DISTRIBUTION OF CEPHALOTHIN TO THE EXUDATE IN COMPARISON WITH THE PLASMA OR LYMPH CONCENTRATIONS IN CROTON OIL-INDUCED INFLAMMATORY RATS

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The transport of cephalothin (CET) to the exudate was investigated in croton oil-induced inflammatory rats following the intramuscular administration of CET-aqueous solution, CET-O/W emulsion, or CET-W/O emulsion. The lymphatic transport of CET was the highest following the administration of W/O emulsion. The exudate level of CET was in a decreasing order of W/O emulsion, O/W emulsion, and aqueous solution.

In the rat whose blood vessels at femoral rectus, the site of administration, were ligated, the plasma level was decreased by the administration of aqueous solution, but not by W/O emulsion. The exudate level of CET was significantly higher in case of W/O emulsion. Possible mechanisms involved are discussed.

Keywords—cephalothin; emulsion; exudate; plasma; lymph; inflammatory rat; protein binding; blood vessel-ligated; resorption

INTRODUCTION

There are a number of factors involved in a higher bioavailability, higher plasma level, longer half-life and lower side-effect following the administration of drugs. It is well known that the onset of the efficacy in the clinical treatment is related to the time for drugs such as antibiotics to reach the tissue fluid of the inflammatory site.1-5)

The fate of cephalothin (CET) has been studied extensively in men and animals.6-8) However, few data are available concerning lymph and exudate concentrations following the administration of the antibiotic.9) It has been recognized that the tissue fluid is connected with the lymphatic fluid.

From these standpoints, we measured the drug concentration in the plasma, lymph and exudate in the inflammatory rats following the administration of CET and investigated whether lymph concentration of the drug has any relation to that of the exudate and whether the antibiotic was transported to the extravascular tissue through the blood vessel taking into consideration the effect of protein binding.10) Effect of dosage form of CET on its distribution was also examined.

EXPERIMENTAL

1) Materials — Sodium cephalothin (CET-Na) was purchased from Shionogi Pharmaceutical Co., Ltd. Croton oil and olive oil were obtained from Kishida Chemical Co., Ltd. Gelatin was obtained from Nakarai Chemical Co., Ltd., and polyoxyethylene derivative of hydrogenated castor oil (HCO-60) and sorbitan sesquioleate (SO-15) were obtained from Nikko Chemical Co., Ltd.

2) Preparation of Emulsions — O/W Emulsion: One ml of sesame oil, 2 ml of CET-Na solution (10 mg/ml) and 2 ml of 10% gelatin solution were mixed and sonicated in ice water for 1-2 minutes by Branson Sonifier B-12 (20 kc).

W/O Emulsion: Three ml of sesame oil containing SO-15 and HCO-60 at the concentration of 2.75% and 0.25%, respectively, was mixed with 2 ml of CET-Na solution (10 mg/ml) and the mixture was sonicated at 60° for 1-2
minutes.

3) Procedure of Animal Experiments — Male Wistar rats weighing about 180 g were anesthetized with sodium pentobarbital. After clipping hair, 20 ml of air through the millipore filter (pore size 0.22 μm) were injected s.c. on the dorsum of a rat. Then 1% croton oil in olive oil was injected into the air pouch. On day 7, the transport experiments were carried out under pentobarbital anesthesia. The thoracic lymph duct was cannulated according to the method of Bollman, et al. A heparin-filled catheter (o.d. 0.8 mm, i.d. 0.5 mm) were inserted into the thoracic lymph duct and fixed with the aid of a drop of tissue cement, Alon Alpha (Sankyo Co., Ltd.). In some experiments, most of blood vessels at the femoral rectus, the site of administration, were ligated, followed by the tearing of skin.

One ml of the drug preparation (10 mg of CET-Na) was injected i.v. or i.m. The lymph was collected periodically in the heparinized tube and the volume was calculated from its weight. Blood specimen was drawn every hour from the tail vein. The settled volume of exudate was collected every hour from the pouch with a syringe. After the examination, the exudate volume was checked and converted into the date of about 8 ml exudate volume.

4) Analytical Methods — The collected plasma, lymph and exudate were analyzed microbiologically by the cylinder plate method of Bennett et al. using Staphylococcus aureus. The concentration of CET was calculated by standard curve of each specimens.

5) Measurement of Binding of CET to Serum, Lymph and Exudate — The binding was measured by utilizing equilibrium dialysis method. A 10 ml aliquot of serum (lymph or exudate) containing CET (20 μg/ml) or 10 ml of aqueous solution containing the same amount of CET was respectively placed into each Visking cellulose bag, and was equilibrated against each materials overnight at room temperature. The concentration of CET in outer phase were measured by the cylinder plate method using Staphylococcus aureus. Binding to serum (lymph and exudate) by the initial concentration of CET was calculated.

RESULTS AND DISCUSSION

Recently, a number of reports have appeared describing the transport of drugs to the interstitial fluid which is induced by the method of silastic tissue cages and skin window technique in rabbits and humans, and to granuloma which is induced by the subcutaneous injection of carrageenin. In this paper, the effect of pharmaceutical modification on the transport of CET to the exudate was examined in comparison with the plasma and lymph levels.

Plasma and Exudate Concentrations of CET after Intravenous or Intramuscular Administration of the Aqueous Solution of Its Sodium Salt

Fig. 1 shows the plasma concentration of CET after the intravenous or intramuscular administration of CET-Na (10 mg in 1 ml of aqueous solution) in inflammatory rats. In case of i.v. administration, the disappearance from plasma was so rapid that no CET was detectable at 3 hr. On the other hand, the disappearance was delayed in case of i.m. administration. Although the time to reach peak plasma level was within 30

![Graph showing plasma concentration over time](image-url)

FIG. 1. Plasma Concentration after Intravenous or Intramuscular Administration of CET-Na

○: solution (i.v.), ●: solution (i.m.). Given values are means ± S.D. (n = 4—6).
min, a considerable plasma level was still detectable even at 8 hr, which may be due to a slow diffusion from the site of injection to the blood stream.

Fig. 2 shows the exudate level of CET after the administration (i.v. or i.m.) of CET-Na (10 mg) aqueous solution. The exudate levels of CET after i.v. administration are significantly higher, about twice at 1–3 hr, than those after i.m. administration, which is maintained at the constant level from 0.5 to 5 hr.

**Plasma and Lymph Concentrations of CET after Intramuscular Administration of CET-Na as O/W or W/O Emulsions**

Plasma and lymph concentrations of CET after i.m. administration of CET-Na (10 mg) emulsions are shown in Fig. 3 and Fig. 4, respectively. Plasma CET levels after the administration emulsion form were significantly lower than those of aqueous solution. Differences in plasma levels between gelatin-emulsified and albumin-emulsified O/W emulsions were not significant. W/O emulsion gave the lowest plasma CET concentration especially in the initial stage.

Contrary to the plasma levels, CET in lymph was the highest in case of W/O emulsion, approximately twice that of the aqueous solution and is in the following order; W/O emulsion > O/W emulsion (albumin) > O/W emulsion (gelatin) > aqueous solution. As is evident from Fig. 3 and Fig. 4, CET concentrations in lymph were higher than those in plasma, namely, lymphatic transport of CET was enhanced by using the emulsion. The lymph-to-plasma concentration ratios 1 hr after the administration of W/O emulsion and O/W

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**FIG. 2. Exudate Concentration after Intravenous or Intramuscular Administration of CET-Na**

〇: solution (i.v.), ●: solution (i.m.). Given values are means ± S.D. (n = 4–6).

**FIG. 3. Plasma Concentration after Intramuscular Administration of CET-Na**

〇: solution (i.m.), △: O/W emulsion (gelatin, i.m.), ■: O/W emulsion (albumin, i.m.), ●: W/O emulsion (i.m.). Given values are means ± S.D. (n = 4–6).

**FIG. 4. Lymph Concentration after Intramuscular Administration of CET-Na**

Keys are the same as in Fig. 3. Given values are means ± S.D. (n = 4–6).
emulsion (albumin) were approximately 13 and 7, respectively.

**Exudate Level of CET after Intramuscular Administration of Various Types of Emulsions**

Fig. 5 shows the CET concentration in the exudate after the *i.m.* administration as described above. CET was rapidly transported to the inflammatory site and the exudate level was maintained for a relatively long period of time. The exudate transport of CET was increased in the order of aqueous solution < O/W emulsion (gelatin) < O/W emulsion (albumin) < W/O emulsion. It has been generally regarded that drugs are transported to the inflammatory site through the blood vessels. However, from these results, it appeared that CET administered *i.m.* as emulsions was absorbed mainly through the lymphatic capillary, reaching the thoracic lymph duct, and migrating to the subclavian vein. As far as we know, the drug injected into interstitial spaces of tissue is transported away from the injected site by the circulating blood, but W/O emulsion reaches the regional lymph nodes to varying degrees.\(^{16,17}\) The slower increases in the exudate level of CET in the form of emulsions coincide with this long pathway. A part of this antibiotic in the lymphatic vessels may be directly transported through the lymph wall at the inflammatory site. In order to clarify this possibility further, protein binding of the drug in the blood, lymph, and exudate was examined by using an equilibrium dialysis technique\(^ {14}\) and the amount in percentage bound was found to be 59.2±4.1, 21.5±3.3, and 70.3±4.8, respectively.

These values indicate that most of CET in the

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**FIG. 5. Exudate Concentration after Intramuscular Administration of CET-Na**

Keys are the same as in Fig. 3. Given values are means ± S.D. (*n* = 4–6).

**FIG. 6. Plasma Concentration after Intrapouch Administration of CET-Na**

〇: solution, ●: W/O emulsion, -----: 10 mg/ml, ---: 30 mg/ml. Given values are means ± S.D. (*n* = 4–6).

**FIG. 7. Lymph Concentration after Intrapouch Administration of CET-Na**

Keys are the same as in Fig. 6. Given values are means ± S.D. (*n* = 4–6).
lymph is unbound whereas most of the drug in the exudate is bound. For the purpose of the observing the mode of dispersion of emulsion after injection, Sudan Blue, a lipophilic dye is dissolved in oily phase prior to emulsification and observed the photomicrograph of section of the thigh muscle and lymph nodes after injection of emulsion.

**Plasma and Lymph Concentration of CET after Intrapouch Administration**

In order to investigate the reabsorption of the inflammatory site, CET concentration in the plasma and lymph was measured after the intrapouch administration of CET-Na in an aqueous solution of W/O emulsion and the data are shown in Fig. 6 and 7, respectively. CET was transferred both in the plasma and in the lymph and levels were maintained for more than 5 hr after the intrapouch administration of CET-Na aqueous solution at a dose of 30 mg. However, at a dose of 10 mg, CET was detected neither in the plasma nor in the lymph. This might be due to the protein binding, the dilution in the exudate and so on. On the other hand, considerable CET was detected in the plasma and the lymph even at the dose of 10 mg emulsion. After administered in the pouch, W/O emulsion appeared to behave as W/O/W in the exudate. The drug in the inner aqueous phase would be hardly bound with proteins, absorbed in the lymphatic capillary with oil particles, or transported into the blood after the oil is trapped in the lymph nodes.\textsuperscript{16,17}

**FIG. 9. Lymph Concentration after Intramuscular Administration of CET-Na to the Intact or Blood-Ligated Rats**

Keys are the same as in Fig. 8. Given values are means ± S.D. (n = 4–6).

**FIG. 8. Plasma Concentration after Intramuscular Administration of CET-Na to the Intact or Blood Vessel-Ligated Rats**

- O: solution, •: W/O emulsion, —: intact, ------: ligated. Given values are means ± S.D. (n = 4–6).

**FIG. 10. Exudate Concentration after Intramuscular Administration of CET-Na to the Intact or Blood-Ligated Rats**

Keys are the same as in Fig. 8. Given values are means ± S.D. (n = 4–6).
Effect of Ligation of Blood Vessels at Femoral Rectus

In order to investigate the exchangeability of a free drug between blood and lymph, the transport of CET was examined in the rat whose blood vessels at the site of administration were ligated. The experiment was to examine whether the drug could be transported from lymph to plasma on its way to the thoracic duct. Fig. 8 shows the plasma concentration of CET after i.m. administration in the blood vessel-ligated rat. It is significantly lower in the case of aqueous solution in the early stage, while significantly higher in case of W/O emulsion. Fig. 9 shows the lymph concentration of CET in the same experiments. When the transport via blood vessels is inhibited, the free drug seems to be transported by the lymph route. In case of W/O emulsion, the lymph concentrations were lower contrary to the plasma concentration. However, the amount transported via lymph, which is estimated by AUC, was not significantly different in both rats. As shown in Fig. 10, the exudate concentration in the blood vessel-ligated rat was negligible within 30 min and increased gradually. This delay in reaching the inflammatory site can be explained by the slow transport of the drug in the lymph to the plasma. However, the continued increase in the exudate concentration even at 3 hr suggests the possibility of the temporary adsorption in the lymph nodes. These data suggest that the transport of drug (CET) is facilitated by emulsion, and a high concentration of drug in lymph (exudate) can be maintained longer by using the W/O emulsion. A slower diffusion of antibiotics from the injection site achieved, and a wide application of these dosage forms for clinical use is anticipated.

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