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The distribution of antibacterial agents between plasma and lymph in the dog

P. ACRED, D. M. BROWN, B. F. CLARK* AND LINDA MIZEN

Beecham Research Laboratories, Brockham Park, Betchworth, Surrey

Summary

1. Plasma, peripheral and thoracic lymph concentrations of penicillin V, phenethicillin, carbenicillin, ampicillin, cloxacillin, penicillin G, chloramphenicol and sulphadiazine were determined at various time intervals up to 6 h following intramuscular administration of 50 mg/kg to dogs.

2. Peak plasma concentrations of the penicillins occurred within half an hour after administration with the peak lymphatic concentrations occurring 1·5 to 3 h afterwards. For the remaining period of the test the concentration in the lymph exceeded the corresponding concentration in the plasma. Sulphadiazine gave concentrations in thoracic lymph equal to the plasma concentration, but the peripheral lymph concentrations were lower while the concentrations of chloramphenicol in both peripheral and thoracic lymph were always lower than the plasma concentrations.

3. After the peak concentrations were reached, the concentration curves for penicillins in lymph followed the same pattern as found in plasma, the penicillin concentrations declining exponentially. Sulphadiazine produced more persistent levels both in lymph and in plasma while the concentrations of chloramphenicol were still rising 6 h after administration.

4. The free concentrations of penicillin in lymph were equal to the free concentrations in plasma, whereas the concentrations of free sulphadiazine and chloramphenicol in lymph were less than those in the plasma.

Introduction

Since bacterial infections are normally localized and confined to specific sites within the body, it is perhaps of greater importance to know the concentrations of antibiotic at these sites rather than the corresponding blood levels. In addition, since it is generally agreed that the protein bound fraction of the antibiotic is inactive the concentration of the free antibiotic in the extravascular fluids should also be known. There have been only a limited number of studies carried out to determine free antibiotic levels in infected tissues because it is technically difficult to get sufficient fluid from such a site to carry out the assays. According to Yoffey & Courtice (1956) there are only minor differences in the composition of peripheral lymph and interstitial fluid from which it is derived. We have therefore carried out experiments to determine the relationship between plasma and peripheral lymph

* Present address: Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2.

concentrations of some antibacterial agents and have noted the effect of serum protein binding on the distribution in blood and extravascular tissues.

Methods

Drugs

The following chemotherapeutic agents were used: cloxacillin (Orbenin, Beecham Research Laboratories) was used as the sodium salt, phenethicillin (Broxil, Beecham Research Laboratories) as the potassium salt, phenoxymethylpenicillin (Penicillin V, Eli Lilly & Co. Ltd.) as the potassium salt, ampicillin (Penbritin, Beecham Research Laboratories) as the sodium salt, benzylpenicillin (Crystapen, Glaxo Laboratories Ltd.) as the sodium salt, carbenicillin as the sodium salt (Pyopen, Beecham Research Laboratories), chloramphenicol (Chloromycetin, Parke Davis & Co.) and sulphadiazine (May & Baker Ltd.).

Solutions of the penicillins were prepared in normal saline. Chloramphenicol solutions were prepared by dissolving the compound in absolute ethanol and making up to volume with normal saline. The resulting solution contained 10% ethanol. The solution of the sulphonamide was prepared by dissolving the agent in 1 N NaOH, neutralizing with 1 N HCl and making up to volume with normal saline.

Doses

Groups of two or three greyhounds (20–30 kg body weight) were used. The compounds were administered intramuscularly into the left hind leg muscle at a dose of 50 mg/kg.

Samples of blood and lymph for assay were taken at 0·5, 1, 2, 3, 4, 5 and 6 h following administration. All samples were stored at 4° C until assayed.

Assays

Penicillins and chloramphenicol were determined in the samples by the hole-plate technique ; *Sarcina lutea* was used as the test organism. The plates were incubated overnight at 29° C. In the assays in which the samples were used undiluted, the solutions for the standard curve were prepared in the same fluid as the test specimen, blood or lymph. When specimens required dilution they were assayed against the standard prepared in saline. The zone diameter (mm) of each standard solution was plotted against the logarithm of the concentration (μg/ml) and from the regression line obtained the concentration of the antibiotic in the sample was estimated by interpolation. Concentrations appearing in plasma were calculated assuming a packed cell volume of 40%. Studies previously carried out had indicated that none of the compounds used in these experiments penetrated red cells or were bound to cells.

The free and total sulphonamide content of the sample was determined using the colorimetric method of Bratton & Marshall (1939).

Protein content

The total protein content (g/100 ml) of lymph and serum was determined by the biuret method. The albumin/globulin ratio was determined by the cellulose acetate electrophoresis technique. 10 μl quantities of the samples were spotted onto the

cellulose strips and a constant current of 0·6 mA/cm width was applied for 3 h. The buffer used was 0·06 M barbitone, pH 8·6. The strips were stained with Ponceau " S " (0·2% in 3% trichloracetic acid) and were scanned by transmitted light using a Chromoscan densitometer.

Ultrafiltration

The amount of binding of the compounds to proteins was determined by ultrafiltration. Samples of lymph and serum were obtained from a number of dogs and pooled. The pooled samples, stored at 4° C, were kept for a maximum period of 2–3 days before use. The pH of the samples was checked before each experiment and where necessary adjusted to pH 7·2–7·4 by bubbling CO_2 through the specimen.

Concentrations of the chemotherapeutic agents of 40 μg/ml were prepared in both serum and lymph, and 2·0 ml samples were placed into 8/32 inch Visking tubing which was suspended in a tube 3 inches × 1 inch. The pressure in the tube was reduced to 15 mmHg (1 mmHg≡1·333 mbar) and the system was sealed to prevent evaporation of the filtrate. The filtrate volume was never allowed to exceed 10% of the original solution for filtration. Control experiments were carried out with aqueous solutions to confirm that free passage of the compounds through the membrane occurred in the absence of protein. All experiments were carried out at room temperature (20°–24° C) over a maximum period of 90 min. The filtrate was assayed and the binding of the compounds to serum and lymph was calculated using the following formula:

$$\%\text{binding} = \frac{\text{Initial concentration } \mu\text{g/ml} - \text{filtrate concentration } \mu\text{g/ml} \times 100}{\text{Initial concentration } \mu\text{g/ml}}$$

Collection of lymph

The method used for the collection of peripheral lymph was similar to that described by Smith, Dunton, Protas, Blocker, Cooley & Lewis (1959) and Verwey & Williams (1962a, b).

The dogs were anaesthetized intravenously with pentobarbitone sodium (veterinary Nembutal) 30 mg/kg. The right hind paw lymphatics and the thoracic duct were cannulated as follows:

(*a*) *Peripheral lymph.* The skin on the dorsal surface of the paw was shaved and an area 5 × 2·5 cm carefully removed from a region extending forward from the base of the metatarsals. To render the lymphatics clearly visible 0·1 ml of Evans Blue dye (0·1%) was injected into the subcutaneous spaces between the metatarsals. About 1 cm of one of the larger paw lymphatic vessels was dissected free and cannulated with a No. 12 hypodermic needle (23 gauge) from which the mount had been removed. It was carefully secured and a length of polythene tubing (Portex polythene No. 46, 0·8 mm external diameter) was then attached. The lymph was collected in tubes containing 20 i.u. of heparin. Collection from the peripheral lymph vessel on the side of the animal opposite the injection site was carried out since the concentration of the antibacterials in the lymph on the injected side could be influenced by drainage from the site of injection. Volumes of peripheral lymph up to 1 ml/h were collected.

(*b*) *Thoracic lymph.* A median longitudinal incision through the skin of the ventral surface of the shaved neck of the dog was made, the trachea and left carotid artery exposed, and a respiratory cannula inserted. At the junction of the subclavian, jugular and common jugular veins the anastomosis of the lymphatic system with the venous system was located. At the anastomosis, lymphatic vesels from the head and left foreleg meet together with the thoracic lymph duct, which ascends from the thoracic region and is identified as a flattened thin walled translucent vessel. The duct was carefully freed from the surrounding tissues and cannulated with a polythene cannula 1·7 mm external diameter. Lymph which flowed freely along the duct was collected in tubes containing heparin (0·05 ml, 1,000 i.u./ml). A mean thoracic lymph flow of 23·6 ml/h was produced.

Results

Protein concentration

The total protein content of serum, thoracic and peripheral lymph in g/100 ml and the albumin globulin ratios are given in Table 1. The electrophoretic studies showed that all the plasma proteins were present in the lymph, but at lower concentrations. The lymph values are in good agreement with reported results (Field, Leigh, Heim & Drinker, 1934–1935 ; Glenn, Gresson, Bauer, Goldstein, Hoffman & Healey, 1949 ; Nix, Mann, Bollman, Grindlay & Flock, 1951 ; Courtice & Morris, 1955 ; Verwey & Williams, 1962a, b).

Protein binding

The degree of binding of the antibacterial agents to serum and lymph proteins is shown in Table 2.

The binding to the serum protein decreased in the following order: phenethicillin, cloxacillin, penicillin V, chloramphenicol, penicillin G, sulphadiazine, carbenicillin and ampicillin. The amount of drug bound to protein in the serum, thoracic and peripheral lymph depended on the protein content of these fluids. The decrease

TABLE 1. *Total protein content (g/100 ml) and albumin globulin ratios of dog serum, thoracic and peripheral lymph*

Fluid	Total protein (g/100 ml)	Albumin/globulin ratio	Calculated albumin concentration (g/100 ml)
Thoracic lymph	3·1	1·3:1	1·8
Peripheral lymph	1·47	0·96:1	0·7
Serum	5·7	1:1	2·8

TABLE 2. *Percentage of drug bound to protein in serum, thoracic and peripheral lymph, as determined by ultrafiltration*

Compound	Serum	Thoracic lymph	Peripheral lymph
Phenethicillin	65·5	58·0	11·1
Cloxacillin	64·5	59·2	36·9
Penicillin V	63·9	55·7	6·3
Penicillin G	33·4	34·1	21·6
Carbenicillin	4·7	0	0
Ampicillin	3·5	0	0
Chloramphenicol	36·1	36·8	21·0
Sulphadiazine	21·7	23·0	6·4

in the binding in the peripheral lymph is therefore due to the lower protein content. The results obtained for the penicillins are in good agreement with those reported by Verwey & Williams (1962b). The percentage protein binding of sulphadiazine to dog serum is in close agreement with that reported by Anton (1960).

Concentration of antibacterial agents in plasma, peripheral and thoracic lymph

The total concentrations of the antibacterial agents appearing in the three body fluids following intramuscular dosing are shown in Table 3. The free (unbound) concentrations (Table 4) were calculated by deducting from the total concentration the fraction bound as estimated by the ultrafiltration experiments.

Since two to three animals were used for each estimation, only the mean antibacterial concentrations have been quoted. The majority of individual values were within 20% of the mean, but a few values varied by as much as 50%. Within these limits the penicillins gave similar concentration patterns in the plasma, thoracic and peripheral lymph, the peak plasma concentration occurring 0·5–2 h after dosing, and the peak lymphatic concentration occurring 1–3 h after dosing. Following the peak values the total thoracic and lymphatic concentrations for the penicillins and sulphadiazine were of the same order as the plasma. The total plasma concentration for chloramphenicol, however, was greater than the lymphatic concentration and did not follow the same pattern as that of the penicillins. The concentration with this antibiotic was still increasing 6 h after the time of administration.

The concentration of free penicillin in the lymph was generally in excess of the plasma following the peak plasma concentration. The free levels of sulphadiazine in thoracic lymph were of the same order as those found in plasma, but the free concentrations in peripheral lymph were less. The free concentrations of chloramphenicol in peripheral and thoracic lymph were less than the plasma concentration.

Discussion

Estimates of the amount of drug passing from the blood into the tissues have in many previous studies been made by determining drug concentrations in macerated organs. The results obtained by this method can be misleading, however, owing to the presence of blood in the tissue, and therefore the apparent concentration of antibiotic in the tissue can vary with the vascularity of the particular tissue. Extravascular tissue fluids are, however, difficult to obtain in sufficient quantities for assay, but Yoffey & Cortice (1956) consider that peripheral lymph, which can be obtained in adequate quantities uncontaminated by blood, is very similar to extravascular fluids. Schachter (1948) was the first to determine the concentration of penicillin G in the lymphatic system of dogs following intramuscular and intravenous administration. He found that penicillin persisted in the thoracic lymph longer than in the blood, but that the peak concentration following intramuscular dosing was less than in blood. A more recent study was made by Verwey & Williams (1962a, b), who determined the concentrations of a number of penicillins in peripheral lymph during constant intravenous infusion in dogs. These authors found that the amounts of the penicillins free in lymph were very similar to the amounts free in plasma and

TABLE 3. *Mean total concentration (μg/ml) of antibacterials appearing in plasma, thoracic and peripheral lymph of dogs (groups of two or three) following intramuscular administration of 50 mg/kg*

Fluid	Compound	Concentration (μg/ml) at hours after administration						
		0·5	1·0	2·0	3·0	4·0	5·0	6·0
Plasma	Cloxacillin	16·7	11·7	7·8	6·0	4·5	4·0	3·3
	Phenethicillin	8·0	13·2	14·0	11·0	8·8	6·7	6·2
	Penicillin V	8·7	7·0	7·2	6·5	4·3	3·2	2·7
	Penicillin G	58·7	65·8	39·2	22·5	15·7	10·5	5·0
	Carbenicillin	70·25	43·0	18·5	7·2	5·5	3·25	0
	Ampicillin	14·7	15·8	12·5	9·2	5·2	4·0	3·5
	Chloramphenicol	10·7	10·0	11·8	15·3	14·3	14·2	20·0
	Sulphadiazine	19·2	16·7	16·6	20·0	16·7	14·7	—
Peripheral lymph	Cloxacillin	0·7	3·6	6·3	4·7	2·5	2·8	1·7
	Phenethicillin	1·9	5·0	8·2	8·0	4·7	4·1	3·8
	Penicillin V	0·9	2·7	3·7	4·5	3·4	2·4	1·8
	Penicillin G	8·9	33·0	37·5	22·5	16·7	11·0	4·2
	Carbenicillin	4·5	25·5	32·5	14·7	6·9	5·8	—
	Ampicillin	4·4	9·7	10·6	8·9	8·7	5·6	—
	Chloramphenicol	2·3	6·5	6·7	6·5	7·6	11·8	14·0
	Sulphadiazine	2·0	3·0	8·0	14·0	13·0	10·0	4·0
Thoracic lymph	Cloxacillin	4·8	11·1	9·3	6·8	7·2	3·2	2·5
	Phenethicillin	0·6	5·4	11·5	13·8	11·9	7·9	7·1
	Penicillin V	1·2	7·8	10·0	6·6	5·0	3·3	1·8
	Penicillin G	23·5	46·5	31·5	27·0	18·6	11·2	6·9
	Carbenicillin	15·5	68·0	50·0	21·75	6·3	4·6	4·3
	Ampicillin	5·3	17·2	19·3	12·9	9·2	6·8	6·7
	Chloramphenicol	2·5	6·0	7·7	8·9	9·3	8·7	13·0
	Sulphadiazine	5·5	16·5	21·5	16·5	19·0	21·3	18·8

TABLE 4. *Calculated free concentrations (μg/ml) of antibacterials in dog plasma, peripheral and thoracic lymph*

Fluid	Compound	Concentration (μg/ml) at hours after administration						
		0·5	1·0	2·0	3·0	4·0	5·0	6·0
Plasma	Cloxacillin	5·9	4·2	2·8	2·1	1·6	1·4	1·2
	Phenethicillin	1·3	4·6	4·8	3·8	3·0	2·3	2·1
	Penicillin V	3·1	2·5	2·6	2·4	1·6	1·2	0·97
	Penicillin G	39·1	43·8	28·2	15·0	10·5	7·0	3·4
	Carbenicillin	66·9	40·9	17·6	6·8	5·2	3·1	0
	Ampicillin	14·2	15·2	12·1	8·9	5·0	3·9	3·4
	Chloramphenicol	6·8	6·4	7·55	9·8	9·15	9·1	12·8
	Sulphadiazine	15·0	13·1	13·0	15·7	13·1	11·5	—
Peripheral lymph	Cloxacillin	0·4	2·3	4·0	3·0	1·6	1·8	1·1
	Phenethicillin	1·7	4·4	7·3	7·1	4·2	3·6	3·4
	Penicillin V	0·8	2·5	3·5	4·2	3·2	2·2	1·7
	Penicillin G	7·0	25·9	29·4	17·6	13·1	8·6	3·3
	Carbenicillin	4·5	25·5	32·5	14·7	6·9	5·8	—
	Ampicillin	4·4	9·7	10·6	8·9	8·7	5·6	—
	Chloramphenicol	1·8	5·1	5·3	5·1	6·0	9·3	11·1
	Sulphadiazine	1·9	2·8	7·5	13·1	12·2	9·4	3·7
Thoracic lymph	Cloxacillin	2·0	4·5	3·8	2·8	2·9	1·3	1·0
	Phenethicillin	0·25	2·3	4·8	5·8	5·0	3·3	3·0
	Penicillin V	0·5	3·4	4·4	2·9	2·2	1·5	0·8
	Penicillin G	15·5	30·6	20·8	17·8	12·3	7·4	4·55
	Carbenicillin	15·5	68·0	50·0	21·75	6·3	4·6	4·3
	Ampicillin	5·3	17·2	19·3	12·9	9·2	6·8	6·7
	Chloramphenicol	1·6	3·8	4·8	5·6	5·9	5·5	8·2
	Sulphadiazine	4·2	12·7	16·55	12·7	14·6	16·4	14·5

they concluded that " the concentration of free penicillin in plasma may be a practical and conservative estimate of the free penicillin concentration in interstitial fluid ". Chisholm, Calnan & Waterworth (1968) determined the concentrations of nitrofurantoin, gentamicin and carbenicillin in plasma, thoracic and renal lymph of dogs. The thoracic lymph concentrations of carbenicillin were found to be in equilibrium with those in plasma 0·5–2 h after intramuscular administration of 20 mg/kg of the penicillin. Peak plasma concentrations were approximately twice the peak thoracic lymph concentrations. In some initial experiments with " tissue cage fluid " these authors found that carbenicillin was still present in the fluid even after the penicillin could not be detected in the plasma. In a preliminary communication (Brown, 1964) we reported on the concentrations of penicillin V, phenethicillin, cloxacillin and ampicillin appearing in the thoracic lymph and whole blood of rats following intramuscular administration of 100 mg/kg. The lymph concentrations were found to be higher than the whole blood concentrations ; however, recalculation to give the plasma concentrations, allowing for the packed cell volume of the blood, showed the concentrations in the thoracic lymph to be lower than those in plasma apart from ampicillin, for which the thoracic lymph and plasma concentrations were approximately equal. The results of our experiments in which single intramuscular injections of the antibacterial were administered to dogs are in good agreement with those of Chisholm *et al.* (1968) and Verwey & Williams (1962a, b) and indicate that penicillins, even those which are highly bound to plasma proteins, penetrate well into extravascular spaces. This observation is supported by other workers (Abraham, Chain, Fletcher, Florey, Gardner, Heatley & Jennings, 1941 ; Brown, 1964 ; Florey, Turton & Duthie, 1946 ; Jawetz, 1946 ; McCune, 1960 ; Nathanson & Liebhold, 1946 ; Ungar, 1950 ; Weinstein, Daikos & Perrin, 1951 ; Werner, Knight, McDermott, Adams & Dubois, 1954 ; White, Lee & Alverson, 1946).

Our experiments therefore indicate that penicillins penetrate readily from plasma to extravascular fluids as represented by peripheral lymph, thereby achieving good antibacterial concentrations of the free active penicillins in the tissues. The extent of binding does not appear to influence the transfer from plasma to interstitial fluid and equilibrium between free concentration in plasma and free concentration in peripheral lymph is achieved. The two non-penicillin antibacterials, sulphadiazine and chloramphenicol, do not pass so readily from plasma to extravascular fluids, possibly indicating a less readily dissociable protein/antibacterial combination.

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