STUDIES ON THE EFFECT OF FREE FATTY ACID ON BINDING OF BENZYL-PENICILLIN BY SERUM PROTEINS

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(Received for publication January 26, 1970)

An effective level of penicillin in serum of healthy individuals is maintained for 6, 8 and 10 hours after oral administration of single doses of 260, 325 and 385 mg of potassium penicillin G respectively. Regardless of variations of doses the antibiotic peak level is achieved one hour after oral administration. Palmitic acid stimulates serum protein and albumin binding of penicillin G. The increased binding of penicillin G with whole human serum in the presence of fatty acid is due to the influence of the latter on the albumin fraction of the serum. Dexamphetamine administration to healthy individuals produces a stimulation of penicillin binding by serum protein which is due to an increase of the free fatty acid (FFA) concentration in blood.

Benzyl penicillin is the most commonly used antibiotic. It is rapidly absorbed from the gastro-intestinal tract when administered orally. Though a considerable portion of this antibiotic is lost as a result of inactivation by the acidity of the stomach, nevertheless effective serum concentration lasting for 8 hours may be achieved by oral administration of 325 mg of penicillin G as potassium salt[1]. Initial demonstration of penicillin binding effect of the serum protein by CHOW and MCKEE[2] was followed by extensive studies on plasma protein binding of the antibiotic, KUNIN[3] demonstrated that not all protein-bound drugs are active inhibitors of serum binding of penicillin. However, benzoic acid, sulphonamides and acetyl salicylic acid are active in this respect.

Palmitic acid, one of the main member of free fatty acids of the plasma, also remains bound with plasma protein, specially with albumin fraction. Since plasma proteins are able to bind both penicillin and free fatty acid simultaneously, it is worthwhile to observe the effect of addition of palmitic acid on the binding of benzyl penicillin to serum protein and serum albumin, when the antibiotic concentration is maintained at a therapeutic level. The effect of *in vivo* stimulation of free fatty acid concentration in blood on the serum protein binding of pencillin was also included in this study.

Materials and Methods

Human-plasma-albumin was purchased from Sigma Chemical Co., U. S A. Dexadrin tablets, containing 5 mg of dexamphetamine sulphate were purchased from S. K. F. Ltd., India. Cellophane dialysing tube was obtained from Viscing Corporation, Ill., U. S. A. Stanpen-500 tablets, containing 325 mg of penicillin G and as well as tablets containing

385 mg and 260 mg of potassium penicillin G were supplied by Standard Pharmaceuticals Ltd., India. The Agla-micrometer Syringe of Burroughs Wellcome, England was used in this study.

Potassium penicillin G tablets were orally administered to groups of 10 individual healthy persons after an overnight fast. Blood was drawn from the anticubital vein 30 minutes after administration of the drug and then at hourly intervals for a period of 10 hours. Serum was separated aseptically within one hour after collection of the blood and was stored at −8°C until used.

Two separate groups each of 8 individual volunteers were allowed to take a single tablet containing 325.0 mg of penicillin G. Two hours before penicillin intake, dexamphetamine sulphate was administered orally to each individual. First samples of blood from these volunteers were drawn one hour after oral administration of penicillin.

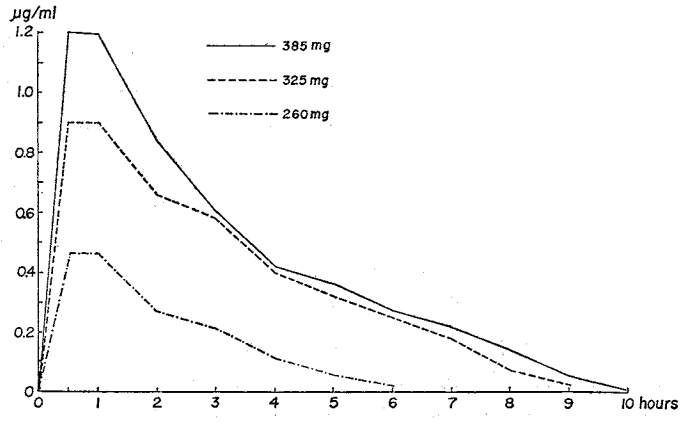
Human serum albumin solution of different concentrations were prepared in KREBS-RINGER phosphate buffer (pH 7.4) containing 3.3 μg of penicillin G per milliliter. Calculated amounts of palmitic acid, dissolved in a minimum quantity of acetone, were added gradually to 10 ml portions of the albumin solution and sterile air was passed through it continuously for a period of 20 minutes in order to remove the acetone. Equal quantities of acetone were added to the same volume of albumin solution in buffer and the operation was repeated to remove the solvent. Potassium penicillin G solution in unhemolysed human serum was prepared and a calculated amount of palmitic acid was added as described. Requisite quantities of KREBS-RINGER phosphate buffer solution were added to the albumin solutions and to the serum in order to compensate for the loss of water due to evaporation which occurred during the removal of acetone. Overall operation was conducted within 30 minutes after addition of penicillin to the respective samples at 25°C.

Penicillin content of the sample was determined following the microbial assay method[4]. Free fatty acid concentration of the sera and of the albumin solutions was measured using the method of DOLE[5]. Ultrafiltration of the serum and albumin solutions was carried out following the method of AMES and SAKANONE[6]. Protein content of the samples was determined according to FOLIN and CIOCALTEU[7].

Results and Discussion

A considerable portion of the antibiotic is absorbed through the gut when potassium penicillin G is administered orally to healthy individuals (Fig. 1). Since the effective concentration of penicillin G is exceedingly small[8] and the bactericidal property of penicillin is not increased[9] by maintaining the higher level of antibiotic concentration, a single oral dose of 325 mg of penicillin G is sufficient to maintain an effective serum level for 8 hours, provided the drug is administered at least 30 minutes before meals. After the oral administration of a single dose of 260 mg of penicillin G to healthy individuals the

Fig. 1. Effect of single dose oral administration of benzyl penicillin on serum penicillin level in healthy individuals.



effective level persist for 6 hours. The effective level is maintained for 10 hours when the dose is increased to 385 mg. Inspite of the variation of doses of penicillin, the peak blood level of the antibiotic is reached between half-and one hour after administration of the drug, indicating that maximum absorption of penicillin occurs within 1 hour after oral administration. This is in accord with a previous observation of BUNN *et al.*[1] who demonstrated that inspite of considerable destruction of penicillin G by the acidity of the stomach, an appreciable portion of the antibiotic if absorbed, producing an effective blood level over a period of 8 hours in their patients.

Table 1. Effect of *in vitro* addition of palmitic acid on the binding of benzyl penicillin with human serum and albumin solution.

Concentration of protein (mg/ml)	% Antibiotic bound			
	Human albumin	Human albumin + Palmitic acid	Human serum	Human serum + Palmitic acid
40+(8)	44.2±2.2	53.1±1.8	40.6±2.6	45.8±2.9
30+(8)	36.0±1.9	43.7±2.9	32.2±2.1	37.6±1.1
20 (7)	29.3±1.9	36.4±1.1	23.2±1.7	29.7±2.0
10 (8)	21.0±0.78	28.6±2.1	18.6±0.8	29.8±1.7

Potassium penicillin G, 3.3 μg/ml solution, was used. Free fatty acid content of the sample was initially measured and requisite quantity of palmitic acid was added to reach 1 μg eq./ml serum and albumin solutions was prepared using KREBS-RINGER phosphate buffer (pH 7.4). Figure in parenthesis represents number of experiments.

The binding of penicillin G by human plasma albumin was studied using different parameter (Table 1) of albumin concentration. When the antibiotic concentration was maintained a therapeutic level, increased binding of penicillin was noticed with an increase of the albumin concentration in solution; with a reduction of 50 % in albumin concentration, the decrease in albumin binding of penicillin is only one third. Approximately a 50 % reduction of the binding is observed when albumin concentration was reduced to a 1 % level. Addition of adequate quantity of palmitic acid to reach the concentration of 1 μg Eq. of the latter per ml of the sample, shifts the equilibrium of binding, irrespective of the concentration of albumin used. The stimulating effect of the fatty acid on the binding of penicillin with albumin is highest with low albumin concentration. When the experiment was carried out using potassium penicillin G in buffer solution along with palmitic acid, the ultrafiltration characteristic observed was similar to that when a pure penicillin solution was used. This indicates that palmitic acid dose not alter the permeability of the cellophane membrane. The influence of palmitic acid on the human albumin present in the solution is responsible for the increase of penicillin G binding.

Although serum albumin appears to be the major determinant of binding of the other types of penicillins, binding of penicillin G to albumin was found to be considerably less than binding to whole serum[3]. However, in this study, when human serum was diluted with KREBS-RINGER phosphate buffer to maintain the protein concentration at a level of 4 % and the binding capacity of this diluted serum is compared with the same level of albumin solution, a relatively greater increase in binding of penicillin in albumin is noticed. Addition of fatty acid of the diluted serum elevates the concentration of bound penicillin, but this stimulation of binding of antibiotic to serum never approached the level observed in albumin solution of equal palmitic acid concentration. Stimulation of the protein binding of penicillin is also observed in whole serum in presence of palmitic acid (Table 2). About 58 % of the total penicillin

Table 2. Effect of palmitic acid on the binding of penicillin G to undiluted human serum.

	Final FFA concentration (μg eq/ml)	% increase in serum binding of benzyl penicillin
Human serum + Palmitic acid	0.61	5.6±0.48(5)
	0.72	12.5±0.62(6)
	1.06	20.4±0.97(6)
	1.41	26.5±1.3 (5)

3.3 μg/ml of potassium penicillin was used with pooled and unhemolysed human serum. FFA content of the pooled serum was measured and a calculated amount of extra palmitic acid was added. Figure in parenthesis represents number of experiments.

Table 3. Effect of oral administration of dexamphetamine on free fatty acid and protein bound penicillin level in serum.

	Number of subject	Dose of drug (mg)	Free fatty acid concentration (μg eq/ml)	% Antibiotic bound
Control	8	—	0.482±0.04	56.8±2.4
Dexamphetamine group	7	10	0.536±0.032	62.1±2.8
Dexamphetamine group	7	20	0.684±0.05	69.3±1.8

Drug was administered 2 hours before penicillin intake. Blood wes drawn from individuals one hour after oral administration of a tablet containing 325 mg of potassium penicillin G.

remains bound to protein fractions of the serum when penicillin G is added to whole serum containing palmitic acid. Since the major part of the total penicillin represents a protein bound form in this study, significant part of this protein binding might be due to the influence of farry acids normally present in the serum. Though addition of palmitic acid to the undiluted serum, containing 3.3 mcg/ml penicillin G enhances the binding, the increment of additional antibiotic binding depends upon to concentration of palmitic acid present. When the fatty acid concentration is increased by 50 % of the original level normally present in serum, the increment of binding is about 12.5 %, while it exceeds 20 % when the fatty acid concentration was doubled. A rise of FFA concentration in plasma to this extent is not unusual in certain pathological conditions, fasting and after administration of sympathomimetic drugs.

Following administration of a single dose of 325 mg of potassium penicillin G to a group of individuals previously treated with dexamphetamine increased serum binding of penicillin is observed (Table 3). The pronounced lipid mobilising effect[10] of decamphetamine seems to be responsible for such a stimulation. The results clearly indicate that increased serum-protein-binding of the antibiotic, observed in volunteers one hour after administration of penicillin, is due to a rise of plasma FFA concentration induced by dexamphetamine sulphate, although the latter does not stimulate the binding of penicillin by serum protein when studied *in vitro*. As FFA in plasma remains bound with albumin, the change induced by FFA might primarily be on the albumin fraction. Since the binding of drugs to protein is determined by the number of binding sites and their affinity towards binding sites which in turn is determined by the dissociation of the drug-protein-combination[11], fatty acid after being bound with plasma albumin might after the dissociation of drug protein combination, thereby increasing the bound penicillin in circulating plasma. STAINLAKE *et al.*[12] also showed a similar increased binding of 11-hydroxy-steoroid by plasma protein in the presence of oxyphenylbutazone. On the contrary, acetyl-salicylic acid may release the hormone from albumin bound state. Serum protein binding appears to be one of the important determinants of distribution of drugs[13,14] in the body. The drugs which are capable of being highly bound with plasma protein, tend to remain in the intravascular compartment, while poorly bound drugs may diffuse more rapidly into interstitial fluid.

Although binding to plasma protein may delay uptake, and, metabolism of the drugs by the liver[15] and retard the rate of renal excretion[16], KUNIN[17] failed to link the mechanism of renal tubular secretion of penicillin to serum binding in rabbits.

The interference of serum protein with antimicrobial activity of penicillins[18,19,20] is well-established. ANTON[21] also demonstrated that only the unbound fraction of sulphonamides is microbiologically active. Since penicillin-serum protein binding is readily reversible, during equilibrium, only the unbound drug determines the rate of diffusion from blood vascular system to the interstitial fluid and is available for antibacterial action. Thus, in this study, instead of displacement of penicillin from protein binding sites, FFA stimulates the binding of antibiotic by plasma albumin both *in vivo* and *in vitro* resulting in reduction of the amount of penicillin available for antimicrobial effect.

Acknowledgements

The author expresses his sincere thanks to Mr. R. DUTTA and Mr. N. PAUL for their technical assistance for measurement of penicillin and ultrafiltration. Sincere thanks are due to the donors who supplied blood during the course of investigation. The author is grateful to Dr. S. MUKHERJEE, Prof. of Applied Nutrition, University of Calcutta for giving necessary permission to publish the article.

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