactive substances, such as PG, leukotriene, thromboxane and platelet-activating factor (PAF), are constituted by varieties of enzymes, such as phospholipase A2, COX and lipoxygenase (LOX), to modify arachidonic acid and related substances. COX is a key enzyme in the production of PGs [45–50]. NSAIDs such as IND work by interfering with the formation of PGs. These are naturally occurring substances in the body that cause inflammation and make nerves more sensitive to pain impulses. PG has a variety of functions in vivo, for example, the production of membrane-bound PAF and TNF-α were reported to be rather augmented by IND administration.

Arachidonic acid is itself an important regulator of specific cellular processes including the regulation of PKC and phospholipase C and modulation of Ca^{2+} transients. Arachidonic acid can also be converted to potent inflammatory lipid mediators, the eicosanoids. This can occur enzymatically through the lipoxygenase or COX pathways for the production of leukotrienes, lipoxins, thromboxanes, or PGs. Arachidonic acid is also subject to non-enzymatic, free radical oxidation to bioactive isoprostanes and isoleukotrienes. Activation of the 5-LOX of leukocytes produces the leukotrienes and these lipid–peptide conjugates and dihydroxyeicosanoids provoke bronchoconstriction and inflammation.

In this study we found augmented production of IFN-γ by IND addition to PEC culture in vitro, probably due to inhibition of the down-regulation system controlled by

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Fig. 5. Immunofluorescence test of granulocytes and macrophages in various tissues. Cells were collected from the liver, spleen and peritoneal cavity of mice which were administered SSG/IND. The surface phenotypes of these cells were analyzed by a two-color immunofluorescence test, using FITC anti-monocyte (Mac-1) mAb and R-PE anti-granulocyte (Gr-1) mAb.
PGE synthesis. In addition, augmentation of IFN-γ might further positively effect the production of COX-2, similar to the self-induction system.

NSAIDs are associated with a number of adverse effects. These include effects on the kidney, and exacerbating asthma in some people, but the most important adverse effect of NSAIDs and aspirin is that on the gastrointestinal tract. NSAIDs and aspirin cause gastric erosions which can become ulcers. These can cause symptoms of an ulcer in some people, the ulcers may bleed, and indeed some people may die of a bleeding ulcer caused by NSAIDs.

Melarange and Rashbrook compared the effect of nabumetone and IND on rat gastric mucosal COX activity ex vivo and in vitro [44]. Nabumetone was less potent and less active in inhibiting the production of gastric mucosal 6-keto-PGF1α compared with IND. Anti-inflammatory doses of nabumetone failed to enhance bile salt-induced gastric erosion or mucosal permeability to dextran whereas IND significantly enhanced gastric erosion and in-
creased dextran permeability. These results suggest that nabumetone fails to promote gastric damage or increase permeability because of minimal effects on gastric mucosal COX. Cardin et al. compared IND and nabumetone on the gastric PG release and mucosal resistance to injury through central vagal pathways using a stable thyrotropin-releasing hormone (TRH) [43]. They have found that, under the conditions of producing a similar acute anti-inflammatory response in the carrageenin-induced paw edema, IND potentiated the acid secretion induced by intracisternal injection of TRH whereas nabumetone did not modify the secretory response. Under the experimental conditions, moderate erosions were observed in 100% of rats treated with the combination of IND and TRH whereas no erosions were observed with TRH in combination with nabumetone. In addition, TRH-mediated reduction of mucosal damage induced by intragastric administration of ethanol was also abolished by IND but not altered by nabumetone pretreatment. These data indicate that at comparable anti-inflammatory doses, nabumetone, unlike IND, neither blocks the protection against ethanol injury induced by low doses of TRH injected intracisternally nor potentiates the gastric acid secretion or lesions induced by a higher dose of TRH. In this study, we found the bacterial translocation in IND-administered mice. This observation could be well correlated with the increased vascular permeability and damage of the gastrointestinal tract by combination use of IND/β-glucan administration, and the effects were negligible in the case of nabumetone.

In IND/β-glucan-administered mice, body temperature as well as serum glucose level were significantly reduced. The body temperature of the IND/β-glucan-administered group was lower than that of the IND-administered group. These mice were morbid also by visual inspection. This is also consistent with the hypoglycemic state of these mice. These facts strongly suggested induction of the systemic effect by IND/β-glucan administration. The leukocyte number, especially monocyte/macrophage, and granulocytes were increased significantly by IND/β-glucan administration. We have shown the enhanced production

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### Table 3

<table>
<thead>
<tr>
<th>Total mouse number</th>
<th>Intrapertioneal bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-negative</td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Nil</td>
<td>5</td>
</tr>
<tr>
<td>SSG</td>
<td>5</td>
</tr>
<tr>
<td>IND</td>
<td>6</td>
</tr>
<tr>
<td>SSG + IND</td>
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</tr>
</tbody>
</table>

The peritoneal cavity was washed with 5 ml of Hanks’ balanced salt solution. Peritoneal washings (100 µl) were spread on dishes of blood agar. The resulting dishes were cultured for 24 h at 37°C. The number of colonies was counted by visual inspection. No colonies, −; 1–100 colonies, +; 100–1000 colonies, ++; 1000–colonies, +++.

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**Fig. 8. Peroxidase activity of spleen cells from SSG/IND-administered mice.** The cell lysates were prepared by freezing and thawing. The cell lysates were adjusted to the same protein concentration and peroxidase activity measured by the TMB substrate system. Peroxidase activity: 1 U is equivalent to 7.8 pg ml⁻¹ avidin-horseradish peroxidase. The significance was evaluated by Student’s t-test against the corresponding Nil. **P < 0.01.**
of IFN-γ in vitro. In the previous paper, we have shown the increased concentration of colony-stimulating factor and IL-6 in sera of IND/β-glucan-administered mice. These cytokines have hematopoietic activity, thus the increment of leukocytes is strongly related to the cytokinemiu.

In this study we used various strains of mice, which include lipopolysaccharide-unresponsive, phospholipase A2-deficient, complement component-deficient, NK cell-deficient, or T cell-deficient. None of the strains were specifically resistant or sensitive to the treatment. These facts strongly suggest that this phenomenon may be induced by the function of multiple gene products.

Most NSAIDs have numerous gastrointestinal side effects that are due mainly to the suppression of PG synthesis and the subsequent reduction in vascular perfusion within the gastric mucosa. These side effects significantly limit the usefulness of NSAIDs in the long-term therapy necessary for the treatment of chronic inflammatory diseases such as rheumatoid arthritis, and Alzheimer’s disease [51–53]. Aspirin is a drug that reduces swelling, pain and fever. In recent years, long-term low-dose aspirin has been recommended to reduce the risk of heart attacks and strokes. In the future aspirin may be recommended to reduce the risk of some cancers. Reye’s syndrome, a rare but serious illness affecting children and teenagers, has been associated with aspirin use [8,54–56]. The results obtained in this study suggest a severe side effect of NSAIDs and aspirin. Much attention must be given to various parameters influenced in the long-term exposure to these drugs.

Acknowledgements

Supported by a Grant for Private Universities (HRC project) provided by the Ministry of Education, Science, Sports and Culture.

References


