PAPERS AND ORIGINALS

Concentration of Antibacterial Agents in Interstitial Tissue Fluid

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British Medical Journal, 1973, 1, 569-573

Summary

The concentration of antibacterial agents in the interstitial tissue fluid has been studied in an experimental model using implanted perforated Silastic capsules (tissue cages). Tissue fluid concentrations were always lower than the initial peak concentration in the serum, but for those drugs that were more slowly excreted the tissue fluid was similar to the serum concentration after six hours. In contrast the concentration in the tissue fluid for those drugs that are excreted rapidly was unpredictable, being either negligible or maintained at concentrations significantly better than in the serum. There was no evidence of accumulation in tissue fluid with regular dosage. These results indicate that the tissues may never be exposed to an adequate concentration of some antibacterial agents. This may be of clinical importance in tissue infections. Antibiotics which are rapidly excreted should be given in large enough doses and often enough for adequate blood levels to be maintained, so allowing equilibrium with tissue fluid to be achieved.

Introduction

Failure of treatment with any antibacterial agent may be due to the ineffective use of the drug, either because it is given in the wrong dosage or because its distribution into body fluids is inadequate. Drugs excreted by the kidney are concentrated in the urine, and it is well recognized that some of those used in

the treatment of urinary tract infections give only low blood levels. It is commonly held that urine levels reflect renal tissue concentrations, but if this is so it might reasonably be expected that the concentration of the drug in the lymph draining from the kidney would be higher than that in the serum. However, in previous studies of antibacterial agents in lymph (Chisholm *et al.*, 1968) this was found not to be so, and only nitrofurantoin, which is reabsorbed from the tubules, produced higher levels in renal lymph, while the other antibacterial drugs studied showed no significant difference between the concentration in the serum and that in lymph from different parts of the body including the kidney.

The concentration of an antibiotic in the fluids of the body is generally inferred from measurement of the drug in blood, tissue homogenates, or lymph (Stamey *et al.*, 1965; Cockett *et al.*, 1967). Some methods using radioisotope-labelled drugs show the distribution but not the concentration (Currie *et al.*, 1966), while others have assumed the concentration in fluids such as inflammatory exudates (Raeburn, 1971). The expressions "tissue fluid," "tissue levels," and "tissue distribution" are often used in a manner that implies a precision that is not justified by the techniques employed. For example, the use of a tissue homogenate to measure drug concentrations is particularly misleading because irrespective of the care taken to prevent contamination by blood or urine the homogenate remains heterogeneous. Tissues are composed of cells, blood, lymph, and interstitial fluid, with blood and lymph representing only a small proportion of the extracellular fluid.

Some workers have based their investigations on the premise that thoracic duct lymph is representative of the extracellular fluid of many tissues of the body (Lithander *et al.*, 1968), but, in fact, little is known of the relation between the drug in blood or lymph and its concentration in the largest part of the extracellular fluid—the interstitial tissue fluid.

It has recently been shown that fluid obtained from a Silastic device known as a "tissue cage" (Guyton, 1963) is tissue fluid, the equivalent of natural interstitial fluid and in direct communication with it (Calnan *et al.*, 1972 a; Calnan *et al.*, 1972 b). The lining within the cage contains young fibroblasts, many capillaries, and obvious tissue spaces. The rate of diffusion into and out of the cage fluid of small molecules, such as sodium, is rapid, whereas for larger molecules, such as albumin,

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it is slower, but the times of transfer agree with what is already known about them. The total protein in the cage fluid, initially at concentrations similar to those of serum, falls to a constant level of 40%. This level is maintained for at least a year unless infection intervenes, when it rises towards serum levels and the usual subatmospheric pressure becomes atmospheric. The concentrations of chloride and potassium in cage fluid differ from those in serum but again their relationships are constant. Methods for demonstrating the size of the extracellular fluid compartment confirm that the cage fluid is part of the compartment and that the rate of exchange of solutes is similar.

In the studies described here the concentrations of antibacterial agents in this fluid were measured, and we present the results together with a discussion of the clinical relevance.

Materials and Methods

The investigations were carried out in dogs whose weights ranged from 23.6 to 31.4 kg. Tissue cages were made from Silastic tubing 5 cm in length and closed at each end. Perforations 0.2 mm in diameter were made so that about 45% of the surface was open (fig. 1). Four to six tissue cages were placed under the abdominal and thigh skin of each dog and left in position for four weeks before use. After this time 0.5-1.0 ml of interstitial tissue fluid could be withdrawn from a cage two or three times a day using a syringe with a fine needle. Blood samples were obtained by venepuncture; the serum was separated and all specimens were frozen until assayed. Two to four studies were made with each dog to determine the reproducibility of the results.

FIG. 1—Tissue cage.

Studies were made in two groups. The first group of experiments were made with a single intramuscular dose with serial blood and cage fluid samples for up to 24 hours. Dose schedules were: sodium ampicillin 250 mg; benzathine ampicillin 250 mg; benzathine ampicillin 250 mg + sodium ampicillin 50 mg; benzathine ampicillin 600 mg + sodium ampicillin 150 mg; gentamicin 80 mg; sulphamethoxazole 500 mg; tobramycin 50 mg; and trimethoprim 200 mg. The second group of experiments were made with repeated (12-hourly) intramuscular doses for five days using a schedule approximating to the recommended clinical dose. Samples of blood and interstitial tissue fluid were taken daily immediately before the next dose was given. Dose schedules were: benzathine ampicillin 250 mg + sodium ampicillin 50 mg; benzathine ampicillin 600 mg + sodium ampicillin 150 mg; gentamicin 1.5 mg/kg; sulphamethoxazole 500 mg; tobramycin 2.5 mg/kg; and trimethoprim 200 mg.

Microbial Assays.—All drugs were assayed by the plate diffusion method using *Sarcina lutea* as the test organism for ampicillin, *Bacillus pumilis* for trimethoprim and sulphamethoxazole, and *B. subtilis* for gentamicin and tobramycin. All specimens from each experiment were assayed together, and corresponding samples of blood and cage fluid were always included on the same plate. It was found that similar results were obtained whether standards were in serum or interstitial tissue fluid. Therefore with all drugs except ampicillin all standards and any dilutions of the specimens which were required were prepared in normal horse serum. With ampicillin these were made in broth.

Results

In the first group of experiments the concentrations in serum and tissue fluid were observed during 24 hours after a single intramuscular dose. The results, shown in figs. 2 to 9, are examples of experiments that were repeated at least twice and usually three or four times. The results for each antibacterial agent were consistent.

The shape of the blood peak curve was similar for gentamicin, ampicillin, and tobramycin, with no drug detected in the serum at 12 hours. In contrast the concentration of gentamicin and ampicillin in the tissue fluid was higher than in the serum after four hours and was present beyond 12 hours. The tissue fluid level of tobramycin remained low throughout the 24-hour study.

The decline of the blood peak curve was less steep for the other antibacterial agents, and the tissue fluid concentrations for these did not reach the level in the serum until six to eight hours after administration. The slopes of the curves and concentrations after 12 hours for trimethoprim, sulphamethoxazole, and benzathine ampicillin (600 mg) plus sodium ampicillin

FIG. 2—Concentration of sodium ampicillin (250 mg I.M.) in serum and tissue fluid.

FIG. 3—Concentration of gentamicin (80 mg I.M.) in serum and tissue fluid.

FIG. 4—Concentration of tobramycin (50 mg I.M.) in serum and tissue fluid.

FIG. 5—Concentration of sulphamethoxazole (500 mg I.M.) in serum and tissue fluid.

FIG. 6—Concentration of trimethoprim (200 mg I.M.) in serum and tissue fluid.

FIG. 7—Concentration of benzathine ampicillin (250 mg I.M.) in serum and tissue fluid.

FIG. 8—Concentration of benzathine ampicillin + sodium ampicillin (250 mg + 50 mg I.M.) in serum and tissue fluid.

FIG. 9—Concentration of benzathine ampicillin + sodium ampicillin (600 mg + 150 mg I.M.) in serum and tissue fluid.

(150 mg) was similar; the drugs were measurable in both serum and tissue fluid at 24 hours. The studies with benzathine ampicillin (250 mg) plus sodium ampicillin (50 mg) showed negligible serum levels after 12 hours but tissue fluid concentrations persisted until 24 hours after administration.

In the second group of experiments the concentrations of six antibacterial agents were studied daily for five days, and the results for duplicate experiments with each drug are presented in table I. These data represent the minimum concentration during the 12-hour period after an intramuscular dose. Each of these antibacterial agents shows consistent concentration in both serum and tissue fluid, not only in individual experiments but also in the duplicate experiment. It is also evident that none of these drugs showed evidence of accumulation in either the serum or the tissue fluid.

Discussion

After parenteral administration blood levels of some drugs are known to change so quickly that it is possible that these are not always representative of either lymph or interstitial tissue fluid levels. Our results show that not only is this so but if small doses of rapidly excreted drugs are given an adequate level may never be achieved in the interstitial tissue fluid.

The blood curves obtained with the antibacterial agents used in this study fall into two classes—those which achieve a high peak in half an hour to one hour and then fall rapidly, usually to nil in eight hours, and those in which the peak is lower and the level falls only slowly over 24 hours—for example, trimethoprim, sulphamethoxazole, and high-dose benzathine ampicillin.

TABLE I—Concentrations of Antibacterial Agents in Serum and Tissue Fluid during 5-day Study with 12-hourly Drug Dosage. All Samples taken Just Before Second Dose Each Day

	Day	Experiment 1		Experiment 2	
		Serum ($\mu\text{g/ml}$)	Tissue Fluid ($\mu\text{g/ml}$)	Serum ($\mu\text{g/ml}$)	Tissue Fluid ($\mu\text{g/ml}$)
Benzathine ampicillin 250 mg + sodium ampicillin 50 mg I.M. 12-hourly	1	0.35	1.1	0.07	1.1
	2	0.23	1.9	0.48	2.3
	3	0.5	1.2	0.11	1.1
	4	0.06	1.1	0.72	1.2
	5	0.33	1.1	0.18	1.1
Benzathine ampicillin 600 mg + sodium ampicillin 150 mg I.M. 12-hourly	1	1.9	1.9	2.5	2.2
	2	1.9	2.3	2.0	2.5
	3	1.5	1.9	1.25	2.2
	4	1.65	1.65	1.0	1.5
	5	1.1	1.5	1.25	2.2
Gentamicin 60 mg I.M. 12-hourly	1	0.1	0.2	0.1	0.37
	2	0.1	0.29	0.1	0.37
	3	0.1	0.3	0.1	0.2
	4	0.1	0.29	0.1	0.29
	5	0.1	0.5	0.1	0.29
Sulphamethoxazole 500 mg I.M. 12-hourly	1	16	16	9	9
	2	40	50	40	50
	3	50	50	40	50
	4	50	50	50	50
	5	50	50	62	50
Tobramycin 2.5 mg/kg body weight I.M. 12-hourly	1	0.1	0.9	0.1	0.9
	2	0.1	1.0	0.1	0.9
	3	0.1	1.0	0.1	0.6
	4	0.1	0.8	0.1	0.66
	5	0.1	0.9	0.1	0.66
Trimethoprim 200 mg I.M. 12-hourly	1	0.9	1.5	0.6	1.3
	2	0.9	2.0	0.7	1.0
	3	1.0	2.0	0.6	1.7
	4	0.9	2.0	0.5	1.2
	5	0.66	2.0	0.5	1.1

In contrast the interstitial tissue fluid curve always produced a much lower peak, but with drugs that were excreted rapidly the curve fell more slowly and, after about four hours was higher than that in the blood, sometimes still being detectable at 24 hours. On the other hand, drugs excreted more slowly achieved an equilibrium at about four hours, and thereafter the levels fell together.

Thus in contrast to lymph the concentrations of antibacterial agents in interstitial tissue fluid are not necessarily similar to those in the serum. A variety of factors influence the distribution of these drugs—the rate of absorption, the blood peak, protein binding, the volume of distribution, the time interval between dose and specimen, and the permeability of the tissues (Orsolini, 1970). Since most of these factors are constant for each study it appears that the main explanation for the difference in concentration between interstitial tissue fluid and lymph lies in the differences in permeability between the tissue cage and the lymphatics. It is suggested that not only do the lymphatics drain the interstitial tissue fluids but their thin walls allow for rapid equilibration. By contrast the equilibration of antibacterial agents throughout the entire interstitial tissue fluid appears to be slower. It might be argued that the movement of these drugs was altered by the artificial nature of the tissue cage. However, in separate studies we have shown the very rapid transport of ^{24}Na both into and out of these cages, while the larger molecule of radioiodinated human serum albumin takes significantly longer to equilibrate (Calnan *et al.*, 1972b). Most antibacterial agents have a molecular weight of about 400, so that some delay in distribution might be expected.

The relevance of protein binding of drugs to tissue distribution and to therapeutic efficacy remains a subject for debate (Brown, 1964; Rolinson, 1967). Protein binding, usually to albumin, is a reversible process with the bound drug being inactive and relatively non-diffusible. Thus it is believed that such binding reduces the free drug available for transport into the tissue fluids, as the concentration in the tissue fluids is dependent on the levels of free drug in the blood (Rolinson, 1967). However, in our studies of the transport of radioiodinated human serum albumin into tissue cages about 30% of the final concentration in the cage was reached in six hours and 50% in 12 hours. There is therefore evidence that some protein-bound drug will enter the interstitial tissue fluid, but it seems unlikely that this factor plays any part in maintaining drug

levels. Our studies of the physiological properties of lymph have shown that while the total protein in lymph varies according to the site of sampling, the total protein in interstitial tissue fluid is constant at about 40% of that in serum (table II).

TABLE II—Comparison of Physicochemical Properties of Arterial Blood, Lymph, and Interstitial Tissue (Cage) Fluid

	Arterial Blood	Lymph	Interstitial Tissue Fluid
Mean pressure (mm Hg)	106	1-8	-6.0 to -8.0
Protein (g/100 ml)	6-7	<div> <div>1.0 (leg)</div> <div>2.3 (kidney)</div> <div>5.0 (thoracic duct)</div> </div>	2-3
Na (mEq/l.)	151	148	152
K (mEq/l.)	4-5	4-5	3-4
Cl (mEq/l.)	107	118	118
pH	7.31	7.50	7.52
O ₂ (mm Hg)	125	135	160
CO ₂ (mm Hg)	39	25	25

If protein binding is responsible for maintaining interstitial tissue fluid levels it might be expected that poorly bound antibiotics such as ampicillin (28%) and gentamicin (30%) would also have poorly maintained tissue levels. But this was not the case, and both of these drugs were shown to have interstitial tissue fluid levels higher than the blood levels after the initial peak.

Further, by including benzathine ampicillin in our studies we have shown that if a rapidly excreted drug is modified to maintain a steady blood level the interstitial tissue fluid level will approximate to it. In contrast, when repeated doses of a rapidly excreted drug were given, although a significant level of the drug remained in the interstitial tissue fluid when the next dose was given, there was no evidence of any accumulation in either the blood or the interstitial tissue fluid.

Although both gentamicin and ampicillin are rapidly excreted in the urine a variable proportion of both drugs remains unaccounted for after 12 hours. In studies at present in progress at University College Hospital it has been found that small quantities of gentamicin are still present in the urine three to four days after a single dose of 60 mg of gentamicin, and it is suggested that this prolonged excretion may be due to the slow removal of the drug from the interstitial tissue fluid.

Conclusions

It is well recognized that the distribution of antibiotics varies between species; nevertheless, we believe that our observations have several therapeutic implications.

(1) The blood peak gives little indication of the tissue peak, which was often less than 50% of the blood peak. It is possible that despite a high blood peak the tissues may never be exposed to an adequate concentration of the drug. Conversely, an antibacterial agent with a low but prolonged peak may have adequate tissue fluid concentrations. Since these concentrations are unpredictable there appears to be no substitute for direct measurement in the study of the distribution of drugs in body fluids.

(2) In order to achieve effective tissue levels in clinical practice most antibacterial agents which are excreted rapidly should be administered either frequently or in sufficiently large doses to ensure that adequate blood levels are maintained and the tissue levels equilibrate. Maintenance of adequate blood levels may be particularly important for the distribution of these drugs in diseased tissues.

(3) Many drugs have negligible blood levels although they are widely used for the treatment of urinary tract infections. Most of these drugs are excreted mainly by glomerular filtration, and the therapeutic effectiveness of such drugs is dependent on the very high drug concentrations in the urine. However, it must be emphasized that the tissue concentrations in these circumstances are, like those in the blood, also negligible. Thus in the treatment of so-called tissue infections, and

especially the tissues of the urinary tract, the choice of drug should be guided not only by the excellence of the urinary concentration but also by the ability to achieve adequate blood levels.

We wish to thank Mr. D. Martin, Miss Lynn Boardman, and Mr. L. M. Taylor for their expert technical help, and the department of medical illustration for the figures. We are grateful for the support for these investigations from Antibioticos S. A., Beecham Research Laboratories, British Schering Ltd., Eli Lilly and Co. Ltd., Roche Products Ltd., and the Wellcome Foundation.

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New Index for Evaluation of Oral Glucose Tolerance Test Results

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British Medical Journal, 1973, **1**, 573-577

Summary

A simple numerical index is presented which describes the shape of an oral glucose tolerance response curve and appears to be a measure of the efficiency of homeostatic control. It is unaffected by the laboratory method of glucose estimation, the site of blood sampling, the amount of glucose given, and whether or not the patient has fasted overnight. The index satisfactorily differentiates clinically diabetic from non-diabetic individuals and seems to provide a sensitive method of describing small changes in glucose homeostatic control such as might occur, for example, in pregnancy.

Introduction

The recommendations by authoritative bodies such as the British Diabetic Association (Fitzgerald and Keen, 1964) and W.H.O. (1965) specify arbitrary concentrations of glucose at

given times after an oral glucose load, above which the response should be regarded as "abnormal."

Apart from the fact that criteria based on absolute levels or on increments of blood glucose may require adjustment in accordance with the source of the blood and whether determinations are made on whole blood or plasma (Lind *et al.*, 1972), the essentially all-or-none nature of the most widely used criteria does not allow accurate description of relatively small changes such as might occur, for example, in normal pregnancy or after medication with, say, oral contraceptive or hypoglycaemic agents.

The total area under the curve provides one simple single figure method of describing the changes in blood glucose after an oral load. That method was used by Jarrett and Graver (1968) to describe changes occurring during the course of the menstrual cycle. The total area, however, is heavily influenced by the portion under the fasting blood glucose level so that a small change in the level has a disproportionately large effect.

Most of the body's control systems, like those in engineering, operate by means of a closed loop or "feedback" mechanism so that displacement from a preset norm, whether for temperature, pH, osmolality, or plasma glucose concentration, brings corrective measures into play which drive the value back towards the norm. When using plasma glucose as an example a rise in concentration or positive "error" invokes corrective measures including the secretion of insulin. And since no control system is perfect the concentration tends to be driven below the norm, a negative error, which in turn invokes mechanisms to send the glucose concentration up again. Even in the fasting state such "hunting" around the norm can be detected,

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