Effect of Gomisin A (TJN-101) on the Arachidonic Acid Cascade in Macrophages

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Abstract—It has been reported that leukotrienes (LTs) may play a role in inflammatory liver diseases, and several inhibitors of LTs show an inhibitory effect on experimental liver injuries. In this study, the effect of Gomisin A (TJN-101), which is a lignan component of schisandra fruits, on the arachidonic acid cascade in macrophages was examined to explain the mechanisms of the inhibitory effect of TJN-101 on liver injuries. The production of leukotriene B₄ was suppressed by treatment with TJN-101, while the activity of 5-lipoxygenase was not affected. The release of arachidonic acid from macrophages stimulated with fMet-Leu-Phe or the Ca²⁺ ionophore A23187 was suppressed by treatment with TJN-101. The activity of phospholipase A₂ was not affected by treatment with TJN-101. These results suggested that TJN-101 produces an inhibitory effect on the biosynthesis of LTs by preventing the release of arachidonic acid, and it was thought that the preventive effect on the arachidonic acid cascade may be partially associated with the inhibitory effect of TJN-101 on liver injuries.

Gomisin A (TJN-101), a lignan component of schisandra fruits (1), has been known to prevent liver injuries induced by hepatotoxic chemicals (2–5). We have previously reported that TJN-101 reduced the mortality of mice with immunological acute hepatic failure experimentally induced by the injection of heat-killed Propionibacterium acnes (P. acnes) and a small dose of lipopolysaccharide (6). TJN-101 also prevented the isolated liver cell injuries caused by the culture supernatant containing cytotoxic factors from the antibody-dependent cell-mediated cytotoxic reaction mixture or activated macrophages reaction mixture in vitro (6, 7). These findings suggest that TJN-101 has a protective effect against liver cell injuries caused by cytotoxic factors from immunological reactions.

Leukotrienes (LTs) are potent inflammatory agents that are synthesized by the lipoxygenase pathway. Recently, it has been reported that LTs may play a role in inflammatory liver diseases (8). In immunological acute hepatic failure, mononuclear cells (e.g., monocytes and macrophages) are infiltrated in the liver lobules by the injection of heat-killed P. acnes (9–12), and these cells are the predominant cells producing LTs (13, 14). Kupffer cells, which are resident hepatic macrophages, have also been shown to release LTs (15, 16). Another report has suggested that the induction of immunological acute hepatic failure is suppressed by treatment with 5-lipoxygenase inhibitors (17). In this study, the effect of TJN-101 on the arachidonic acid cascade in macrophages was examined to explain the mechanisms of the inhibitory effect of TJN-101 on liver injuries.

Materials and Methods

Materials

The chemical structure of TJN-101 is shown in Fig. 1. This component was isolated from schisandra fruits by the method reported
by Ikeya et al. (1) and dissolved in ethanol. The LTB₄ [³H] RIA kit and L-α-1-palmitoyl-2-(1-¹⁴C)arachidonyl - phosphatidylcholine (specific activity, 54.5 mCi/mmol) were purchased from New England Nuclear, Boston, Massachusetts. (1-¹⁴C)Arachidonic acid (specific activity, 59.6 mCi/mmol) was purchased from the Radiochemical Center, Amersham, United Kingdom. Ca²⁺ ionophore A23187, arachidonic acid, penicillin, streptomycin, 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE, 15-HETE and porcine pancreatic phospholipase A₂ (PLA₂) were purchased from Sigma Chemical Co., St. Louis, Missouri. The biologically active peptide fMet-Leu-Phe was purchased from the Peptide Institute, Inc., Osaka, Japan. Marcol 52 was purchased from Esso Sekiyu K.K., Tokyo, Japan. Hank’s solution and RPMI 1640 were purchased from Nissui Pharmaceutical Co., Ltd., Tokyo, Japan.

Methods

1.LTB₄ production: Male Wistar strain rats, weighing about 350 g, were used. Ten milliliters of sterilized Marcol 52 was injected into the peritoneal cavity. Peritoneal exudate cells were collected 4 days later by perfusing the peritoneal cavity with 200 ml of Hank’s solution. After the oil phase was removed, a cell population consisting of more than 90% the macrophages was obtained by density gradient centrifugation (18). The cells were washed and suspended in RPMI 1640 solution containing 1% BSA, 100 units/ml of penicillin, and 100 µg/ml of streptomycin. The cell concentration was adjusted to 1×10⁷ cells/ml, and 10 µl of ethanol or various concentrations of TJN-101 solution was added to 1 ml of cell suspension. After incubation at 37°C for 12 hr in a humidified cell-incubator with aeration by 5% CO₂ in air, the cells were washed and resuspended in Gey’s buffer containing 1% BSA. The amount of LTB₄ was measured with the LTB₄ [³H] RIA kit after stimulation of the cells with 1 µM of Ca²⁺ ionophore A23187 at 37°C for 20 min.

2. 5-Lipoxygenase activity: 5-Lipoxygenase activity was measured by the previously described method (19). Rat peritoneal macrophages, prepared as described above, were suspended in 0.05 M phosphate buffer containing 1 mM EDTA and 0.1% gelatin. The cells were homogenized and centrifuged at 105,000 g for 10 min. The resulting supernatant was incubated with 0.2 µCi of (1-¹⁴C)-arachidonic acid, 0.8 mM of CaCl₂, 2×10⁻⁵ M of indomethacin, and various concentrations of TJN-101 solution at 37°C for 5 min. After the incubation, the reaction was terminated by adjusting the pH of the mixture to 3.0 with HCl, and the mixture was extracted with 8 volumes of ethyl acetate. The lipid extract, after evaporation under a stream of N₂, was chromatographed on a silica gel G plate and was separated using the solvent system of petroleum ether/ether/acetic acid (50:50:1). Labeled substrate and standards were localized by autoradiography or iodine vapor and scraped off for liquid scintillation counting. The activity of 5-lipoxygenase was expressed as the radioactivity of 5-HETE.

3. Release of arachidonic acid: The release of arachidonic acid was measured according to the method of Hirata et al. (20). Male Hartley strain guinea pigs, weighing about 450 g, were used. Five milliliters of Marcol 52 was injected into the peritoneal cavity. Peritoneal exudate cells were collected 4 days later by the method described above. The cells were suspended at a concentration of 5×10⁶ cells/ml and incubated for 12 hr. After incubation, the cells were washed with Gey’s buffer containing 1% BSA and resuspended in the same buffer. Each cell suspension was incubated with 0.25 µCi of (1-¹⁴C)arachidonic acid for another 1 hr, and then the cells were washed. The cells were resuspended in the same buffer, and each cell suspension was incubated with 10 nM of biologically active peptide, fMet-Leu-Phe (20), or 1 µM of Ca²⁺ ions.
ionophore A23187 for 10 min. Five percent of trichloroacetic acid solution (0.5 ml) was added to each suspension and centrifuged at 10,000 g for 20 min. The radioactivity of the resulting supernatant was measured by a liquid scintillation counter.

4. PLA₂ activity: The activity of PLA₂ was measured using L-α-1-palmitoyl-2-(1-1⁴C)-arachidonyl phosphatidylcholine as the substrate. The reaction mixture containing 0.2 μM of radioactive substrate, 0.05 μg of porcine pancreatic PLA₂, and various concentrations of TJN-101 solution were incubated at 37°C for 5 min. After incubation, the reaction was terminated by 3 ml of Dole’s reagent (21, 22). The activity of PLA₂ was determined by the release of (1-1⁴C)arachidonic acid from the substrate.

5. Statistical analysis: Results were expressed as means±S.E. Statistical evaluation was performed by an analysis of variance. If the F values indicated statistically significance, a statistical analysis of the data was made by Student’s t-test.

Results

1. Effect on the production of LTB₄: A cell population consisting of more than 90% macrophages was used, and cell viability was approx. 90–95%, as assessed by the trypan blue test. When the cells were incubated for 12 hr, cell viability was maintained at more than 90%, and it was not affected by treatment with TJN-101 (data not shown).

The production of LTB₄ in the macrophages is shown in Fig. 2. The production without stimulation by the Ca²⁺ ionophore A23187 was 2.6±0.1×1⁴ pg/1⁷ cells (n=5), and this production was increased to 11.4±0.2×1⁴ pg/1⁷ cells (n=5) by stimulation with the ionophore. Treatment with 1×1⁴ g/ml of TJN-101 reduced this increase by about 50%.

2. Effect on the activity of 5-lipoxygenase: When the lipid extract, which was extracted from the reaction mixture of the cytosolic fraction of macrophages and ¹⁴C-arachidonic acid, was separated using TLC, the radioactive spots obtained corresponded to standard 5-HETE (Rf=0.37), 12- or 15-HETE (Rf=0.69), arachidonic acid and other lipids. 12-HETE and 15-HETE were not separated in this system. The amount of 5-HETE produced was 2.2±0.3×1⁴ cpm/ml (n=5), and it was about 2.5-fold higher than the amounts of 12- and 15-HETE. The effect of TJN-101 on the activity of 5-lipoxygenase was expressed as the production of 5-HETE. The production of 5-HETE was not affected by treatment with TJN-101 (Fig. 3).

3. Effect on the release of arachidonic acid: Arachidonic acid is released from the cell membrane by stimulation with fMet-Leu-Phe (20) or Ca²⁺ ionophore A23187, and the release of arachidonic acid from the cell membrane was studied using a radiolabeled substrate. (1-¹⁴C)arachidonic acid, which was incubated with macrophages for 1 hr; About 25% of the radiolabeled substrate was in-
Table 1. Effect of TJN-101 on the release of 14C-arachidonic acid induced by fMet-Leu-Phe or Ca++ ionophore A23187 in guinea pig peritoneal exudate cells

<table>
<thead>
<tr>
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<th>Released by fMet-Leu-Phe</th>
<th>Released by Ca++ ionophore A23187</th>
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<tr>
<td></td>
<td>Release of 14C-arachidonic acid (cpm/5×10^6 cells)</td>
<td>inhibition (%)</td>
</tr>
<tr>
<td>Control</td>
<td>4663.5±161.8</td>
<td></td>
</tr>
<tr>
<td>TJN-101, 10^-6 g/ml</td>
<td>3564.8±153.8**</td>
<td>23.6</td>
</tr>
<tr>
<td>TJN-101, 10^-7 g/ml</td>
<td>3064.5±149.7**</td>
<td>34.3</td>
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<tr>
<td>TJN-101, 10^-8 g/ml</td>
<td>2285.0±32.7**</td>
<td>51.0</td>
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Each datum represents the mean±S.E. of 5–6 different experiments. Statistically significant difference from the control group at *P<0.05 and **P<0.01, respectively.

Fig. 4. Effect of TJN-101 on the activity of phospholipase A2. Each column represents the mean±S.E. of 6 different experiments.

corporated into the cells, and this was not affected by treatment with TJN-101 (data not shown). In the control group, the release of (1-14C)arachidonic acid stimulated with fMet-Leu-Phe was 4.7±0.2×10^3 cpm/5×10^6 cells (n=6). This release of arachidonic acid from macrophages was markedly suppressed by TJN-101 treatment. As higher concentrations of TJN-101 were added, the release was more suppressed. When the macrophages were stimulated with the Ca++ ionophore A23187, the release of (1-14C)-arachidonic acid in the control group was 3.1±0.4×10^3 cpm/5×10^6 cells (n=6). This release was prevented by treatment with TJN-101 (Table 1).

4. Effect on the activity of PLA2: Arachidonic acid is hydrolyzed from the β-position of phosphatidylcholine by incubation with PLA2 in vitro. This hydrolysis was 4.2±1.6×10^6 cpm/mg protein/min (n=6), and it was not affected by treatment with TJN-101 at the dose of 1×10^-7 to 1×10^-4 g/ml (Fig. 4).

Discussion

TJN-101 shows an inhibitory effect on liver cell injuries induced by various hepatotoxic chemicals (2–5), and it also prevents liver injuries caused by immunological reactions in vivo and in vitro (6, 7). From these findings, it was thought that TJN-101 protects the liver cells from cytoxicities. LTs are synthesized by the lipoxygenase pathway in various tissues (13, 14) and thought to be the primary mediator of inflammation. Recently, it has been reported that LTs may play a role in inflammatory liver diseases (8). Several inhibitors of LTs synthesis were found to suppress liver injuries induced by D-galactosamine (23) or α-amanitine (18). Another report has shown that immunological acute hepatic failure is inhibited by treatment with a 5-lipoxygenase inhibitor (17), and TJN-101 also has an inhibitory effect on this hepatic failure (6). This injury is induced by cytotoxic factors from mononuclear cells (e.g., monocytes and macrophages), which are infiltrated in the liver lobules, and these cells are the predominant cells producing LTs (13, 14). In this study, the effect of TJN-101 on the arachidonic acid cascade using macrophages, which is a one of these infiltrated mononuclear cells, was examined to clarify the mechanisms of the inhibitory effect of TJN-101 on liver injuries.

When the macrophages were incubated with TJN-101, the production of LTB4 caused by stimulation with the Ca++ ionophore A23187 was reduced. The activity of 5-lipoxygenase was not affected by treatment with TJN-101. LTs are mainly derived from arachidonic acid.
which is released from the \( \beta \)-position of phospholipids by the predominant enzyme PLA\(_2\). TJN-101 suppressed the release of arachidonic acid from macrophages induced by treatment with fMet-Leu-Phe and Ca\(^{2+}\) ionophore A23187. The activity of PLA\(_2\) was not affected by treatment with TJN-101. From these findings, it was suggested that TJN-101 inhibited the biosynthesis of LTs in macrophages by preventing the release of arachidonic acid. Furthermore, it was reported that TJN-101 has a stabilizing effect on the plasma membrane, and it shows an inhibitory effect on the influx of \( ^{45}\)Ca\(^{2+}\) into liver cells (24). These effects of TJN-101 may be related to its inhibitory effect on the release of arachidonic acid.

LTs may play a role in inflammatory liver diseases, and lipoxygenase inhibitor shows a preventive effect on experimental liver injuries. From our findings, it is suggested that the preventive effect of TJN-101 on the arachidonic acid cascade may be associated with its inhibitory effect on liver injuries.

References
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