Caries-inducing Activity of the Hydrogenated Derivative of an Isomaltooligosaccharide Mixture in Rats

Jun Tsunehiro, Takashi Matsukubo,* Masao Shiota,** and Yoshinori Takesu*

Research and Development Center, Showa Sangyo Co., Ltd., Hinode, Funabashi, Chiba 273, Japan
*Department of Hygiene and Community Dentistry, Tokyo Dental College, Masago, Mihama-ku, Chiba 261, Japan
**Technical Division, Showa Sangyo Co., Ltd., Higashi Fukushiba, Kamisu, Kashima-gun, Ibaraki 314-02, Japan

Received January 29, 1997

The caries-inducing activity of the hydrogenated derivative of an isomaltooligosaccharide mixture (IMO-H) was evaluated in vitro for its acidogenicity and in vivo an experimental caries system with specific-pathogen-free (SPF) rats. Streptococcus sobrinus 6715 (serotype g) did not produce a significant amount of acid from IMO-H, whereas Streptococcus mutans MT8148 (serotype c) gradually produced a small amount of acid, although the degree was less than that of sucrose. In vivo experiments were conducted on rats which were provided with the test sugars at two different times: at the time of organism inoculation, and after the organisms had become completely established. IMO-H did not induce significant dental caries in rats infected with the S. sobrinus 6715 or S. mutans MT8148R strain.

Key words: cariogenicity; hydrogenated isomaltooligosaccharide; sugar substitute; mutans streptococci

Dental caries is a disease in which there is an interplay of three principal factors: the host, the microflora, and the substrate.18 Evidence to support the importance of sucrose in the pathogenesis of dental caries has been obtained from epidemiological investigations on human subjects.2-4 Mutans streptococci are accepted to be one of the microorganisms responsible for dental caries, and their cariogenicity depends on the availability of sucrose.5 Therefore, it has been suggested that one major method for preventing dental caries is to control sucrose consumption. Several investigators have examined the possibility of substituting sucrose by non-cariogenic sugars or sugar alcohols.6-9

Isomaltooligosaccharides, a mixture of z1→6 glucosides, are produced enzymatically from starch and are widely used in various foods and drinks as a sweetener. Various physiological effects have been reported.10-14 To evaluate the cariogenicity of a commercially available isomaltooligosaccharide mixture (IMO), a series of experiments were performed. These studies demonstrated that glucose and maltose, minor components of IMO, were utilized as a substrate for acid production15,16 and induced weak caries in rats.15 To improve this defect, we prepared the hydrogenated derivative of an isomaltooligosaccharide mixture (IMO-H) from IMO by hydrogenation. This procedure converts glucose and maltose to sorbitol and maltitol, respectively, which have a very low cariogenic potential.

Dental caries occurs as the result of enamel and dentine decalcification of the teeth by organic acid that is produced by bacteria in the dental plaque.17 This acid production from sugars is directly associated with their cariogenic potential. To measure the pH response under dental plaque, an in-dwelling electrode method has been used for an in situ investigation.18 Matsukubo et al.18 have studied the acidogenicity of sugars and foods by using similar intra-oral pH ion-sensitive field effect transistor apparatus which was further improved. When evaluating the cariogenicity of sugars in an animal experiment, it is important to consider the existence of dental plaque. The purpose of the present study is to examine the caries-inducing activities of IMO-H. In addition, we tried to evaluate the cariogenicity of test sugars by using rats in which the organisms had become completely established.

Materials and Methods

Sugars. The hydrogenated derivative of an isomaltooligosaccharide mixture (IMO-H) was prepared from the isomaltooligosaccharide mixture (IMO) by hydrogenation in a laboratory with the Raney nickel method.20 Isomalto-900® (Showa Sangyo Co., Tokyo, Japan) was used as IMO and contained 90.6% of isomaltooligosaccharides (29.9% of isomaltose, 14.9% of isomaltotriose, 11.6% of panose, and 34.2% of others) and 9.4% of other saccharides (3.4% of glucose, 4.0% of maltose, and 2.0% of maltotriose). IMO-H contained the individual sugar alcohols of IMO components (Table I). The purity and composition of each test sugar were analyzed by HPLC.12 Sucrose used for the in vitro tests was a reagent-grade product.

Bacterial strains. Streptococcus mutans MT8148 (serotype c) and MT8148R (serotype c), and Streptococcus sobrinus 6715 (serotype g) were used in the present study. S. mutans MT8148R and S. sobrinus 6715 were streptomycin-resistant.

Acid production by a resting-cell suspension. S. mutans MT8148 and S. sobrinus 6715 were cultured at 37°C for 18 h in brain heart infusion broth, centrifuged, and washed twice with phosphate-buffered saline (PBS, 1 mM, pH 7.0). The collected cells were suspended again in PBS. The original suspension was then diluted with PBS, adjusting the 200-times dilution of the cell suspension to an optical density of 0.15 at 550 nm. This cell suspension (0.6 ml) was then added to 2.4 ml of each sugar solution at 5%. The acidity of the reaction mixture was periodically measured with a pH meter by the method of Ooshima et al.34

Caries induction in rats. SPF male Sprague-Dawley rats (15 days of age; Japan Clea Lab., Tokyo, Japan) were used in this study. The animals were kept in polycarbonate cages (3 rats/cage) fitted with filter cap in a semi-SPF...

Abbreviations: IMO-H, hydrogenated derivative of an isomaltooligosaccharide mixture; IMO, isomaltooligosaccharide mixture; CFU, colony-forming unit; S. mutans, Streptococcus mutans; S. sobrinus, Streptococcus sobrinus; SPF, specific-pathogen-free; PBS, phosphate-buffered saline; MS, Mitis-Salivarius.
room (23 ± 2°C and 55 ± 5% RH). During the experimental period, the rats were fed with the diet and water ad libitum.

The rats were randomly divided into 6 groups, A to F, as shown in Tables I and IV and provided with a modified diet (Table II) containing different test sugars to replace the 56% sucrose in diet 2000.22 Group A received 56% cornstarch (cons-diet); group B, 36% cornstarch and 20% sucrose (suc-diet); and group C, 36% cornstarch and 20% IMO-H (IMO-H-diet). The rats in these three groups were fed on each diet throughout the experimental period (49 days for strain 6715 and 56 days for strain MT8148R). On the other hand, the rats belonging to groups D, E, and F were fed on the suc-diet early in the experimental period (to the 21st day for strain 6715 and to the 28th day for strain MT8148R). The suc-diet was then replaced with the cons-diet (group D) or IMO-H-diet (group E) until the end of the experimental period. The rats in group F were sacrificed when the suc-diet of groups D and E was replaced. All sugars incorporated into the diet were finely pulverized.

The protocol of the rat experiment for strain 6715 is shown in Fig. 1. At the beginning of the experiment, the oral flora of the animals was depressed by administering penicillin G in the drinking water (400 units/ml of water), inoculating orally with 100 μl (4000 units/ml of water) and administering tetracycline (4 mg/g of diet) in the ordinary powdered diet (MF; Oriental Yeast Co., Tokyo, Japan) for two days to facilitate the establishment of the inoculated bacteria in the oral cavity. In order to induce dental caries, the animals were inoculated orally either with 0.2 ml of a suspension of S. sobrinus 6715 or S. mutans MT8148R concentrated at about 10⁶ colony-forming units (CFUs)/ml for the first 5 days of the experimental period. The oral flora of the dental plaque was checked every week. A plaque sample from the molar dentition was collected with a sterile cotton stick and then shaken vigorously in a tube containing 1 ml of sterile physiological saline. The sample was serially diluted in the same solution, and 0.2 ml portions were dropped on Mitis-Salivarius (MS) agar for streptococci and on the same agar containing streptomycin (500 μg/ml of medium) for the infected strain. All plates were incubated anaerobically in a nitrogen-carbon dioxide gas mixture at 37°C for 2 days. All CFUs were carefully counted for both media, and the percentage of the infected strain’s CFUs versus the total number of CFUs was calculated. These procedures were performed for half of the rats in each group.

At the end of the experimental period, the animals were sacrificed under ether anesthesia, and their jaws were removed. Both mandibles were immersed in 10 ml of physiological saline, and the solution was ultrasonicated for 60 s. Each suspension was diluted, and portions were dropped on MS-agar containing streptomycin. The recovery of the infected organism from the mandibles was measured as already described. The molar teeth in the maxilla were stained, and the dental plaque scores on the smooth surface (buccal and lingual) of the first and second molars were evaluated by the methods of Regolati and Hotz.23 After scoring the dental plaque accumulation, the jaws were autoclaved for 5 min at 121°C to facilitate the removal of the soft tissues, and stained with murexide. Dental caries was scored on the sectioned jaws according to the method of Keyes.24 The dental plaque and caries scores were judged by an examiner who was

Table I. Composition of the IMO-H* (% as Dry Matter)

<table>
<thead>
<tr>
<th>Hydrogenated isomaltooligosaccharides</th>
<th>90.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP2 Isomaltitol</td>
<td>29.8</td>
</tr>
<tr>
<td>Others</td>
<td>5.6</td>
</tr>
<tr>
<td>DP3 Isomaltotriitol</td>
<td>14.9</td>
</tr>
<tr>
<td>Panitol</td>
<td>11.6</td>
</tr>
<tr>
<td>Others</td>
<td>3.1</td>
</tr>
<tr>
<td>DP4 Isomaltotetraitol and others</td>
<td>16.8</td>
</tr>
<tr>
<td>DP5 Isomaltopentaitol and others</td>
<td>6.8</td>
</tr>
<tr>
<td>DP6, 7</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Other sugar alcohols 9.4

DP, degree of polymerization.

* Hydrogenated derivative of the isomaltooligosaccharide mixture.

a Main components were nigerioitol and kojibioitol.

Table II. Composition of Diets Used in the Rat Experiment (% as Dry Matter)

<table>
<thead>
<tr>
<th>Cons-diet</th>
<th>Suc-diet</th>
<th>IMO-H-diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>56</td>
<td>36</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>IMO-H*</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Othersb</td>
<td>6715</td>
<td>7514</td>
</tr>
</tbody>
</table>

a Hydrogenated derivative of the isomaltooligosaccharide mixture.
b Components of diet 2000 except for 56% sucrose.

Fig. 1. Protocol of the Rat Experiments with the S. sobrinus 6715 Strain.

a: The constituents of the three different diets (cons-diet, suc-diet, and IMO-H-diet) are shown in Table II.
b: Ordinary powdered diet (Oriental Yeast Co.)
not informed of the coding. Statistical differences were calculated by the one-way ANOVA followed by the Tukey-Kramer methods.

Results

Acid production by mutants streptococci in vitro

Both resting-cell suspensions of S. sobrinus 6715 and S. mutans MT8148 did produce acid from sucrose. The pH of the reaction mixtures dropped markedly within several minutes, whereas the pH of the reaction mixtures of IMO-H decreased slowly. After 40 min, the pH of the reaction mixture, when incubated with either strain 6715 or MT8148, was 6.35 or 5.93, respectively (Fig. 2).

Caries induction in rats

Figure 3 shows the transition of the ratio of S. sobrinus 6715 to total streptococci in dental plaque samples. Strain 6715 was detected in all animals up to 3 weeks after inoculation. The ratio for group A (the rats fed on the cons-diet) and for group C (IMO-H-diets) remained below 10% throughout the experiment, while the ratio for group B (suc-diet) increased steadily. Statistical differences were found between group B and group A or C. Replacement of the suc-diet with the cons-diet or IMO-H-diets (groups D and E) resulted in a decreased ratio for strain 6715 to total streptococci. In the 5th and 6th weeks of the experimental period, the differences were statistically significant.

The caries score and recovery of S. sobrinus 6715 are presented in Table III. The caries score of the rats in group C (IMO-H-diets) was similar to that of the rats in group A (cons-diet, negative control). These rats developed only baseline caries, whereas the rats in group B (suc-diet, positive control) developed notable caries. There was no statistical difference between groups A and C. However, the difference in the caries score between group B and group A or C was statistically significant. On the other hand, in groups D and E, where sucrose in the diet was replaced with the test sugar, there was no more induction of caries.

Table III. Cariogenicity and Recovery of Infected S. sobrinus 6715 in SPF Rats Provided with IMO-H* and Other Sugars

<table>
<thead>
<tr>
<th>Group</th>
<th>Test sugar</th>
<th>n</th>
<th>Caries score (mean ± S.E.)</th>
<th>Recovery of S. sobrinus 6715 log(mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Provided with the test sugar at the time of organism inoculation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Cornstarch</td>
<td>12</td>
<td>9.8 ± 2.1</td>
<td>4.97 ± 0.47</td>
</tr>
<tr>
<td>B</td>
<td>Sucrose</td>
<td>12</td>
<td>39.0 ± 5.0</td>
<td>6.78 ± 0.10</td>
</tr>
<tr>
<td>C</td>
<td>IMO-H</td>
<td>12</td>
<td>11.2 ± 1.4</td>
<td>5.29 ± 0.24</td>
</tr>
<tr>
<td>(Provided with the test sugar after the organisms had become completely established)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Cornstarch</td>
<td>12</td>
<td>25.5 ± 3.3</td>
<td>5.23 ± 0.11</td>
</tr>
<tr>
<td>E</td>
<td>IMO-H</td>
<td>12</td>
<td>21.1 ± 1.9</td>
<td>5.46 ± 0.23</td>
</tr>
<tr>
<td>F</td>
<td>Control*</td>
<td>6</td>
<td>23.2 ± 3.5</td>
<td>N.T.*</td>
</tr>
</tbody>
</table>

* Hydrogenated derivative of the isomaltooligosaccharide mixture.
* The rats were sacrificed when the suc-diet of groups D and E were replaced.
* Not tested.
* p<0.01 (one-way ANOVA followed by the Tukey-Kramer method).

(a) Acidogenicity of IMO-H* and Sucrose by the Resting-cell Suspension of S. mutans MT8148 and S. sobrinus 6715.

A resting-cell suspension of S. sobrinus 6715 (——) or S. mutans MT8148 (····) was prepared and mixed in a 5% sugar solution. The starting pH of the medium was adjusted to 7.0. A, control (cells only); □ sucrose; ● IMO-H.

(a) Hydrogenated derivative of the isomaltooligosaccharide mixture.

(b)
There was no statistical difference among groups D, E, and F. When examining the recovery of strain 6715 from mandibles at the end of the experiment, the number of colonies obtained from the rats fed on the suc-diet (group B) was higher ($p < 0.01$) than those obtained from the rats in the other groups.

There were some differences in the degree of bacterial establishment between strains 6715 and MT8148R. The transition in the ratio of *S. mutans* MT8148R to total streptococci in the dental plaque is presented in Fig. 4. There was no significant difference among the groups, except for group C (the rats fed on the IMOH-diet), which was lower ($p < 0.05$) than group B in the 3rd and 6th weeks of the experimental period. Similar findings were observed in the recovery of this strain at the end of the experimental period (Table IV). There was no statistical difference among the groups, except between groups B (suc-diet) and C (IMOH-diet).

According to the dental caries development, the results of the experiment with *S. mutans* MT8148R were almost the same as those with strain 6715. The caries score is presented in Table IV. There were statistical differences between groups B and A or C, but there were no statistical differences between groups A and C and between groups D, E, and F.

The mean total food intake of the rats in the different groups was not significantly different, but the mean weight gain of the rats fed on the IMOH-diet (group C) was slightly lower than those of the other control groups. With regard to replacing the suc-diet with the cons-diet (group D) or IMOH-diet (group E) in the middle of experimental period, there were no statistical differences between groups D and E.

**Discussion**

In the present study, we used two kinds of mutants streptococci, *S. sobrinus* 6715 and *S. mutans* MT8148, which had been isolated from the human oral cavity and are believed to be cariogenic in humans. There were some differences in the acidogenicity of IMOH-H between strains 6715 and MT8148. Differences in the fermentation characteristics of serotypes of these organisms have been observed with other sugars. Minami et al. have reported that serotype c of *S. mutans* MT8148 did ferment isomaltooligosaccharide, while serotype g of *S. sobrinus* 6715 did not. In addition, serotype c of mutants streptococci could metabolize sorbitol, a minor component of IMOH-H.

In the rat experiment, the ratio of infected organisms to total streptococci in the dental plaque was measured semi-quantitatively by swabbing. The ratio obtained in the 6th week of the experimental period (in the 7th week for strain MT8148R) was almost correlated with the recovery of

### Table IV. Cariogenicity and Recovery of Infected *S. mutans* MT8148R in SPF Rats Provided with IMOH-H and Other Sugars

<table>
<thead>
<tr>
<th>Group</th>
<th>Test sugar</th>
<th>$n$</th>
<th>Caries score (mean ± S.E.)</th>
<th>Recovery of <em>S. mutans</em> MT8148R (log(mean ± S.E.))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Provided with the test sugar at the time of organism inoculation)</td>
</tr>
<tr>
<td>A</td>
<td>Cornstarch</td>
<td>12</td>
<td>4.1 ± 0.7**</td>
<td>8.17 ± 0.09</td>
</tr>
<tr>
<td>B</td>
<td>Sucrose</td>
<td>12</td>
<td>29.5 ± 3.2**</td>
<td>8.23 ± 0.06</td>
</tr>
<tr>
<td>C</td>
<td>IMOH-H</td>
<td>12</td>
<td>5.7 ± 0.9**</td>
<td>7.93 ± 0.12*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Provided with the test sugar after the organisms had become completely established)</td>
</tr>
<tr>
<td>D</td>
<td>Cornstarch</td>
<td>12</td>
<td>16.3 ± 2.6</td>
<td>8.20 ± 0.13</td>
</tr>
<tr>
<td>E</td>
<td>IMOH-H</td>
<td>12</td>
<td>17.3 ± 3.6</td>
<td>8.05 ± 0.10</td>
</tr>
<tr>
<td>F</td>
<td>Control</td>
<td>12</td>
<td>22.9 ± 3.3</td>
<td>N.T.*</td>
</tr>
</tbody>
</table>

- a Hydrogenated derivative of the isomaltooligosaccharide mixture.
- b The rats were sacrificed when the suc-diet of groups D and E were replaced.
- c Not tested.
- *$p < 0.05$, **$p < 0.01$* (one-way ANOVA followed by the Tukey–Kramer method).
infected organisms from the rat mandibles at the end of the experiment. There was significant difference in the transition of ratios between the rats infected with S. sobrinus 6715 and S. mutans MT8148R. The ratio for strain 6715 was low for the rats receiving the IMOH-diet, and high for those receiving the suc-diät. On the other hand, the ratio for S. mutans MT8148R was established in the oral cavity of rats fed on the diets containing different sugars, in spite of the presence of sucrose. A similar finding from animal experiments was recognized in the previous report. Gibbons et al. have demonstrated findings which support the sucrose-dependency of the strain of S. sobrinus 6715. In their description, strain 6715 possessed an adherin which bound to glucan synthesized from sucrose, whereas the strain of S. mutans did not.

In the in vivo cariogenicity test, we adopted two experiments in which the rats were provided with the test sugars at two different times: at the time of organism inoculation, and after the organisms had become completely established. The cariogenicity of sugar substitutes is associated with their ability to establish the organisms and the production of acid by the established organisms. Most sugar substitutes have a lower potential to establish the organism because water-insoluble glucan, by which the cariogenic bacteria adheres to the tooth surface, is synthesized from sucrose. In the former experiment, therefore, the cariogenicity of the sugar substitutes would not be directly reflected by their acidogenicity. Indeed, the cariogenicity of glucose, one of the most acidogenic sugars, was significantly lower than that of sucrose in the rat experiment. The recovery of the organisms in the rats which had received glucose was low until three weeks after inoculation, when the tooth enamel was maturing and more sensitive to acid. In addition, Washizu et al. have examined the effect of coupling sugar (a mixture of glucosylated sucrose) on caries advance by using infected rats. The percentage caries score for the coupling sugar group versus the positive control group, which was obtained in an experiment using infected rats, was about twice as high as the one using non-infected rats. This result suggests that the latter experiment with infected rats was significant in the evaluation of the cariogenicity of sugar substitutes.

Both experiments, we adopted in the present study, showed that the caries score of IMOH was as low as that of cornstarch, the negative control used in our experiments. The caries scores for group C (IMO-H) and group A (cornstarch) were at the baseline level, and the caries scores for group E (IMO-H) and group D (cornstarch) were almost the same as that for group F, whose rats were sacrificed when the suc-diät of groups D and E were replaced. These results indicate that IMOH did not induce caries, even under the conditions of the organisms being completely established.

Although S. mutans MT8148R could produce some acid from IMOH in vitro, no caries was induced in vivo in the experiment with this strain. This might be ascribable to the low amount and speed of acid production. Thus, the saliva of the rats could wash and neutralize the acid derived from IMOH before demineralization of the enamel could occur. Yamamoto et al. have evaluated the acidogenicity of sugar substitutes by using a resting-cell suspension. They demonstrated the relationship between the pH decrease and the acid production by measuring the volume of an alkaline solution needed to neutralize the acid. The terminal pH (after 20 min incubation) for sucrose was 4.11, while for galactose, in which the pH decreased slowly, it was 5.02. The volume of alkaline solution needed to neutralize was about 4 times more for sucrose as that for galactose. These results suggest that the acid produced from IMOH was probably much lower than that from sucrose. Furthermore, Kaneko et al. have reported the acidogenicity of IMOH in situ under human dental plaque by using intra-oral apparatus. They demonstrated that the acidogenicity of IMOH was similar to that of maltitol, which is ranked as a non-acidogenic sugar substitute. IMOH did not induce any pH change below the critical value of 5.7.

Lycasin, a commercially available hydrogenated maltooligosaccharide mixture, is similar to IMOH in its molecular weight distribution. The most commonly used type is Lycasin® containing about 7% of sorbitol, 52% of maltitol, 15% of maltotriitol, 8% of tetra-to-hepta saccharide alcohol, and 18% of higher-saccharides alcohol. A series of experiments has ranked Lycasin® as hypo-acidogenic and as non-cariogenic. In addition to these findings, Grenby has demonstrated that, as the chain length decreased, less acidogenicity and cariogenicity was apparent by comparing the two kinds of Lycasin®. Compared to Lycasin®, IMOH consists of lower-molecular-weight compounds (Table I).

The effects of IMOH on the formation of dental plaque were not judged in this animal experiment. A notable accumulation of plaque was not observed with all rats, even those fed on the diet containing sucrose. This result seems to have been due to the low content of sucrose in the diet. More plaque formation was observed on the occlusal surfaces and in fissures than on the smooth buccal and lingual surfaces, in which most of the caries lesions were induced. This decline was significant in the rats fed on the diet containing sucrose. Further experiments will be needed to elucidate the value of IMOH as a sugar substitute for decreasing dental plaque formation.

Side effects such as diarrhea have often been observed in rat caries experiments with diets involving a high content of a sugar substitute, especially sugar alcohol. In the present study, the animals fed on IMOH showed the symptom of diarrhea and had a decreased food intake for almost a week after IMOH was provided, especially the young rats. However, their condition gradually improved and their health had recovered to normal after 2 or 3 weeks. As for the body weight, the rats fed on the IMOH-diet throughout the experimental period tended to lose weight compared with the other control groups. This phenomenon seems to have been due to the low digestibility and calorific value of IMOH which is common to sugar alcohols.

In conclusion, the results of the in vitro acidogenicity and rat experiments strongly suggest that IMOH has no or very low cariogenic potential, similar to cornstarch in this respect. The use of IMOH as a sugar substitute might be an advantage in preventing dental caries.

Acknowledgments. The authors thank Drs. S. Hamada and T. Ooshima of Osaka University Faculty of Dentistry for providing microorganisms used in the present study.
References