The Effects of M-protein Fraction of Hemolytic Streptococci on Embryonic Heart in the Rat

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Wistar rats were administered M-protein fraction (MP) of group A type 12 hemolytic streptococci on day 9 of gestation. When 9 mg/kg of MP was injected, the incidence of malformations was 11.8%, and the incidence of cardiovascular malformations was 7.9%. The main malformations were ventricular septal defect, microphthalmia, and hydrocephalus. Light and electron microscopic studies revealed no myocarditis nor endocarditis in either the maternal or fetal heart. Fluorescent antibody technique demonstrated no immunological cross-reaction between MP and rat heart tissue. The results suggest that MP is slightly teratogenic in the rat.

INFECTION is an important environmental factor in teratogenesis. Streptococcal infection common in humans occasionally, causes successive rheumatic fever. Of the suggested pathogenetic mechanisms of rheumatic fever, the immunological cross-reaction between group A streptococcal cells and human heart tissue has been strongly considered. Matsumura and his colleagues reported that mothers who had born babies with congenital heart diseases had higher anti-streptolysin O and anti-streptokinase titers than control mothers with normal babies. Although an association between streptococcal infection and cardiovascular malformations is suspected, there have been no teratological experiments utilizing the M-protein fraction (MP) of hemolytic streptococci. For this reason, we studied the effects of MP on embryonic heart in the rat.

MATERIALS AND METHODS
MP was obtained from group A type 12 hemolytic streptococci by Lancefield's method. Pregnant rats (12 weeks old, weighing approximately 250g) were divided into 2 groups. After confirmation of the number of embryos by laparotomy on day 9 of gestation (plug day = day 0), each agent was injected intraperitoneally following the operative procedure of Brent. Group MP received varying doses of MP diluted with saline (1 mg/ml). Control group was injected with saline. On day 21, the rats were killed, and the number of resorbed, dead, and live fetuses were counted. Each fetus was fixed in Bouin's fixative and dissected following the technique of Barrow and Taylor. Maternal and some fetal hearts were removed and preserved in 10% formaldehyde solutions. Hematoxylin-Eosin stained preparations were used for observations. Portions of maternal and some fetal myocardia were fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in epoxy resin for electron microscopic examination. In order to detect the immunological cross-reaction between MP and rat heart tissue, the fluorescent antibody technique was employed.

Key Words:
Experimental teratology
M-protein of hemolytic streptococci
Cardiovascular malformations

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TABLE I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of litters</th>
<th>No. of embryos on day 9</th>
<th>Mortality (%)</th>
<th>Malformations (%)</th>
<th>Cardiovascular malformations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP 3 mg/kg</td>
<td>6</td>
<td>85</td>
<td>2.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MP 4.5 mg/kg</td>
<td>6</td>
<td>84</td>
<td>6.0</td>
<td>5.1</td>
<td>2.5</td>
</tr>
<tr>
<td>MP 6 mg/kg</td>
<td>6</td>
<td>73</td>
<td>8.2</td>
<td>7.5</td>
<td>4.5</td>
</tr>
<tr>
<td>MP 9 mg/kg</td>
<td>6</td>
<td>86</td>
<td>11.6</td>
<td>11.8*</td>
<td>7.9</td>
</tr>
<tr>
<td>Saline 9 ml/kg</td>
<td>6</td>
<td>79</td>
<td>3.8</td>
<td>2.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Significantly different from the control at p < 0.05

RESULTS

Table I summarizes the number of litters and fetuses, as well as mortality and malformations. Group MP (9 mg/kg) showed a significant increase (p < 0.05) in malformations compared with the control group. The types and number of malformations in both groups are shown in Table II. The incidence of cardiovascular malformations was 7.9% in group MP (9 mg/kg). In the maternal hearts in group MP, the infiltrations of eosinophils into the myocardial interstices and slight infiltrations of mononuclear cells and lymphocytes in perivascular locations were observed. No significant changes were found in the fetal myocardia. Mitochondrial changes (mitochondriosis, disappearing of cristae) were observed in the maternal myocardia by electron microscopic examination, though in the fetal myocardia they remained unchanged. The fluorescent antibody technique demonstrated no cross-reaction between MP and rat heart tissue.

DISCUSSION

Previously, we reported that the incidence of malformations was low following administration of anti-heart serum alone, while in combination with subteratogenic dose of anti-kidney serum, the incidence of cardiovascular malformations increased markedly. Previous reports have supported the concept of an autoimmune mechanism in the pathogenesis of rheumatic heart disease, and some possibility of autoimmune mechanism in teratogenesis. However, no animal experiments have been reported concerning streptococcal infection and congenital heart diseases.

In the present study, the administration of MP (9 mg/kg) increased the incidence of malformations compared to the control group. MP is a component of cell walls of hemolytic streptococci and the most important factor affecting virulence. The effects of MP on the maternal hearts were slight cellular infiltrations in myocardial interstices and perivascular locations, but there was no significant myocarditis or endocarditis. Light and electron microscopic studies revealed no cellular infiltration in the fetal hearts. The fluorescent antibody technique revealed no immunological cross-reaction between MP and rat heart tissue. In the group MP, the incidence of cardiovascular malformations was relatively high, and microphthalmia and hydrocephaly were also noted. The types of malformations were roughly the same as that following anti-kidney serum administration. Therefore, these findings demonstrated that the principal factor in the teratogenesis was not an infiltration in the fetal heart caused by an auto-

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immune mechanism. It seems appropriate that the teratogenic mechanism in this experiment might be considered according to Brent's view. In humans, however, there is a possibility for autoimmune mechanism because immunological cross-reaction between MP and human heart tissue have been reported. The results suggest that MP is slightly teratogenic.

REFERENCES