Effects of Lactic Acid-Forming Bacteria on *Vibrio comma* Inoculated into Intestinal Segments of Rabbits

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**Abstract**

HATTORI, H. (Sankyo Co., Ltd., Tokyo, Japan), H. MISAWA, I. IGARASHI, AND Y. SUGIYA. Effects of lactic acid-forming bacteria on *Vibrio comma* KC-4 and various lactic acid-forming bacteria were injected into the intestinal segments of rabbits (De and Chatterje, 1953) to observe the effects of the latter agents in altering the changes produced by strain KC-4. The animals were sacrificed 10 and 20 hr after inoculation. The inoculated intestinal segments were first examined grossly, and the amount of exudate in the segments, if any, was measured, after which the tissues were subjected to pathological examination. When KC-4 cells together with spore-bearing lactic acid-forming bacilli, strain P-22, or *Lactobacillus casei* were introduced, the intestinal segments showed few or no macroscopic and microscopic changes, and no accumulation of exudate. With mixed inoculation with lactic acid bacteria such as *L. bulgaricus*, *L. acidophilus*, *Streptococcus lactis*, and *S. faecalis*, changes were produced by strain KC-4. Macroscopically, no difference was discernible between the changes caused by mixed inoculation and those produced by single inoculation of KC-4. Upon pathological examination, however, it was seen that changes resulting from mixed inoculation were slightly less severe than those produced by inoculation with strain KC-4 only.

Experimental inoculation into animals of infectious intestinal agents of human origin has been considered relatively difficult. Reported studies include cholera infection in infant rabbits (Dutta and Habbu, 1955), dysentery infection in guinea pigs after fatty metamorphosis caused by starvation or subcutaneous infection of carbon tetrachloride (Fomal et al., 1958, 1959), dysentery infection in monkeys (Hayashi and Iwahara, 1962), cholera infection in ligated gut segments (De and Chatterje, 1953; Jenkins and Rowley, 1959), dysentery infection produced by De's method (Kasuga et al., 1963), infection with *Escherichia coli* (Taylor, Maltby, and Payne, 1958), and *Vibrio comma* infection.

Each of the above studies was undertaken to investigate the virulence of the respective intestinal pathogens. We, however, have considered the possibility that lactic acid-forming bacteria, which make up a part of the intestinal flora, might modify the effect of a pathogen. Accordingly, we inoculated a mixture of *V. comma* KC-4 and lactic acid-forming bacteria, employing the ligated rabbit intestinal segment method of De and Chatterje (1953), and examined the segments subsequently for evidence of pathology.

**Materials and Methods**

*V. comma* KC-4 was supplied by Aiso, Institute of Food Microbiology, Chiba University, Chiba, Japan. *V. comma* KC-4 is a new hemolytic marine strain. The optimal concentration of sodium chloride is 3%, and optimal temperature for growth of the organism is 37°C. This strain has been presumed to be the causative agent of an enteritis type of food poisoning by many investigators in Japan (Fujino, 1953; Takigawa, 1958).

Among the various lactic acid-forming bacteria tested for mixed inoculation was the spore-bearing lactic acid-forming *Bacillus coagulans* P-22 (Nakayama and Sakaguchi, 1950). Other lactic acid-forming bacteria employed were *Lactobacillus casei* IAM 1118, *L. bulgaricus* IAM 1120, *L. acidophilus* IAM 1043, *Streptococcus lactis* IAM 1175, and *S. faecalis* IAM 1262. After fasting for 24 hr, the animals were anesthetized with ether; the temperature of the operating room was kept at 20 ± 5°C. From a point about 5 cm below the sternum, a vertical incision 5 cm long was made in the abdominal wall through which as much of the small intestine as possible was exposed. Next, 20 ml of sterile saline were injected into the upper part of the jejunum, which had been ligated just below the site of injection to prevent downward flow of gastric contents. Then
FIG. 1. Diagram of a ligated small intestine. The letter "a" indicates sites of injection. The reaction loops are identified by roman numerals.

The contents of the intestinal tract were washed out. The method of ligation of the intestinal tract is shown in Fig. 1. Small intestine was ligated about 30 cm from the point of adhesion of the cecum. In each animal, three loops were made, separated by at least 15 cm.

The cultures under test were introduced into the loop with a sterile syringe fitted with a small-bore needle.

Cultures of bacteria for inoculation were grown in nutrient broth with 3% sodium chloride for V. comma KC-4, and with 1% glucose for lactic acid-forming bacteria, at 37 C for 24 hr. The KC-4 inoculum contained 0.5 ml of culture and 0.5 ml of the nutrient broth. The mixed inoculum of KC-4 and lactic acid-forming bacteria contained 0.5 ml of each. Nutrient broth (1 ml) was inoculated into the control loop. In each of the experiments, the middle loop was used for control.

After inoculation, the exposed intestine was carefully placed back into the peritoneal cavity, and the incision was closed. The animals were not supplied with food and water for 10 or 20 hr, and were killed by the intravenous injection of sodium pentobarbital.

The intestinal segments were observed macroscopically, and then were histologically examined with hematoxylin-eosin stain. The exudate in the distended parts of small intestine was aspirated with a sterile syringe, and its volume was measured.

FIG. 2. Macroscopic findings after mixed inoculation with strain KC-4 and strain P-22.

FIG. 3. Macroscopic findings after mixed inoculation with strain KC-4 and Lactobacillus casei.
EFFECTS OF LACTIC BACTERIA ON V. COMMA

RESULTS

The ligated rabbit intestinal segments inoculated with strain KC-4 alone showed distension and accumulation of exudate, with thinner intestinal walls and submucous edema in both 10- and 20-hr specimens. Gross bleeding and necrosis were also observed after 20 hr.

By contrast, the segments injected with the mixed inoculum (P-22 and KC-4), and with nutrient broth, were free from macroscopic lesions in both 10- and 20-hr specimens (Fig. 2).

A very small amount of exudate was demonstrated after 10 hr in the intestinal segments inoculated with L. casei and KC-4, but the 20-hr specimen was free from lesions and was similar to the control loop (Fig. 3).

Mixed inoculation with strain KC-4 and L. bulgaricus, L. acidophilus, S. lactis, or S. faecalis produced, in each case, a lesion similar to that of strain KC-4 alone (Fig. 4-7).

The patho-histological findings in the inoculated intestinal segments were graded according to the criteria given by Kasuga et al. (1963) in their experimental dysentery studies. The results, in comparison with the macroscopic findings, are given in Table 1.

As shown in Table 2, 7 to 12 ml of the exudate were accumulated in loops of about 10 cm with strain KC-4 or with mixed inocula, but no exudate was observed with mixed inoculation with strain P-22 or with L. casei.

DISCUSSION

In this study, a spore-bearing lactic acid-forming bacillus (B. coagulans P-22) and L. casei in-
HIBITATED the production of macroscopic local changes due to strain KC-4, but *L. bulgaricus, L. acidophilus, S. lactis, and S. faecalis* were not effective.

The histological appearance and the macroscopic findings were almost parallel in our experiments.

Even when macroscopic changes similar to those produced by strain KC-4 were found in the segments inoculated with mixtures, patho-histological examination revealed that the damage was milder in the segments receiving mixed inocula than in those receiving strain KC-4 alone.

The pH of the exudate was between 8.5 and 9.5.

**Table 1. Changes in the loops 20 hr after inoculation**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Macroscopic*</th>
<th>Histological†</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC-4</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>KC-4 + P-22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KC-4 + <em>Lactobacillus casei</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KC-4 + <em>L. bulgaricus</em></td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>KC-4 + <em>L. acidophilus</em></td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>KC-4 + <em>Streptococcus lactis</em></td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>KC-4 + <em>S. faecalis</em></td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Symbols: +++ = distension of intraintestinal cavity, thinning of intestinal wall, and marked hyperemia; ++ = distension of intraintestinal cavity, thinning of intestinal wall, but no hyperemia; + = slight distension of intraintestinal cavity with mild hypertrophy of intestinal wall; - = no distension of intraintestinal cavity.

† Symbols: +++ = almost complete destruction of epithelial cells and severe bleeding of mucosa; ++ = marked derangement of epithelial cells, anasarca of mucosal and submucosal layers, and extensive cell infiltration; + = shortening of intestinal villi, with slight derangement of epithelial cells; - = normal intestinal villi, with normal arrangement of epithelial cells.

The bacteriostatic activity of cultures of lactic acid-forming bacteria has chiefly been attributed to the reduction in pH by lactic acid. The results of pH determinations in this experimental study, however, would seem to suggest that the inhibition of lesions is not due to production of lactic acid. Explanation of the mechanism involved in the inhibition of production of lesions by certain lactic acid-forming bacteria will have to await further studies.
Table 2. Amounts of exudate in the loops

<table>
<thead>
<tr>
<th>Organism</th>
<th>Time after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 hr</td>
</tr>
<tr>
<td>KC-4</td>
<td>ml</td>
</tr>
<tr>
<td>KC-4 + P-22</td>
<td>9</td>
</tr>
<tr>
<td>KC-4 + Lactobacillus casei</td>
<td>3</td>
</tr>
<tr>
<td>KC-4 + L. bulgaricus</td>
<td>11.5</td>
</tr>
<tr>
<td>KC-4 + L. acidophilus</td>
<td>8</td>
</tr>
<tr>
<td>KC-4 + Streptococcus lactis</td>
<td>12</td>
</tr>
<tr>
<td>KC-4 + S. faecalis</td>
<td>10</td>
</tr>
<tr>
<td>Control (nutrient broth)</td>
<td>0</td>
</tr>
</tbody>
</table>

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Literature Cited