**Short Communication**

**Sulfatide, a Specific Sugar Ligand for L-Selectin, Blocks CCl₄-induced Liver Inflammation in Rats**

Jun-ichi KAJIHARA, Ye GUOH, Kazuo KATO, and Yasuo SUZUKI*

Biochemistry Research Laboratories, JCR Pharmaceuticals Co., Ltd., 2–2–10 Murotani, Nishi-ku, Kobe 651–22, Japan
*Department of Biochemistry, University of Shizuoka School of Pharmaceutical Science, Shizuoka 422, Japan

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The effects of sulfatide, which is a specific sugar ligand for L-selectin (LECAM-1), on CCl₄-induced liver inflammation was studied in rats. Intramuscular pretreatment with sulfatide suppressed the levels of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) that were increased by CCl₄ injection, but galactosylceramide, a desulfated form of sulfatide, did not. A light-microscopic analysis found that the extent and the severity of lesions of the liver cells induced by CCl₄ injection were significantly less in the rats treated with sulfatide. These results show that sulfatide suppresses the CCl₄-induced liver inflammation by inhibiting the attachment of L-selectin expressing lymphocytes to their native sugar ligands.

Selectins, which include L-selectin, E-selectin, and P-selectin, are well characterized by a similar structural motif consisting of a lectin domain, followed by an epidermal growth factor (EGF) domain, a complement-regulatory domain, and a transmembrane region. The carbohydrate ligands for the selectin family were first identified as sialyl Leα or sialyl Leα,4–7 Suzuki et al.,8 however, reported that native sulfatide purified from bovine brain bound to Rat L-selectin more strongly than did sialyl Leα, and its binding was Ca²⁺-independent. Further, Watanabe et al.9 and Imai et al.10 reported that sulfatides were a ligand for mouse L-selectin.

L-selectin is a cell adhesion molecule that mediates leukocyte rolling and leukocyte adhesion to endothelium at sites of inflammation,11,12 so that the use of a specific sugar ligand such as sulfatide is expected to prevent the inflammation. There are, however, no reports concerning the effects of sulfatide on animal models.

In this paper, the effects of native sulfatide on CCl₄-induced liver inflammation in rats were compared with those of galactosylceramide, a desulfated form of the sulfatide.

Native sulfatide was isolated from fresh bovine brain by the method described before.8) CCl₄ was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Female Wistar rats weighing about 150–200g were obtained from Japan SLC (Hamamatsu, Japan). In the experiment, the rats received an intramuscular injection of sulfatide (0.3, 1.0, 3.0 mg/kg body weight) or galactosylceramide (1.0 mg/kg body weight) and after 15h, acute liver inflammation was induced by intraperitoneal administration of 50% (v/v) of CCl₄ in olive oil at a dose of 0.1 ml per 100g body weight. In another experiment, the rats were treated with an intramuscular injection of sulfatide (1.0 mg/kg) just after the injection of 50% CCl₄. All rats were anaesthetised with ether and bled from the hearts. Measurement of serum GPT and GOT, and light microscopic observation of the liver were done 24h after the injection of CCl₄. Serum GPT and GOT were measured with kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Portions of each liver were fixed in 10% formalin. Paraffin sections were stained with haematoxylin and eosin, and examined under light microscopy. All results are shown as mean ± standard error, and the statistically significant differences (p < 0.05, p < 0.005) were tested by Student’s t-test.

An injection of CCl₄ induced acute liver inflammation in rats and the serum GPT and GOT levels increased greatly after 24h (Table I). However, the intramuscular pretreatment with sulfatide (0.3–3.0 mg/kg) significantly suppressed the serum GPT and GOT levels in a dose-dependent manner, and its maximum suppression rate (70%) was obtained when injected at a dose of 3.0 mg/kg (Table I). The light microscopic examination showed that focal necrosis occurred in the center of the hepatic lobules and the remaining hepatocytes were small and denatured in the rats which were received with only CCl₄. Further, ballooned foamy cells, which are seen as white points in Fig. (b), were observed in the intermediate zone (Fig. (b)). In the rats pretreated with sulfatide, these changes were clearly suppressed (Fig. (c)), indicating that the effects of sulfatide were also confirmed histologically.

The timing of treatment with sulfatide was examined. The effects of sulfatide were clearly observed when the treatment was 15h before an injection of CCl₄, as mentioned above. It had, however, no effect when administered just before the injection of CCl₄.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GOT Suppression rate (%)</th>
<th>GPT Suppression rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>52 ± 3</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>CCl₄ only</td>
<td>2787 ± 491</td>
<td>0</td>
</tr>
<tr>
<td>CCl₄ + Sulfatide (0.3 mg/kg)</td>
<td>2520 ± 635</td>
<td>10</td>
</tr>
<tr>
<td>CCl₄ + Sulfatide (1.0 mg/kg)</td>
<td>1013 ± 483**</td>
<td>64</td>
</tr>
<tr>
<td>CCl₄ + Sulfatide (3.0 mg/kg)</td>
<td>855 ± 273**</td>
<td>70</td>
</tr>
</tbody>
</table>

(Sulfatide was administered intramuscularly at 15h before CCl₄ injection.)

| CCl₄ + Sulfatide (1.0 mg/kg) | 2369 ± 947               | 15                       | 1424 ± 697 |

(Sulfatide was administered intramuscularly just after CCl₄ injection.)

In experiment, five rats were used for each group. *p < 0.05. **p < 0.005.
with sulfatide (1.0 mg/kg) clearly suppressed the serum GPT and GOT levels, that with galactosylceramide (1.0 mg/kg) had no effect. This suggests that the effects of sulfatide in this animal model depends on its sulfate group. Generally, CCl₄ is known to be converted to the CCl₃ radical, which is a highly toxic chemical species, by the NADPH-cytochrome P-450 drug-metabolizing enzyme system.12) The inflammation of this model is induced by the injury of liver cells associated with CCl₃ radical, followed by the development through the influx of neutrophil or lymphocyte. Previously, Abe et al. reported that saikosaponin a or d, which was obtained from the extracts of the roots of Bupleurum falcatum L., was effective on CCl₄-induced liver injury by reducing the liver NADPH-cytochrome P-450 metabolizing enzymes activities,13) however, sulfatide did not affect those activities in our experimental results (data not shown), suggesting that sulfatide has its biological activity through some mechanism distinct from that by saikosaponin.

Our experiment clearly indicated that sulfatide specifically suppressed the liver inflammation induced by CCl₄ since galactosylceramide had no effect. Sulfatide is considered to be one of the specific ligands for L-selectin. For example, its ability to bind to L-selectin was demonstrated using an ELISA or TLC/binding assay system,8) and Green et al. reported that L-selectin binding to endothelium was inhibited by sulfatide in vitro.14) L-Selectin is reported to mediate leukocyte rolling and leukocyte adhesion to endothelium at sites of inflammation,15) so that it is considered that L-selectin is associated with the initial step of inflammation. Our results indicated that only pretreatment with sulfatide had this effect, which supported the hypothesis described above.

In consideration of several pieces of evidence described here, it is suggested that sulfatide may suppress the CCl₄-induced liver inflammation by inhibiting the binding of L-selectin to the native ligand expressed on endothelium.

Table II. The Effects of Sulfatide and Galactosylceramide on CCl₄-induced Liver Inflammation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GOT</th>
<th>Suppression rate (%)</th>
<th>GPT</th>
<th>Suppression rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>52±3</td>
<td>28±2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CCl₄ only</td>
<td>3816±542</td>
<td>0</td>
<td>2016±290</td>
<td>0</td>
</tr>
<tr>
<td>CCl₄ + Sulfatide (1.0 mg/kg)</td>
<td>1492±724</td>
<td>61</td>
<td>808±311*</td>
<td>60</td>
</tr>
<tr>
<td>CCl₄ + Galactosylceramide (1.0 mg/kg)</td>
<td>3280±745</td>
<td>14</td>
<td>1760±390</td>
<td>13</td>
</tr>
</tbody>
</table>

Sulfatide and galactosylceramide were administered intramuscularly at 15 h before CCl₄ injection.

In the experiment, five rats were used for each group.

* p<0.05.

References
5) M. L. Philipis, E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal,
Sulfatide Blocks Liver Inflammation in Rats