Prevention of Human Rotavirus Infection with Chicken Egg Yolk Immunoglobulins Containing Rotavirus Antibody in Cat

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Key words: Human rotavirus, Bovine rotavirus, Passive protection, Egg yolk immunoglobulins

Summary

A study was made on the passive protection against rotavirus-induced diarrhea. Chickens were immunized with bovine rotavirus (serotype 1) and the egg yolk immunoglobulins containing a high titer anti-rotavirus neutralizing antibody (CEYI) was obtained. The CEYI was then orally administered to specific-pathogen-free cats, and the cats were infected with human rotavirus. The cats treated with the CEYI remained clinically healthy after challenge, whereas diarrhea occurred in the placebo-fed cats as control. Virus antigens were detected in feces in all the diarrheal cases in the placebo-fed cats but were only sporadically detected in the CEYI-fed cats. However, the cats were only protected against rotavirus infection by the presence in the gut at the time of injection of the antibody. These results suggested that continuous administration of the CEYI is capable of preventing children from diarrhea induced by human rotavirus infection and viral shedding.

Introduction

Rotaviruses are important etiological agents of diarrhea disease in human as well as in various animal species throughout the world. One of the most important aspects of rotavirus infections is the problem of their prevention. Two approaches have been used in attempts to this problem; one is direct vaccination of infants to elicit active immunity, using live attenuated heterologous rotaviral vaccines12), and other is passive immune protection by oral administration of infants with antirotavirus-antibody-containing preparations56-7). Nevertheless, the effects of active immunization of infants with live virus vaccine has been questioned, because of problem arising from the practicability of actively immunizing breast-fed infants and from treating diarrheal disease caused by rotavirus infection in hospitalized infants. Therefore, passive immunization also remains of major importance. Passive immunity may have a useful role in the prophylaxis of rotavirus infection.

The authors have reported that the successful infection of specific pathogen free (SPF) cats with human rotavirus8). This model system may serve as experimental animals in the study of human rotavirus infections. This present study was designed to see if the passive immunization due to oral administration of the chicken egg yolk immunoglobulin G with potent antiviral activity could protect cats against experimental human rotavirus infection.
Chiken Egg Yolk Immunoglobulins to Rotavirus

Materials and Methods

Viruses:
Shimane strain of bovine rotavirus (serotype 1) was grown in MA-104 cells and propagated with $10^9$ TCID$_{50}$/ml in the growth medium. YO strain of human rotavirus, which belong to the third serotype defined for human rotavirus, was kindly supplied by Dr. S. Matsuno, National Institute of health, Tokyo, Japan, and used for oral challenge.

Chickens:
Five month old white leghorn hens were obtained from the poultry farm in our laboratories and were kept in individual cages throughout the experiment.

Cats:
SPF cats$^9$, aged 5—7 weeks after birth, of the litter were used. Cats 5—7 weeks old, infected orally with human rotavirus, consistently developed diarrhea.

Immunization of chickens and isolation of immunoglobulin G from egg yolks:

Chickens were immunized with bovine rotavirus Shimane strain as follows: bovine rotavirus antigen inactivated with formalin was intramuscularly immunized into hens at a dose of $10^9$ TCID$_{50}$/hen. Freund’s complete adjuvant was used in conjunction with killed antigen. Six weeks after the initial immunization, the same antigen and adjuvant were employed for booster injections of hens in the same manner. Samples of the yolks of eggs from these hens were tested for determining virus neutralizing antibody level. When the antibody titer in the yolks reached a maximum level (more than 1:1,024), the eggs laid by the immunized hens were collected in order to produce antibody containing samples as follows: the eggs were pooled and yolks of these eggs were separated and combined together. Crude immunoglobulin G was extracted from these yolks with chloroform, and these extracts were directly spray dried.

Table 1 Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of litters</th>
<th>No. of cats</th>
<th>Sample</th>
<th>Days after administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>CEYI*</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>Placebo</td>
<td>P</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>7</td>
<td>CEYI</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9</td>
<td>Placebo</td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>11</td>
<td>CEYI</td>
<td>VC</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13</td>
<td>Placebo</td>
<td>VP</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>14</td>
<td>CEYI</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>16</td>
<td>Placebo</td>
<td>P</td>
</tr>
</tbody>
</table>

a : Chicken egg yolk immunoglobulin G containing antibody (immunoglobulin G was extracted from egg yolks of chicken, hyper immunized against bovine rotavirus).
b : CEYI were administered orally twice a day.
c : Human rotavirus was given orally after a feed.
d : Non-immunized chicken egg yolk immunoglobulin G (immunoglobulin G was extracted from egg yolks of chicken, seronegative for rotavirus).
e : Placebo were administered orally twice a day.
Table 2  Clinical symptoms and viral shedding in feces of cats treated with immunoglobulins from chicken egg yolk after challenge with human rotavirus

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cats</th>
<th>Sample</th>
<th>Days after challenge</th>
<th>Clinical result</th>
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<tbody>
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<td></td>
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<tr>
<td>1</td>
<td>2</td>
<td>CEYI</td>
<td>-  -  -  -  -  -  -  -  -</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td></td>
<td>-  -  -  -  -  -  -  -  -</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>Placebo</td>
<td>-  -  -  +  +  +  +  +  +</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td></td>
<td>-  -  -  +  +  +  +  +  +</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>CEYI</td>
<td>-  -  -  -  -  -  -  -  -</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td></td>
<td>-  -  -  -  -  -  -  -  -</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Placebo</td>
<td>-  -  -  -  -  -  -  -  -</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>CEYI</td>
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<tr>
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<td>-  -  -  -  -  -  -  -  -</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>CEYI</td>
<td>-  -  -  -  -  -  -  -  -</td>
<td>Diarrhea</td>
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<td>4</td>
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<td>-  -  -  -  -  -  -  -  -</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>Placebo</td>
<td>-  -  -  -  -  -  -  -  -</td>
<td>Diarrhea</td>
</tr>
</tbody>
</table>

a; Chicken egg yolk immunoglobulin G (immunoglobulin G was extracted from egg yolks of chicken, hyper immunized against bovine rotavirus). b; Virus not detected. c; Virus detected. d; Non-immunized chicken egg yolk immunoglobulin G (immunoglobulin G was extracted from egg yolks of chicken, seronegative for rotavirus). e; Died.

Neutralizing antibody titer in CEYI:

The titer of neutralizing antibody against bovine the neutralizing antibody titer was ≥1,024 TCID₅₀/ml.

Treatment:

The sixteen cats were allocated into four treatment groups of 3—5, receiving CEYI or non-immunized chicken egg yolk immunoglobulins (placebo) as control and virus as detailed in Table 1. Briefly, after reconstitution, 1 ml of the CEYI or placebo was orally administered twice a day for three to eight days. As for viral infection, 10⁷ PFU/ml of human rotavirus YO strain was given 30 min-2 hours after a feed.

Observations:

Cats were examined clinically at least twice daily, particular attention being paid to the consistency of the feces. Fecal samples were collected at the time of clinical examinations.

Detection of rotavirus antigen in feces:

Detection tests were performed on feces before and after virus inoculation by a commercial reverse passive hemagglutination (RPHA) test kit (ROTA CELL: Nissui Seiyaku Co. Ltd).

Serology:

Serum samples were collected from each cat before and on days 21 after virus inoculation and were tested for rotavirus antibody by the CF test, as described¹⁰.

Results

Group 1:

Of 5 cats of the litter, 3 animals as the CEYI administration group were orally administered consecutively 2 ml a day for a total of 8 days, i.e. 4 days before and 4 days after virus inoculation. In consequence diarrhea attributable to viral infection was not observed in the CEYI administration group, whereas onsets of severe diarrhea were observed in the placebo administration group as control and one of the animals died on day 4 of rotavirus inoculation. The virus antigen was detected in an animal of the
CEYI administration group but it was transient. Both animals of the placebo administration group showed excretion of the viral antigen accompanying the onsets of diarrhea.

Moreover, body weights showed smooth increased in the CEYI administration group, whereas decreases were observed in the placebo group. An increase in body weight was observed in the surviving animal from week 4 after virus inoculation.

Group 2:

Four animals of the litter were divided into 2 groups each consisting of 2 animals. Two ml of the CEYI was oral administered consecutively for 4 days before and 4 days after virus inoculation in the CEYI administration group. One animal of the CEYI administration group showed the onset of diarrhea and viral excretion on day 4 after challenge with YO strain but they were transient. Both animals of the placebo administration group showed the onset of diarrhea on day 2 after challenge and viral antigens were detected in feces consecutively for 3 days.

The body weights in the placebo administration group showed a decreasing tendency but increased from day 7.

Group 3:

Four animals of the litter were divided into 2 groups each consisting of 2 animals. In this experiment the first CEYI administration was started 30 min after virus inoculation. Thereafter, the CEYI administration was continued for 5 days. Similarly placebo administration was performed. As a result no abnormality was observed in the CEYI administration group. On the other hand, diarrhea appeared in one of 2 animals of the placebo administration group on day 4 after challenge, and viral antigens were detected in feces of the 2 animals.

Body weights showed smooth increases in the CEYI administration group, whereas no increase was observed in 10 days after virus inoculation in the placebo administration.

Group 4:

Three animals of litter were divided into 2 groups. Two ml of the CEYI was oral administered consecutively daily for 3 days before challenge in the CEYI administration group. Similarly placebo administration group was performed. Then, animals in the both groups were challenged with YO strain 3 days after ingesting the final dose of the CEYI or the placebo.

All cats in both group were susceptible to infection when challenged with YO strain developing diarrhea and excreting virus that persisted for 2 to 3 days. The body weights in the both groups showed a decreasing tendency but increased from day 8.

serum antibody:

At the time of infection, none of the cats had serum antibody to rotavirus of human or feline origin; and was not detected in the serum of any cat when sampled on days 21 after infection.

Discussion

In the present study, we have demonstrated that cats can be protected from infection by human rotavirus by continuous administration of the chicken egg yolk immunoglobulin G with neutralizing-antibody activity against bovine rotavirus. In four experiments the incidence rate of diarrhea in the CEYI administration group was 33.3%, 3 in 9 case. On the other hand, it was 85.7%, 6 in 7 case of the placebo administration group as control. The lasting days of diarrhea were 1 day in case of the CEYI administration group but were long as 1 to 4 days, mean being 2. 4 days in the placebo administration group. Viral antigens were detected in all the diarrheal cases in the placebo administration group, but were only sporadically detected in the CEYI administration group. However, such protection was transient, and the cats were susceptible to rotaviral diarrhea when challenged at 42 hours after CEYI feeding (Group 4).
The cats were only protected against rotavirus infection by the presence in the gut at the time of infection of the antibody (Group 1, 2, 3). These results suggest that the presence of antibody to rotavirus in the gut partially neutralize both the initial challenge virus and virus subsequently released from infected cells.

The Shimane strain of bovine rotavirus is able to grow in large quantities in MA-104 cells, which is a suitable substrate as inoculum for immunization of hens. This strain is antigenically very closely related to human rotavirus serotype 3111). In the preliminary experiments, we found that human rotavirus serotype 1 and 3 were neutralized by the CEYI. Numerous investigators have shown that serum and milk obtained from a bovine rotavirus-immunized cows can neutralize human rotavirus12-14). The most effective protection to rotavirus infection was provided by the continued secretion of IgA in milk, although IgG has been shown to be protective when in high concentrations15). In humans, antibody titers are generally low in breast milk16) and many infants are no longer breast-fed when they are most susceptible to rotavirus infection. Therefore, continuous administration of the CEYI would provide a rational method of protection during this period. It would appear to have potential for protecting children against rotavirus infections, particularly in an outbreak in hospitalized children. It seems that more favorable prophylactic effects can be obtained by the antibody with a higher neutralizing antibody titer.

References
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Chiken Egg Yolk Immunoglobulins to Rotavirus


抗トウイルス鶏卵抗体のヒトトウイルス実験感染ネコにおける下痢発症予防効果

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2) 株式会社ゲンコーボレーション岐阜ラボラトリ
3) わかもと製薬株式会社

平賀 千兼1) 児玉 義勝2) 杉山 剛3) 市川 洋一1)

(平成1年7月7日受付)
(平成1年8月4日受理)

要 旨
ロタウイルス性下痢症に対する受身感染防御について検討を行った。ニワトリをウシロタウイルス（血清型・1）で免疫し、高力価の抗ロタウイルス中和抗体を含む卵黄免疫グロブリンGを得た。この抗ロタウイルス鶏卵抗体（CEYI）をSPFネコに経口投与し、ヒトロタウイルスで攻撃をおこなかった。CEYI処置ネコはウイルス攻撃後も健康状態を維持したが、コントロール群では下痢発症がみられた。コントロール群のうち下痢発症がみられたネコ便からはウイルス抗原が検出されたが、CEYI抗群におけるウイルス抗原の検出は散発的であった。しかしながら、感染防御はその抗体がウイルスの感染と同時に腸管内に存在した場合にみられた。以上の結果から、CEYIの連続投与によりヒトロタウイルス感染による子供の下痢発症とウイルス排泄を予防しうることが示唆された。

平成2年1月20日