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Lymphatic transport of sodium cefoxitin in the presence of sodium 5-methoxysalicylate after injection into rat rectal connective tissue, femoral muscle and femoral vein

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Lymphatic uptake of sodium cefoxitin after injection into rectal connective tissue was greater than after injection into the femoral muscle of rats. Coadministration with sodium 5-methoxysalicylate enhanced lymphatic drug uptake at both sites. This enhancement may be an indirect result of 5-methoxysalicylate's suppression of vascular permeation of the cefoxitin. An adjuvant-induced increase in lymphatic fluid flow may also be partially involved in the enhancement of cefoxitin lymphatic transport.

de Boer & Breimer (1980) have shown that if a drug is taken up by the lower rectal venous blood supply, it enters the systemic circulation directly avoiding first-pass metabolism in the liver. Recently, a second rectal route involving the gut-associated lymphatic system has been shown to transport significant amounts of drug and also permitting drugs to bypass liver metabolism. Caldwell et al (1982) reported that recovery of sodium cefoxitin, a highly water-soluble drug, from lymphatic fluid collected from rat thoracic ducts after rectal administration of a microenema containing sodium cefoxitin and the adjuvant sodium 5-methoxysalicylate, was significantly higher than cefoxitin recovery after intravenous injection. The present report compares the extent of lymphatic transport of cefoxitin in rats after its injection into rectal connective tissue with that after either intravenous or intramuscular injection with or without sodium 5-methoxysalicylate. In addition, the mechanism underlying the enhancing action of 5-methoxysalicylate on cefoxitin lymphatic transport is examined.

Materials and methods

Sodium cefoxitin was supplied by Merck Sharp & Dohme (Rahway, NJ, USA). Sodium 5-methoxysalicylate was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). 50 μl of a solution containing 1·56 mg of cefoxitin alone or with additives was injected into the rectal connective tissue, femoral muscle or femoral vein of male Sprague-Dawley rats, 225–250 g that had been fasted for 16 h before the experiments but had free access to water. Administration into rectal connective tissue involved exposing the rectum by a dorsal incision near the anus, injecting the solution and closing with sutures. For this, rats were anaesthetized with 60 mg kg^{-1} sodium pentobarbitone; temperature was maintained at 37 °C. After injection, lymphatic fluid was collected from the thoracic duct according to Bollman et al (1948) and blood was taken from the jugular vein at designated times. Plasma was isolated by centrifugation. The assay of cefoxitin in lymph and plasma was by a high pressure liquid chromatographic technique (Nishihata et al 1984a).

Results and discussion

One hour after injection into the rectal connective tissue, lymph cefoxitin concentrations were higher than those in plasma while the opposite was seen in the intramuscular and intravenous studies (Fig. 1). A comparison of lymph cefoxitin concentrations at each site showed them to be higher after rectal injection than after intramuscular or intravenous injection. Furthermore, recovery of cefoxitin from lymphatic fluid collected over 4 h after injection into rectal connective tissue was significantly higher than recoveries after intramuscular or intravenous injection (Table 1). Intramuscular injection resulted in greater plasma cefoxitin values than those obtained after rectal administration. Although the network of lymph flow in the rectum is not known, the difference in drug uptake between the rectal and intramuscular administration sites may be due to a higher ratio of lymph flow versus blood flow in the rectal area compared with that in the femoral muscle.

Sodium 5-methoxysalicylate has been shown to improve rectal absorption of several drugs (Nishihata et al 1981a, 1982a) as has sodium salicylate (Nishihata et al 1980, 1981b, 1982b). In the present study on cefoxitin lymphatic transport, the addition of 5-methoxysalicylate, 0·65 M, to the injected solution resulted in a significant increase in lymph cefoxitin concentration 1 h after injection into the rectal or intramuscular site (Fig. 1). As also shown in Fig. 1, when 5-methoxysalicylate was injected with cefoxitin, the rate of appearance of the drug in the plasma was slower than in absence of adjuvant. No noticeable change was seen in either the lymph or plasma cefoxitin values after intravenous injection of drug with adjuvant.

When sodium chloride (0·65 M) replaced sodium 5-methoxysalicylate, the concentration of cefoxitin in the plasma and lymphatic fluid after injection into rectal

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Table 1. Recovery of cefoxitin in lymph and lymphatic fluid volume collected from rat thoracic duct after an injection of cefoxitin (6·25 mg kg^{-1}) with or without additives at various administration sites (n ≥ 4).

Additives in cefoxitin injection (M)	% recovery* of cefoxitin in lymph (mean ± s.d.) 1 h	4 h	Ratio A† at 4 h	Lymphatic fluid volume, ml (mean ± s.d.) 1 h	4 h	Ratio B† at 4 h	A/B
Rectal connective tissue							
None	0·38 ± 0·10 ‖	0·69 ± 0·14 ‖	1	0·46 ± 0·11	1·48 ± 0·27	1	1
5MSA‡ (0·65)	0·81 ± 0·12¶	2·03 ± 0·36¶	2·94	0·61 ± 0·12	2·13 ± 0·31¶¶	1·43	2·06
NaCl (0·65)	0·45 ± 0·12	0·86 ± 0·19	1·25	0·57 ± 0·11	2·02 ± 0·26	1·36	0·92
Ethanol (5·0)	1·03 ± 0·21¶	2·60 ± 0·32¶	3·77	0·66 ± 0·08	2·32 ± 0·41¶	1·58	2·39
ε-ACA§ (0·1)	0·18 ± 0·07	0·59 ± 0·08	0·86	0·26 ± 0·08	0·87 ± 0·08¶	0·59	1·46
Femoral muscle							
None	0·18 ± 0·04	0·39 ± 0·08	1	0·38 ± 0·12	1·37 ± 0·24	1	1
5MSA (0·65)	0·39 ± 0·09¶	1·03 ± 0·21¶	2·64	0·48 ± 0·06	1·77 ± 0·21	1·29	2·05
NaCl (0·65)	0·23 ± 0·07	0·51 ± 0·09	1·31	0·46 ± 0·09	1·81 ± 0·26	1·32	0·99
Ethanol (5·0)	0·69 ± 0·20¶	1·43 ± 0·38¶	3·67	0·56 ± 0·21	2·13 ± 0·36¶	1·55	2·36
ε-ACA (0·1)	0·11 ± 0·04	0·28 ± 0·04	0·72	0·21 ± 0·09	0·76 ± 0·09¶	0·55	1·31
Femoral vein							
None	0·13 ± 0·05	0·24 ± 0·06		0·34 ± 0·08	1·19 ± 0·24		
5MSA (0·65)	0·14 ± 0·04	0·28 ± 0·04		0·36 ± 0·09	1·24 ± 0·32		

* Percent recovery relative to initial dose. † Ratio of value with additive to value without additive. ‡ Sodium 5-methoxysalicylate. § ε-Aminocaproic acid. ‖ $P < 0·01$ versus intravenous and intramuscular injection. ¶ $P < 0·01$ versus no additive. ¶¶ $P < 0·05$ versus no additive.

connective tissue or femoral muscle was similar to that when cefoxitin alone was injected (Fig. 2 and Table 1). After injection with sodium chloride at either the rectal or muscular site, cefoxitin recovered from lymphatic fluid, showed only a slight increase over its recovery after administration alone (Table 1). These results suggest that the enhancing action of sodium 5-methoxysalicylate on the lymphatic transport of cefoxitin is probably not related to hypertonicity or isotonic strength of the solution administered. However, the small increase in lymphatic fluid volume and recovery of cefoxitin seen after its coadministration with sodium chloride implies a possible correlation between lymph volume and drug uptake.

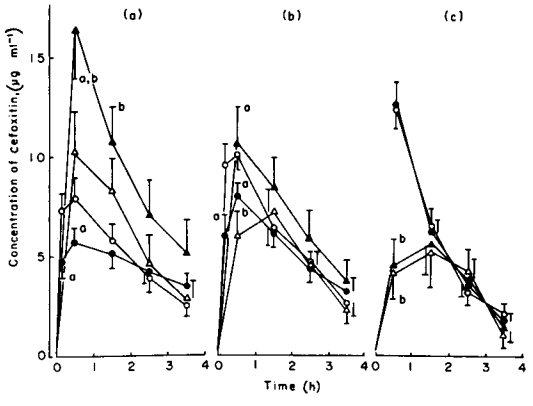

FIG. 1. Concentration of cefoxitin in lymph (△ and ▲) collected from the thoracic duct and in plasma (○ and ●) after an injection of cefoxitin (6·25 mg kg^{-1}) alone (△ and ○) or with 0·65 M sodium 5-methoxysalicylate (▲ and ●) into (a) rectal connective tissue, (b) femoral muscle and (c) femoral vein. Concentration of cefoxitin in lymph was determined at 1 h intervals for 4 h. Each value represents mean ± s.d. (n ≥ 4). For 'a', $P < 0·001$ versus without additives; for 'b', $P < 0·001$ versus cefoxitin concentration in plasma.

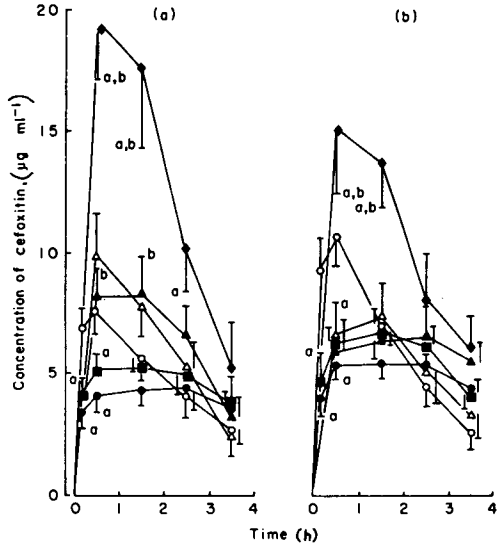

FIG. 2. Concentration of cefoxitin in lymph (△, ■ and ▲) collected from thoracic duct and plasma (○, □ and ●) after an injection of cefoxitin (6·25 mg kg^{-1}) with either 0·65 M sodium chloride (○ and △), 5·0 M ethanol (□ and ■) or 0·1 M ε-aminocaproic acid (● and ▲) into (a) rectal connective tissue and (b) femoral muscle. Concentration of cefoxitin in lymph was determined at 1 h intervals for 4 h. Each value represents the mean ± s.d. (n ≥ 4). For 'a', $P < 0·001$ versus without additives (see Fig. 1); for 'b', $P < 0·001$ versus cefoxitin concentration in plasma.

If A equals the ratio of cefoxitin recovery when sodium chloride is present in the injection to cefoxitin recovery in absence of sodium chloride, and B equals the ratio of lymphatic fluid volume collected in the presence of sodium chloride to lymphatic fluid volume collected without sodium chloride, Table 1 shows that the ratio A/B approaches 1. If 5-methoxysalicylate's adjuvant action was responsible for the increase in lymphatic fluid flow the A/B value for 5-methoxysalicylate should also be near 1 but, as shown in Table 1, it is twice that of the A/B ratio for sodium chloride. This suggests that the effect of 5-methoxysalicylate on cefoxitin uptake from rectal connective tissue and femoral muscle involves some mechanism other than an increase in lymphatic fluid volume.

This could be its suppresion of cefoxitin uptake into the blood. If the drug is prevented from entering the blood, more could be available to the lymphatic system. This possibility was examined using ε-aminocaproic acid, which is known to suppress vascular permeation (Nishihata et al 1984b). Injection of cefoxitin with ε-aminocaproic acid into either the rectal connective tissue or femoral muscle, significantly inhibited drug uptake into the blood and slightly suppressed uptake into the lymph (fig. 2). Since the A/B ratio for ε-aminocaproic acid was significantly greater than 1, suppression of vascular permeation may be involved in the adjuvant-enhanced lymphatic uptake of cefoxitin.

Ethanol also enhances rectal cefoxitin absorption (unpublished data). As shown in Fig. 2 and Table 1, injection of cefoxitin with ethanol significantly enhanced lymphatic cefoxitin concentrations, increased the lymph fluid volume and suppressed cefoxitin uptake into blood. These results are similar to those obtained with 5-methoxysalicylate. The A/B ratio for ethanol was similar to that for 5-methoxysalicylate (i.e. >2·0) which suggests that ethanol and 5-methoxysalicylate behave similarly. In a recent investigation of the effect of non-protein sulfhydryls on membrane permeability (unpublished data), we found that salicylate, a 5-methoxysalicylate analogue, and ethanol, decreased reduced non-protein sulfhydryl levels in rat rectal mucosa and enhanced cefoxitin permeation of the tissue. Hence non-protein sulfhydryls may also be involved in the adjuvant-drug response.

In conclusion, lymphatic transport of cefoxitin appears to occur more readily from the rectal connective tissue than from femoral muscle. A faster rate of lymphatic fluid flow than blood flow in the rectal tissue may account for the difference. The connection between lymphatic fluid flow and lymphatic drug transport was evident after injection of cefoxitin with sodium chloride. The enhancing effect of 5-methoxysalicylate on lymphatic uptake of cefoxitin from the rectal and muscular sites does not appear to be due solely to an adjuvant-induced increase in lymphatic fluid flow. Evidence from this study suggests that 5-methoxysalicylate may act to suppress the uptake of cefoxitin into the blood than by increasing the amount of drug available to the lymphatic system.

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