The Enhancement of Vascular Permeability by DTPA

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It was demonstrated that DTPA (diethylenetriaminepentaacetic acid) enhanced the vascular permeability by the intradermal injection test in rats. This change was inhibited by the pretreatment of Epinephrine (vasoconstrictor). The enhancement of vascular permeability was also observed in the small intestinal tract when DTPA was injected intravenously and introduced into the gastro-intestinal tract of rats.

As a result, it was considered that the enhancement of vascular permeability could induce side effects such as the decrease of blood pressure, vasodilation, hemorrhage or congestion in various organs after DTPA administration. Therefore, it was suggested that DTPA should be cautiously treated to humans.

KEY WORDS: DTPA, enhanced vascular permeability, intradermal injection test, rats, injected intravenously, introduced into the gastro-intestinal tract, side effects

I INTRODUCTION

DTPA (diethylenetriaminepentaacetic acid) has been known as the chelating agent that can dekorporate effectively plutonium and other actinides.1-4) However, it is necessary to determine whether DTPA has side effects and to establish the method of prevention against side effects, before it is applied clinically to the human. This problem must be solved as soon as possible, because the actual accidents had already occurred in which DTPA had been administered to the human.5,6)

The oral administration of DTPA has the possibility to be advantages because patients could take it immediately and easily by themselves for a long period when they encountered radiation accidents. We have examined the side effects of DTPA in the cases of oral administration to the experimental animals such as rats and beagle dogs. When more than 50 human dose (H.D.; 1 H.D. = 30 μmol/kg body weight) of Zn-DTPA or 10 H.D. of Ca-DTPA was administered orally to rats, the congestion and hemorrhage in the small intestine were observed.7) The similar changes and the hemosiderin deposition outside the vessels in the lamina propria of the small intestine were also seen in beagle dogs when 1 H.D. of Zn-DTPA was administered orally.8) Various side effects of DTPA after variable administration routes with regard to the vascular system have been reported by many investigators.9-11) From these findings, it is likely that DTPA could have some actions to affect the vascular system.

In the present study, it was elucidated, in the first place, that DTPA enhanced the vascular permeability when the test methods was applied which had been used in the passive cutaneous anaphylaxis test of drugs.12) In the next place, it was observed that the enhancement of vascular permeability was induced by the intravenous injection of DTPA and the direct administration of DTPA into the gastro-intestinal tract of rats, to be considered as practical administration routes to humans. The relationships between the enhanced vascular permeability and the side effects with congestion and hemorrhage were discussed.
II MATERIALS AND METHODS

1. Experimental animals
Female Wistar rats (about 250 g, 10 months old), from the animal facility of our institute, were used for tests.

2. DTPA solution
Na$_3$Ca-DTPA and Na$_3$Zn-DTPA purchased from DOJINDO Laboratory were dissolved in the isotonic saline solution, adjusted to pH 7.2. The concentration of stock solution was 450 $\mu$mol/ml. These solutions of Ca-DTPA and Zn-DTPA were diluted to 22.5 ($\times$1/1), 11.3 ($\times$1/2), 7.5 ($\times$1/3), 5.6 ($\times$1/4) and 2.8 ($\times$1/8) $\mu$mol in 0.05 ml, respectively.

3. Tests for vascular permeability
The following four tests were carried out after rats were anesthetized with ketamine hydrochloride (Sankyo Co., Ltd.).

(1) In order to clarify that DTPA enhances the vascular permeability, this test was carried out, according to the test method of the passive cutaneous anaphylaxis test of drugs.12)
Either Ca-DTPA and Zn-DTPA solution of various dilutions or isotonic saline solution as control was injected intradermally in 0.05 ml into the shaved dorsal skin of rats. In order to observe clearly the vascular permeability, rats were injected intravenously in 0.5 ml of 1% solution of Evans Blue dye (E. Merk A G.), 10 and 30 min, 1, 3 and 5 hr after the intradermal injection of DTPA solutions. Rats were sacrificed 20 min after the administration of dye. The dorsal skin was reflexed, and the degree of vascular permeability change was determined by measuring the mean diameter of blueing site induced by the extravasation of dye and its intensity was checked.

(2) In order to assess that the increase of vascular permeability is caused by the direct action to vessel of DTPA. Epinephrine (0.08 mg/kg body weight), as a vasoconstrictor, was injected intramuscularly to rats. Five or 20 min following the pretreatment of Epinephrine, serial dilutions of DTPA and isotonic saline solution were injected intradermally to rats. After 10 min, rats were injected intravenously in 0.5 ml of 1% Evans Blue dye. Thereafter, the degree of vascular permeability change was observed in the same manner as the described above.

(3) This test was done to observe whether the vascular permeability increased by the intravenous injection of DTPA or not.
Either Zn-DTPA solution including doses of 188 $\mu$mol of 0.42 ml and 375 $\mu$mol in 0.83 ml, or 0.80 ml of isotonic saline solution was injected intravenously to rats. A half ml of 1% Evans Blue dye was injected intravenously 10 min later. Thereafter, the changes of blueing site in the gastro-intestinal tract were observed for 10 min, and compared to the control.

(4) This test was done to observe whether the vascular permeability increases by the oral administration of DTPA or not.
The abdominal wall of rat was incised, and the region of cardia of stomach and rectum were tied with thread excluding surrounding the vessels. Immediately, either about 20-22 ml of Zn-DTPA solution (450 $\mu$mol/ml) or isotonic saline solution was injected into the gastro-intestinal tract. Ten minutes after DTPA instillation, 0.5 ml of 1% Even Blue dye was injected intravenously. Animals were sacrificed 10 min after the injection of dye. The gastro-intestinal tract was removed, and the degree of extravasation of dye was compared to that of control.

III RESULTS

(1) The vascular permeability changes, which showed blueing induced by the extravasation of Evans Blue dye in the rat skin, were observed by the intradermal injection of DTPA (Fig. 1). The
mean diameters of blueing circles induced by each DTPA were summarized in Table 1 and Fig. 2. The maximal change (diameter: Zn-DTPA 8.9±1.3 mm, Ca-DTPA 10.6±1.0 mm) was observed 10 min after the intradermal injection of DTPA. The mean diameters of blueing circles induced by Ca-DTPA were greater than those of Zn-DTPA. The intensive changes were observed at doses of 22.5, 11.3 and 7.5 µmol in a dose dependent manner (Fig. 2). The blueing changes were observed at the injection site of a high dose (22.5 µmol) up to 5 hr after the intradermal injection of DTPA, although they were reduced. Such changes were not observed by the isotonic saline solution, except for 1 case of 10 min after DTPA injection which might be induced by the technical error of injection.

(2) The enhanced vascular permeability induced by both Ca-DTPA and Zn-DTPA were inhibited by the treatment with Epinephrine prior to DTPA intradermal injections (Table 2 and Fig. 3). The enhancement of vascular permeability of Ca-DTPA was suppressed more strongly than that of Zn-DTPA.

(3) When Zn-DTPA was injected intravenously to rats, the increase of vascular permeability was seen in the duodenum, jejunum and ileum (30–50

Table 1 The mean diameters of blueing circles and its intensity showing the degrees of vascular permeability changes induced by DTPA when injected intradermally to rats.

| Evans Blue dye intravenous injection time after DTPA intradermal injection | Number of rats used | DTPA concentration in 0.05 ml volumes injected intradermally | Isotonic saline solution |
|---|---|---|---|---|
| Ca-DTPA | 10 min | 5 | 10.6±1.0 | 7.8±0.8 | 6.2±0.9 | 5.5±0.4 | 4.8±1.1 | 0 |
| | 30 min | 5 | 5.3±0.6 | 0 | 0 | 0 | 0 | 0 |
| | 1 hr | 5 | 4.5±1.2 | 0 | 0 | 0 | 0 | 0 |
| | 3 hr | 5 | 3.9±0.1 | 0 | 0 | 0 | 0 | 0 |
| | 5 hr | 5 | 4.1±0.4 | 0 | 0 | 0 | 0 | 0 |

Intensity of the extravasation of blue dye indicated by + (pale) or ++ (strong). Number of parentheses presented animals showing blueing changes.
Table 2 The suppression of vascular permeability by the treatment of Epinephrine prior to DTPA intradermal injection.

<table>
<thead>
<tr>
<th>Time pretreated by Epinephrine prior to DTPA intradermal injection</th>
<th>Number of rats used</th>
<th>DTPA concentration in 0.05 ml volumes injected intradermally</th>
<th>Isotonic saline solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn-DTPA</td>
<td>5</td>
<td>8.9±1.3</td>
<td>22.5 μmol (×1/1) 11.3 (×1/2) 7.5 (×1/3) 5.6 (×1/4) 2.8 (×1/8)</td>
</tr>
<tr>
<td>Zn-DTPA + Epinephrine (0.08 mg/kg)</td>
<td>5</td>
<td>7.4±0.9</td>
<td>4.7±0.2</td>
</tr>
<tr>
<td>5 min</td>
<td>5</td>
<td>6.5±0.4</td>
<td>4.7±0.4</td>
</tr>
<tr>
<td>20 min</td>
<td>5</td>
<td>6.5±0.4</td>
<td>4.7±0.6</td>
</tr>
<tr>
<td>Ca-DTPA</td>
<td>5</td>
<td>10.6±1.0</td>
<td>7.8±0.8</td>
</tr>
<tr>
<td>Ca-DTPA + Epinephrine (0.08 mg/kg)</td>
<td>5</td>
<td>6.5±0.6</td>
<td>4.9±0.6</td>
</tr>
<tr>
<td>5 min</td>
<td>5</td>
<td>8.3±0.4</td>
<td>5.1±1.1(4) 5.1±0.3(4) 3.7±0.4(2) 2.1(1)</td>
</tr>
</tbody>
</table>

Number of parentheses presented animals showing blueing changes.

Fig. 3 The suppression of vascular permeability by the treatment of Epinephrine (0.08 mg/kg body weight) 5 min prior to DTPA intradermal injection.

Fig. 4 The intensity of extravasation of dye in the gastro-intestinal tract induced by Zn-DTPA when injected intravenously to rats. Three animals were used in each group. Intensity of extravasation of dye indicated by – (negative), + (mild), ++ (moderate), respectively.

Fig. 5 Infusion of dye surrounding the vessels of stomach, duodenum and cecum when Zn-DTPA was injected intravenously.
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(4) When Zn-DTPA was introduced into the gastro-intestinal tract, the prominent blueing was observed particularly in the small intestine, as compared to the control (Figs. 6 and 7). This change might be estimated to occur by the following process. DTPA removed into the vessel through the mucosa of small intestine and enhanced the permeability of vessel. Thereafter, the dye extravasated into the intestinal mucosa.

IV DISCUSSIONS

The extravasation of Evans Blue dye in the skin of rats to which DTPA solution was injected intradermally and the inhibition of this change by the pretreatment of Epinephrine indicated that DTPA enhanced the vascular permeability through the direct action to the vasculature. The blueing circles reached the maximum within 10 min after the intradermal injection of DTPA, and were observed 5 hr later (Table 1). This shows that the effects of DTPA on vascular permeability appear immediately after DTPA administration and continue for a long time.

The enhancement of vascular permeability by Ca-DTPA was stronger than that of Zn-DTPA (Table 2). This supported that Ca-DTPA was more toxic than Zn-DTPA, as many investigators already reported.4,10,13,14) The vascular permeability of Ca-DTPA was inhibited more strongly by Epinephrine than that of Zn-DTPA. This might reflect the differences in kinetics of Ca2+ ions the vasculature.15)

Most noticeable finding was the blueing changes in the small intestine when DTPA was injected intravenously to rats. Because the intravenous injection of DTPA is considered to be a safe administration route to humans. TAYLOR et al. reported that the hemorrhages in the mucosa of duodenum were observed in the beagle dogs to which DTPA was injected intravenously and subcutaneously.11) Therefore, it was estimated that the hemorrhage in the gastro-intestinal tract could be induced by the vascular permeability action of DTPA.

The intensive extravasation of dye was observed in the small intestine by the direct administration of Zn-DTPA. BALLOU and PALOTAY reported the absorbed sites of DTPA in the rat administered orally were the jejunum and ileum.16) We observed the vasodilation, exudation of the blood and deposition of hemosiderin outside the vessels in the lamina propria of the small intestine of rats and beagle dogs administered orally.7,8) The intestinal
disturbances and its related clinical signs, i.e., loss of appetite, diarrhea, vomiting, melena were observed regardless of administration routes of DTPA, although those causes had been obscure. It is likely that the gastro-intestinal tract is more sensitive to DTPA.

SPENCER and ROSOFF observed the decrease of blood pressure with bradycardia continued 2-4 hr after DTPA intravenous injection in the patient. This finding is corresponding to our results in which the increase of vascular permeability changes continued for 5 hr after the intradermal injection of DTPA. TAYLOR et al. observed the hemorrhages, ranged from small petechiae to frank effusions in the lung, adrenal and thymus, and hyperemia in the medullary of kidney and congestion in the liver of beagle dogs injected intravenously or subcutaneously. Although no evidences were proposed, the medical staffs took care of cardiac status of the patient in Hanford americium accident when tried the protracted therapy of DTPA administration (1-2 g/day). Hence, the detailed pharmacological evidence on the enhancement of vascular permeability of DTPA and the relationship between clinical signs and schedule of DTPA administration should be necessary to be investigated as soon as possible.

V CONCLUSIONS

It was found that DTPA enhanced the vascular permeability by the extravasation test of Evans Blue dye. It is estimated that this change will be related to various side effects with hemorrhage and congestion induced by DTPA. Therefore, it was suggested that DTPA should be cautiously treated to humans.

REFERENCES

8) S. FUKUDA, H. IIDA and Y. OGHISO; to be published.