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<tr>
<td>作者</td>
<td>一ノ瀬 休生 岩永 正明</td>
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<td>期刊</td>
<td>熱帯医学 岡山大学医学部 熱帯医学研究室</td>
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Experimental Study on the Parenteral Use of Antibiotics for the Treatment of Bacterial Diarrheal Diseases

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Abstract: The movement of parenterally given antibiotics to the diarrheal stool was studied by using two kinds of adult rabbit model for experimental cholera (T-model and Intestinal loop model). Four antibiotics (cefotiam=CTM, 20mg/kg; gentamicin=GM, 3mg/kg; minocycline=MINO, 10mg/kg; rifampicin=RFP, 5mg/kg) were administered with intravenous shot to the animals infected with cholera. All drugs appeared in the stool soon after medication. The concentration of the drugs in the stool was highest in GM, and lowest in CTM. RFP was detected from the stool for more than 24 hours after the single dose, but CTM was not detected after 8 hours. The pathway of GM from the blood vessels to the intestinal lumen was almost totally through the intestinal mucosa (mucosal pathway), while that of RFP was mainly through the bile system (hepatic pathway). Mucosal pathway was predominant in the movement of MINO. Hepatic pathway was predominant in CTM, although the movement of this drug was not much directed to the intestine. Different from antibacterial activity in vitro, GM did not work against the pathogen at all in vivo. Vibrio cholerae in the intestine kept on proliferating and producing cholera toxin under the presence of GM in high concentration. RFP showed an excellent bactericidal effect as well as in vitro, but a problem of naturally resistant strain with the frequency of 1 to 10⁶-10⁷ remained. The necessity of abundant defecation to eliminate the pathogen was suggested in the case with bacteriostatic antibiotics such as MINO. The benefit of parenteral use of antibiotics for the treatment of bacterial diarrheal diseases was discussed.

Key Words: Experimental cholera, T-model, Antibiotics.

INTRODUCTION

Acute diarrheal diseases are recognized as a major cause of mortality and morbidity of young children in the tropical area. In many countries, it is said that over one-third of the beds in children's hospital or wards are occupied by the patients suffering from diarrhea (Wkly. Epidem. Rec. WHO, 1982, No. 22). For the treatment of ba-
cterial diarrheal diseases, the use of antibiotics is one of the beneficial maneuvers except some cases with salmonella (Aserkoff and Bennett, 1969). But in any case, antibiotic therapy should be given under the knowledge of metabolism and distribution of the drugs in the body. It is required that the given drugs get effectively into the infection site. When a certain antibiotics is coming up to the market, detail studies on the toxicity, the metabolism, the clinical efficacy etc. are carried out. The drug distribution in the body (blood, urine, bile, cerebrospinal fluid, ascites, and even in the tissue of organs) is also studied. But in general, the drug distribution in the stool is almost not determined. This is unreasonable, because there are many infectious diseases in the intestine which need antibiotic therapy. Iwanaga et al. (1970) reported on the concentration of chloramphenicol (CP) in cholera stool. And they described that when CP was given intravenously, it soon appeared in the stool with high concentration, and the vibrio in the stool disappeared within 6 hours after medication. But when CP was given by mouth, the drug was almost not detected in the stool and the number of viable vibrio in the stool did not decrease. It was clarified later on that orally given CP was inactivated with glucuronidation (data not published). In the following year, Nakatomi (1971) confirmed the movement of CP in cholera patients. Except these two papers, the concentration of antibiotics in the stool after parenteral administration has not been described so far. In this paper, the movement of parenterally given antibiotics in the animals with experimental diarrhea, especially the concentration in the stool, is described.

MATERIALS AND METHODS

Animals: Rabbits of either sexes (Japan White), weighing 2 to 3 kg were used. All rabbits were apparently healthy, and were starved for 24 hours but water ad lib. prior to challenge.

Models for experimental diarrhea: 1. Ileal tube method (T-model). This model procedure was previously described by Iwanaga and Ichinose in this volume. 2. Intestinal loop model. Four, five, or six intestinal loops originated by De and Chatterjee (1953) were made. The length of the loops was various from 10 to 30 cm, and about the same length of uninoculated portions were taken between the loops. Inoculation was done with about $10^8$ of freshly cultured pathogen in 0.1 ml of heart infusion broth (Eiken) per loop.

Pathogen: *Vibrio cholerae* O1 isolated from human stool in Kenya in 1980 (our laboratory No. A-66) was used throughout this study. Since the isolation, the strain has been maintained in a butt of nutrient agar (Eiken) with subcultures every 3 to 4 months at room temperature.

Antibiotics: Cefotiam (Takeda), gentamicin (Shionogi), minocycline (Lederle Japan), and rifampicin (Dai-ichi) were used with the dosages of 20, 3, 10, 5 mg/kg, re-
spectively. The minimum inhibitory concentration (MIC) of these drugs against the pathogen used (strain A-66) in vitro with agar plate dilution method were 0.39 mcg/ml of minocycline and 0.78 mcg/ml of the other three drugs.

Medication and treatment for the infected animals: In the animals of T-model, continuous intravenous fluid infusion therapy with lactated Ringer's solution containing 5% glucose was started when the collapsed blood vessels in the rabbit ears were observed. The antibiotics were given with intravenous shot after the development of diarrhea was confirmed by sucking the diarrheal fluid through the tube. In the animals of loop model, the antibiotics were given with intravenous shot in 8 hours after challenge. The animals were sacrificed in 3 hours thereafter. Fluid therapy was not given.

Determination of antibiotics: The concentration of antibiotics in the stool (sucked fluid through the tube, and accumulated fluid in the loops), blood, urine and bile in the bladders were determined by bioassay technique with thin layered cup method using heart infusion agar (Eiken) plates. As the indicator organism, Bacillus subtilis strain ATCC 6633 was used.

Titration of cholera toxin: Cholera toxin (CT) in the stool samples were titlated with reversed passive latex agglutination method. The preparations of purified CT (chol-etox; chem-sero therapeutic Institute) and antiCT sensitized latex particles (V-ET RPLA; Denka seiken Co.) were commercially obtained. Stool samples were centrifuged and filtered through 0.45 μ milipore filter, and the filtrates were titlated for CT.

RESULTS

T-model: In the case with cefotiam (CTM), the amount of sucked stool during the first 5 hours after medication was 138 ml. The drug concentration in the stool reached a maximum level of 1.6 mcg/ml in 2 hours after medication with 20 mg/kg. The number of live vibrio in the stool was steadily going down from the level of 10⁹/ml to 10⁶/ml, until the drug in the stool disappeared. After the disappearance of the drug in the stool, the number of live vibrio went up to the initial level (Fig. 1). In the case with gentamicin (GM), the amount of sucked stool during the first 5 hours was 102 ml. The drug appeared in the stool very soon after medication, and it maintained high concentration for a long time. The peak concentration was seen in 8.5 hours after medication, but the slopes before and after were not steep. Nevertheless, the number of live vibrio in the stool did not decrease at all. In this case, the second dose was given at the 16th hour from the first dose, but the antibacterial effect was not observed. CT concentration in the stool kept high level during the time process in spite of large amount of of the stool was extracted (Fig. 2). In the case with minocycline (MINO), the amount of sucked stool during the first 5 hours after medication was 39 ml. The concentration of the drug reached a maximum of 2.7 mcg/ml in 1 hour and a half after the medication with 10 mg/kg. In 8 hours, only a trace of the
Fig. 1. ○ CTM concentration in the stool (mcg/ml)
○ Number of viable vibrio in the stool (log/ml)
○ CT concentration in the stool (ng/ml)

Fig. 2. ○ GM concentration in the stool (mcg/ml)
↑ second dose (GM 3mg/kg)
Fig. 3.  
- MINO concentration in the stool (mcg/ml)
- Number of viable vibrio in the stool (log/ml)
- CT concentration in the stool (ng/ml)

Fig. 4.  
- RFP concentration in the stool (mcg/ml)
Table 1. The loop test for Cefotiam (20mg/kg iv)

<table>
<thead>
<tr>
<th>Loop No.</th>
<th>Length (cm)</th>
<th>Fluid (ml)</th>
<th>FAR</th>
<th>Cefotiam mcg/ml</th>
<th>total (mcg)</th>
<th>VCC/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>34</td>
<td>1.700</td>
<td>0.49</td>
<td>16.7</td>
<td>5 x 10^8</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>1.3</td>
<td>0.065</td>
<td>1.25</td>
<td>1.6</td>
<td>2 x 10^9</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>9</td>
<td>0.243</td>
<td>0.93</td>
<td>8.4</td>
<td>5 x 10^8</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>20</td>
<td>0.625</td>
<td>0.74</td>
<td>14.8</td>
<td>2 x 10^8</td>
</tr>
<tr>
<td>No.0</td>
<td>12</td>
<td>++</td>
<td></td>
<td>1.55</td>
<td></td>
<td>(-)</td>
</tr>
</tbody>
</table>

CTM concentrations in: Blood 0.5, Urine 1,200, Bile 26. (mcg/ml)

Table 2. The loop test for Gentamicin (3mg/kg iv)

<table>
<thead>
<tr>
<th>Loop No.</th>
<th>Length (cm)</th>
<th>Fluid (ml)</th>
<th>FAR</th>
<th>Gentamicin mcg/ml</th>
<th>total (mcg)</th>
<th>VCC/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>30</td>
<td>2.308</td>
<td>12.0</td>
<td>360.0</td>
<td>1.2 x 10^8</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>5</td>
<td>0.333</td>
<td>13.0</td>
<td>65.0</td>
<td>2.2 x 10^8</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>4</td>
<td>0.190</td>
<td>15.0</td>
<td>60.0</td>
<td>3.2 x 10^8</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>5</td>
<td>0.268</td>
<td>11.7</td>
<td>58.5</td>
<td>3.0 x 10^8</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>14</td>
<td>0.518</td>
<td>7.6</td>
<td>106.4</td>
<td>2.0 x 10^9</td>
</tr>
<tr>
<td>No.0</td>
<td>24</td>
<td>++</td>
<td></td>
<td>1.7</td>
<td></td>
<td>(-)</td>
</tr>
</tbody>
</table>

GM concentrations in: Blood 9.3, Urine 590, Bile 8.8. (mcg/ml)

drug was detected. Following the maximum level, the number of vibrio in the stool began to decrease. It decreased from 10^9/ml to 10^6/ml. But in accordance with the disappearance of the drug, the number of vibrio increased again and kept the level of 10^9/ml thereafter (Fig. 3). In the case with rifampicin (RFP), the amount of sucked stool during the first 5 hours was 36 ml. The concentration of the drug reached a maximum of 11.0 mcg/ml in 2 hours. It decreased steadily thereafter and once it almost disappeared from the stool in 10 hours. But the drug appeared again in the stool. And the concentration was increasing and decreasing repeatedly with lowering the level.
Table 3. The loop test for Minocycline (10mg/kg iv)

The location of the loops

<table>
<thead>
<tr>
<th>Loop No.</th>
<th>Length (cm)</th>
<th>Fluid (ml)</th>
<th>FAR</th>
<th>Minocycline</th>
<th>VCC/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mcg/ml</td>
<td>total (mcg)</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>5</td>
<td>0.250</td>
<td>3.8</td>
<td>19.0</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>14</td>
<td>0.875</td>
<td>3.2</td>
<td>44.8</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>20</td>
<td>1.176</td>
<td>2.2</td>
<td>44.0</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>30</td>
<td>1.250</td>
<td>1.6</td>
<td>48.0</td>
</tr>
<tr>
<td>No.0</td>
<td>48</td>
<td>++</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MINO concentrations in: Blood 17, Urine 48, Bile 34. (mcg/ml)

Table 4. The loop test for Rifampicin (5mg/kg iv)

The location of the loops

<table>
<thead>
<tr>
<th>Loop No.</th>
<th>Length (cm)</th>
<th>Fluid (ml)</th>
<th>FAR</th>
<th>Rifampicin</th>
<th>VCC/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mcg/ml</td>
<td>total (mcg)</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>15</td>
<td>1.500</td>
<td>2.2</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>10</td>
<td>1.111</td>
<td>1.3</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>9</td>
<td>0.900</td>
<td>2.2</td>
<td>19.8</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>2.5</td>
<td>0.192</td>
<td>1.6</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>7</td>
<td>0.875</td>
<td>1.1</td>
<td>7.7</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>7</td>
<td>0.583</td>
<td>1.7</td>
<td>11.9</td>
</tr>
<tr>
<td>No.0</td>
<td>15</td>
<td>++</td>
<td></td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>

RFP concentrations in: Blood 1.3, Urine 9.6, Bile 39. (mcg/ml)

In the beginning, the volume of extracted stool was small, nevertheless, the number of vibrio decreased rapidly to undetectable level (less than $10^4$/ml). But after a few hours observation of vibrio free stool, the organism appeared again and increased their number without any suppression by RFP still present in the stool. This reappeared vibrio was highly resistant to RFP (MIC of RFP >100 mcg/ml).

Loop model: The results were summarized in Table 1 to 4 with an illustration of
the anatomical location of the loops in each case. No. 0 portion is the duodenum between the stomach and the first loop. Greenish, rather mucous fluid was accumulated there because of ileus condition. Of course, vibrio was not inoculated there and not detected when the animals were sacrificed. The drugs in this portion was regarded as that came with bile secretion. The concentration of CTM in the loops was lowest of all four drugs tested, although large amount was administered. The loop No. 2 which showed the concentration of 1.3 mcg/ml was almost negative in the fluid accumulation (FA ratio = 0.065). Therefore, the data may not be reliable. The concentration in No. 0 portion was definitely higher than that in the loops (Tab. 1). When GM was given, the concentration in the loops got very high, but that in No. 0 portion was much lower than that in the loops. Even in the gall bladder bile, the concentration was lower than that in the loops. The vibrios in the loops were not suppressed at all in this high concentration of GM (Tab. 2). In the case with MINO, the concentration in No. 0 portion was also lower than that in the loops. The drug retained in the blood with high level (Tab. 3). The concentration of RFP in the loops were relatively low, comparing with that seen in the T-model. But almost all vibrio in the loops were killed with 1 to 2 mcg/ml of this drug. The concentration in the bile was much higher than that in the urine (Tab. 4).

DISCUSSION

This study proved that parenterally given antibiotics appeared in the intestinal lumen, and it gave a warranty to the treatment with parenteral use of antibiotics for bacterial diarrheal diseases. And the two pathways of the drugs from the blood vessels to the intestinal lumen were clarified. Getting into the intestinal lumen, GM almost totally depends on the pathway through the intestinal mucosa (mucosal pathway), and RFP mainly depends on the pathway through the bile system (hepatic pathway). MINO is a bacteriostatic antibiotics, therefore, the decrease of viable vibrio in the stool depends on the amount of defecation. In this presented case, the volume of sucked stool was not large, but 99.9% of vibrio was once eliminated with one dose of MINO. Of course, this degree of elimination is not satisfactory from bacteriological point of view. Much larger amount of defecation is necessary for the complete elimination of vibrio from the stool, as seen in the human cases with chloramphenicol which was reported by Iwanaga et al. (1970). Or second dose of MINO should be given. In the intestinal loop model, MINO passed through the mucosa and appeared in the loops. Although the concentration in No. 0 portion was lower than that in the infected loops, greenish fluid accumulated in that portion and high concentration in the bile suggested that MINO in the stool of the T-model partially came from the hepatic pathway. The different concentration in each loop may depend on the time of medication and fluid accumulating stage, most likely same as the other three drugs. If the fluid was already
accumulated to some extent at the time of medication, the drug would be diluted by
the fluid already there.

GM has a bactericidal activity against the used pathogen (strain A-66) in the
concentration of 4MIC [MIC=0.78 mcg/ml, 4MIC= 3.13 mcg/ml] as far as the study
in vitro is concerned. GM concentration in the stool was very high in both models, and
the high level was maintained for a long time. Besides, a large amount of stool was
intermittently sucked. Nevertheless, the number of vibrio and amount of CT in the
stool kept high level throughout the experiment. It suggested that the vibrio kept on
proliferating and producing CT even under the presence of GM with high concentration.
GM did not work at all against the pathogen Vibrio cholerae. It is known that antiba-
cterial activity of GM is very weakened under the acid condition (Mori and Iwanaga,
1980). But pH of the stool in this case ranged from 7.15 to 8.20 during the first 20
hours of the disease. GM concentration in the bile and No.0 portion was very low,
which suggested that almost all amount of GM in the stool had come through the mu-
cosal pathway.

CTM is a bactericidal antibiotics of beta–lactams. The routine clinical use of
amount but relatively large dose comparing with the other drugs were given, but the
movement of CTM was not much directed to the intestine. Especially the drug was
poorly permeable through the intestinal mucosa, and did not show the effective elimi-
nation of vibrio in the stool.

When RFP was given intravenously, although the preparation of RFP for the par-
enteral use is not available in clinic, it appeared in the stool with high concentration.
RFP maintained the significant level in the stool for a long time. RFP has a strong
bactericidal activity against Vibrio cholerae, but unfortunately, one per $10^6–10^7$ of the
vibrio was naturally resistant to RFP (data not shown). It was definite that RFP in the
stool after intravenous administration was mainly passed through the hepatic pathway be-
cause of low concentration in the loops and high concentration in the bile and No.0
portion. If RFP is absorbed from the intestine with cholera, it must be secreted again
into the intestine through the bile. It will repeat absorption and secretion running on
this entero–hepatic circulation. This idea is the reason why we tried RFP for the tre-
atment of cholera in this experiment. We expected to succeed “one dose therapy with
RFP” for cholera. But when RFP was given by mouth, the stool with high concentra-
tion of the drug was seen only for a short duration (Fig.5). When the animal was sac-
crificed, the RFP concentration in the blood, the urine, and the bile were very low,
which suggested poor absorption of RFP in cholera case.

It is known that glucose raise the absorptive ability of the intestine with cholera
(Fisher and Parsons, 1953; Phillips, 1964). And this mechanism has been applied to
the oral rehydration therapy (Barua and Burrows, 1974). We did not try to give RFP
with glucose by mouth, but anyway, “one dose therapy” has become hopeless because
of resistant strain.

In the patients with gastroenteritis, the oral administration of the drugs is not always favourable because of nausea and vomiting, or unconsciousness. Besides, these patients may have absorptive dysfunction. In the cases with cholera, the antibiotics given by mouth may pass through the intestinal lumen without immersing in the deep crypt already filled with transudative cholera fluid. In the cases with dysentery, the site of the infection (lamina propria mucosa, colon) is covered with fibrinous pseudomembrane. Exudate movement is directed from the tissue to the lumen. In such condition, not much of the antibiotics in the intestinal lumen can reach the infection site. Even in these cases, parenterally given antibiotics are distributed by the blood stream and diffusely immersed the whole intestinal tissue in the drugs. Then the drugs ooze out to the intestinal lumen with the exudate or transudate. This pattern of the antibiotic movement in the body sounds like ideal. The methods of the use of parenteral antibiotics for bacterial diarrheal diseases should be studied further.
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28–43.


細菌性下痢症における抗生剤の非腸口的投与について －ウサギ下痢症における実験的検討－
ノタム休生, 岩永正明 (長崎大学熱帯医学研究所病原細菌学部門)

我々が最近新しく開発した感染症モデル（ウサギコレラ）を用いて, 非腸口的に投与された抗
生剤の便中への移行とその効果並びに移行経路について検討した。非腸口的に投与された各種抗
生剤は, 急性期コレラにおいて, まもなく便中に出現した。その移行率はセロチジンでは非
常に高率であり, セロチジンでは僅かな移行しかみられなかった。ミノサイクリンは中等度で
あり, リファンピシンの移行は良好であった。1回投与の後, ミノサイクリンとセロチジンは
8 ～10時間で便中から消失したが, セロチジンとリファンピシンは長時間にわたって便中か
ら検出された。腸管ルーペテストとの結果と比較することによって, 便中のセロチジンはそ
の溶と全てが腸粘膜を通過してきたものであり, リファンピシンはその大半が肝・胆系を通じて
便中に現われるということが判明した。ミノサイクリンでは腸粘膜の差が少ない程腸粘膜をと
るものが多く, セロチジンは肝・胆系をとる量の方がやや多かった。便中のセロチジン
濃度は, 使用したコレラ菌に対する in vitro での最小発育阻止濃度を10～20倍も上まわって
いたが, コレラ菌の増殖と毒素産生は全く抑制されていたかった。リファンピシンの殺菌効果は
卓越していたが, 10^{4}～10^{7} の割合で自然耐性菌が存在し, 一旦便中のコレラ菌が消失したと推
見してもまもなく高度耐性菌による増殖がみとめられた。

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