Note

Fatty Degeneration of the Liver Induced by HS-6 Toxin Produced by *Nocardia otitidiscaviarum* from Human Cutaneous Nocardiosis

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It has been confirmed that pathogenic actinomycetes do not produce low molecular weight toxic substances, although the isolation of toxic cell wall components such as trehalose mycolate, arabinogalactan mycolate, and peptidoglycolipid has been reported.1) Recently, Mikami et al. found that *Nocardia* strain IFM 0273 isolated from the cutaneous nocardiosis of an 82-year-old male patient in Fukuoka, Japan, produced a substance toxic to cultured cells.2,3) That toxic substance was called HS-6 toxin, and the structure of HS-6 toxin was found to belong to the 16-membered macrocyclic group with a molecular formula of C_{43}H_{68}O_{12}.3) Nocardial infections in the immunocompromised host are well known.4) Since the *Nocardia* strain was found to produce a toxic substance, it would be of considerable interest to find what influences the HS-6 toxin have on animals. In this paper, HS-6 was administered to mice and tissues were then observed morphologically. Furthermore, the liver lipids of mice were analyzed because marked fatty degeneration appeared in the livers of these mice.

HS-6 toxin was prepared from the mycelia of *N. otitidiscaviarum* strain IFM 0273 as described in a previous paper.3) Male ICR mice (5 weeks old) were purchased from Charles River Japan Inc. Graded amounts (515, 625, and 735 μg/kg body weight) of HS-6 toxin dissolved in 0.5% ethanol were given i.p. to the mice in a single dose. The LD_{50} of HS-6 was 1250 μg/kg body weight. The same amount of 0.5% ethanol was given alone as a control. The mice were killed twenty-four hr after the dose of HS-6 toxin, and a sample of liver was stored for use in the following lipid analyses.

All internal organs of the mice were examined macroscopically and then fixed in 10% neutral formalin. The organs were prepared for light microscopy by the hematoxylin-eosin and periodic acid Schiff-staining.

Fig. 1. An Electron Micrograph of the Liver from a Mouse Given 735 μg/kg of Body Weight of HS-6 Toxin.

24 hr after the treatment. F, fat droplet; N, nucleus; A, autophagosome; B, bile capillary. (× 5500)
Fig. 2. Effects of 735 \( \mu \)g/kg Body Weight of HS-6 Toxin Injection on Content of Total Lipids and TBA Reactants in Liver.

O, indicates the control mice; ▲, indicates the mice with weak fatty degeneration; ●, indicates the mice with strong fatty degeneration; ◊, indicates the mice with extreme fatty degeneration in the liver after the injection of 735 \( \mu \)g/kg body weight of HS-6 toxin.

methods. For electron microscopy, pieces of liver were fixed in a cold 2% paraformaldehyde-glutaraldehyde solution for 12 hr and then postfixed in OsO\(_4\) for 2 hr. After dehydration, the specimens were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H 700 H transmission electron microscope.

Thiobarbituric acid (TBA) reactants in liver homogenates were measured following the method of Ohkawa et al.\(^5\)). Total lipids in the mice liver were extracted by the method of Folch et al.\(^6\)) and separated on Chromarods-S (silica gel, Iatron Co., Tokyo, Japan).\(^7\)) The lipid extract was developed with hexane-ethyl ether-formic acid (90:10:0.2, by volume) for the separation of lipid classes. The lipid constituents separated on the rods were scanned with a hydrogen flame ionization detector (Iatroscan TH-10, Iatron Co., Tokyo, Japan). Statistical calculations were done by Student's t-test.

When 735 \( \mu \)g/kg of HS-6 toxin had been administered, 67% of the mice showed strong and extreme fatty degenerations in the centrilobular, midzonal, and periportal areas of the liver. In addition, electron microscopic observations showed numerous fat droplets in hepatocytes after the dose of 735 \( \mu \)g/kg of the toxin (Fig. 1). Numerous autophagosomes were also observed in several hepatocytes. Mice given 515 or 625 \( \mu \)g/kg of HS-6 toxin did not show fatty degeneration in the liver. After the treatment with 735 \( \mu \)g/kg of toxin, the content of total liver lipids of mice with strong and extreme fatty degenerations was markedly increased as compared with that of control mice (Fig. 2).

The TBA reactants in the liver of mice with strong and extreme fatty degenerations were also significantly higher than those of control mice. Following the dose of toxin (735 \( \mu \)g/kg body weight), the content of triglycerides in liver lipid components was significantly increased as compared with that of control mice (Table I). Fatty degeneration is caused by an increase of neutral lipid synthesis and/or a decrease in the discharge of lipids from the liver.\(^8\)) The HS-6 toxin transported to the liver and/or the metabolites of this toxin would cause these disorders in the hepatocytes. The TBA reactants indicate lipoperoxide levels in tissues. Some kinds of liver injuries such as chronic hepatitis and adiposis hepatica accompany the induction of lipoperoxidation in plasma and liver.\(^9\)) The induction of liver lipoperoxidation caused by the dose of HS-6 toxin may be correlated with the appearance of numerous fat droplets in hepatocytes.

This study shows that HS-6 toxin produced by Nocardia otitidiscaviarum caused significant fatty degeneration in the liver of mice. Additional work will be needed to clarify the toxic effects of the HS-6 toxin on the patients who are infected with Nocardia.

### Table I. EFFECTS OF HS-6 TOXIN INJECTION ON CONTENT OF LIVER LIPID COMPONENTS

<table>
<thead>
<tr>
<th>Dose</th>
<th>PL(^{a})</th>
<th>FC</th>
<th>FA</th>
<th>TG</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>54±4</td>
<td>3±1</td>
<td>0±0</td>
<td>7±1(^{c})</td>
<td>0±0</td>
</tr>
<tr>
<td>HS-6 toxin(^{a})</td>
<td>52±3</td>
<td>3±1</td>
<td>1±1</td>
<td>70±46(^{d})</td>
<td>0±0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. Means not followed by c or d are significantly different \( (p<0.05) \).

\(^{a}\) 735 \( \mu \)g/kg body weight.

\(^{b}\) PL, phospholipids; FC, cholesterol; FA, free fatty acids; TG, triglycerides; CE, cholesterol esters.

### References

Toxic Effects of HS-6 Toxin Produced by Nocardia Strain