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Human Blood Preservation: Effect on *In Vitro* Protein Binding

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Abstract □ *In vitro* plasma protein binding for phenytoin, meperidine, and bretylium tosylate was affected by the type of preserved human blood used for its estimation. Fresh heparinized plasma and serum gave equivalent fractions bound at the concentrations studied for all three drugs. However, the *in vitro* plasma binding of phenytoin and meperidine decreased ~9–50% when estimated in fresh citrated plasma or commercially available lyophylized human serum at the concentration levels investigated. The fraction of bretylium tosylate bound to plasma protein decreased ~30–40% when estimated in fresh citrated plasma but was unchanged when estimated in the lyophylized human serum.

Keyphrases □ Binding, plasma protein—effect of blood preservation, humans □ Blood—preservation, effect on *in vitro* plasma protein binding □ Pharmacokinetics—effect of human blood preservation on *in vitro* protein binding

The influence of plasma protein binding on the distribution and elimination of drugs was recently reviewed (1). Accurate evaluation of the fraction of drug bound is essential for a thorough understanding of a drug's pharmacokinetics. Plasma/serum protein binding is normally estimated using several *in vitro* techniques such as equilibrium dialysis, ultrafiltration, gel filtration, differential spectrophotometry, and electrophoresis (2). Several investigators determined plasma protein binding in fresh plasma from subjects participating in pharmacokinetic studies (3, 4), and other sources of blood were utilized in other studies. A frequently reported source is fresh blood bank plasma (5, 6). Blood bank plasma is normally preserved with a standard anticoagulant/preservative, citrate phosphate dextrose USP. However, the presence of preservative has been implicated in lowering the protein binding of quinidine (5). Therefore, it is important that the effect of blood constituents and preservatives be evaluated.

The purpose of this investigation was to determine if a standard anticoagulant/preservative would interfere with the binding of other drugs and the suitability of a commercially available lyophilized serum[1] as a substitute for blood bank plasma when large volumes of plasma/serum are required. The drugs studied, phenytoin, meperidine, and bretylium tosylate, are chemically different and have been reported[2] to exhibit a binding of 90, 60, and 10%[2], respectively (3, 7).

EXPERIMENTAL

Blood was obtained from one healthy male volunteer, 27 years of age, who was not using any medication. Blood was collected in a clean glass flask containing either 400 IU of heparin/20 ml of blood or 63 ml of citrate phosphate dextrose/450 ml of blood. The blood to be used as serum was collected in clean glass test tubes and allowed to clot. Lyophilized serum[1], reconstituted according to the manufacturer's suggestion, was used as the commercial source of lyophilized human serum.

Table I—Effect of Blood Preservation Procedure on Fraction of Drug Bound [a]

Drug	Concentration, μg/ml	Fresh Plasma Heparin	Fresh Plasma Citrate	Fresh Serum	Lyophilized Serum
Phenytoin	15.0	0.89 (0.90, 0.88)	0.80 (0.76, 0.84)	0.89 (0.89, 0.89)	0.80 (0.79, 0.81)
	5.0	0.90 (0.89, 0.90)	0.81 (0.79, 0.83)	0.90 (0.91, 0.89)	0.80 (0.80, 0.80)
Meperidine	0.98	0.58 (0.56, 0.60)	0.25 (0.23, 0.27)	0.54 (0.52, 0.56)	0.26 (0.22, 0.30)
	0.12	0.60 (0.58, 0.62)	0.27 (0.22, 0.28)	0.58 (0.52, 0.62)	0.23 (0.18, 0.28)
Bretylium tosylate	1.2	0.13 (0.14, 0.12)	0.04 (0.04, 0.04)	0.10 (0.12, 0.09)	0.12 (0.12, 0.12)
	0.3	0.11 (0.12, 0.11)	0.06 (0.06, 0.05)	0.11 (0.10, 0.12)	0.10 (0.08, 0.12)

[a] Mean of two determinations; individual values are given in parentheses.

A standard solution of [^{14}C]meperidine, [^{14}C]bretylium tosylate, or [^{14}C]phenytoin was added to plasma at two concentrations within the respective therapeutic ranges. Protein binding was then determined by equilibrium dialysis. The samples were dialyzed across a cellophane membrane against phosphate buffer (0.067 *M*, pH 7.4) at 37° using a dialysis cell. Equilibrium was achieved for each drug by 24 hr. A control

[1] Sera Chem, Fisher Scientific, Orangeburg, NY 10962.
[2] A. Yacobi, personal communication, American Critical Care, McGaw Park, IL.

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dialysis was run at each concentration. Following equilibration, aliquots of the buffer and plasma compartments were sampled and analyzed by liquid scintillation spectrometry. Sample efficiency was estimated by external standardization.

RESULTS AND DISCUSSION

Data presented in Table I indicate agreement of the binding values in fresh serum and heparinized plasma with those reported earlier[2] (3, 7). However, the binding of all three drugs significantly decreased in the fresh blood bank plasma, thereby indicating drug displacement from binding sites by the citrate phosphate dextrose preservative. The decreased binding was not due to a change in pH since the pH was maintained at 7.4 after addition of the preservative. The binding of phenytoin and meperidine was also lowered in lyophilized serum while that of bretylium tosylate was not affected. The manufacturer reports that salicylates are added to lyophilized serum. Therefore, a possible explanation may be a change in protein conformation due to freezing and reconstitution, which would affect the more heavily bound drugs (phenytoin and meperidine) or competitive interaction with the salicylates. Bretylium tosylate would be least affected since it is only 10% bound.

Based on these results, studies done with commercial lyophilized serum should be verified by binding studies in fresh plasma/serum. Furthermore, when blood bank plasma is used for binding studies, the type of preservative used should be stated to allow rigorous evaluation of the pharmacokinetic parameters related to the free fraction and to facilitate duplication of protein binding studies.

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Acyclic Puromycin Analogs: 9-[(2-Phenylalanylamidoethoxy)methyl]adenine and 9-(3-Phenylalanylamidopropyl)adenine

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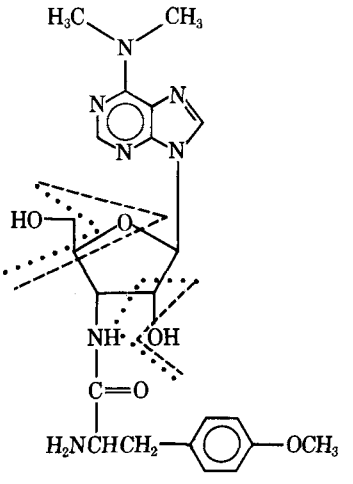
Abstract □ Two acyclic puromycin analogs, in which the 2′- and 5′-hydroxymethyl groups or the 2′-hydroxyl and 5′-hydroxyethoxy portions of the cyclic carbohydrate ring were excised, were synthesized and evaluated for inhibition of an *in vitro* protein-synthesizing system and for antiviral and antibacterial activity. No puromycin-like activity was seen with these conformationally free compounds.

Keyphrases □ Puromycin—acyclic analogs, synthesis and analysis, evaluation for inhibition of protein synthesis and antiviral and antibacterial activity □ Analogs—of puromycin, acyclic, synthesis and analysis, evaluation for inhibition of protein synthesis and antiviral and antibacterial activity □ Antineoplastics—puromycin, synthesis and analysis of acyclic analogs, evaluation for inhibition of protein synthesis and antiviral and antibacterial activity

A program initiated to synthesize nucleoside analogs, in which the cyclic carbohydrate moiety is replaced by an acyclic side chain, led to the discovery of the potent antiherpetic drug acyclovir, 9-[(2-hydroxyethoxy)methyl]-guanine[1] (1). This approach to nucleoside modification has been extended to the synthesis of two acyclic analogs of the broad spectrum antibiotic puromycin (I). This nucleoside antibiotic, which is an inhibitor of protein biosynthesis, has not been useful in the treatment of human disease because of its nephrotoxicity (2). It was shown (3–5), however, that toxicity could be alleviated by removal of the 5′-hydroxy group.

This paper reports an approach to separation of toxicity from activity involving the synthesis of distinct fragments of puromycin in which parts of the cyclic carbohydrate moiety are excised. Elimination of the 2′- and 5′-hydroxymethyl groups (dotted lines in I) or the 2′-hydroxyl and 5′-hydroxyethoxy portions (broken lines) would leave side chains as in II*a* or II*b*, respectively[2]. Both II*a* and II*b* can assume a conformation that is superimposable on puromycin, but they lack the 5′-hydroxyl group implicated in puromycin toxicity (3, 6). Open chain analogs similar to



I

[1] Zovirax.

[2] In this work, the readily available DL-phenylalanine was used since the phenylalanyl analog of puromycin is nearly as active as puromycin (7, 8). In addition, the *N* 6-methyl groups of puromycin were shown to be unnecessary for puromycin-like activity (9).

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