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PRELIMINARY COMMUNICATIONS

ABSENCE OF EFFECT OF HEPARIN ON THE BINDING OF PRAZOSIN AND PHENYTOIN TO PLASMA PROTEINS

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The effect of heparin on the binding of drugs to plasma proteins has been the subject of many studies in the recent literature (1-9). Administration of heparin has been reported to decrease (1,3-9) or occasionally increase (2) the binding of both acidic and basic drugs to plasma proteins. It has been proposed that the mechanism by which heparin alters the binding of drugs to plasma proteins is related to its effect on lipase activity in plasma. Presumably, lipases which are released and activated after the *in vivo* administration of heparin hydrolyze plasma triglycerides and produce an increase in the plasma concentrations of non-esterified fatty acids (NEFAs). The NEFAs, in turn, displace drugs from their binding sites to plasma proteins.

Recently, we have provided evidence that much of the reported increases in NEFA concentrations after administration of heparin are due to continued *in vitro* hydrolysis of plasma triglycerides (10). The true *in vivo* increases in concentrations of NEFAs following heparin administration were small (less than 20 percent) and not statistically significant from pre-heparin values. These results suggested that the reported effect of heparin on the binding of drugs might also be an *in vitro* phenomenon; therefore, the present study was designed to re-examine the question of whether administration of heparin alters the binding of drugs to plasma proteins *in vivo*. An inhibitor of lipase activity was used to separate *in vivo* from *in vitro* effects, and the binding of two model compounds (phenytoin, a weak acid, and prazosin, a weak base) was studied.

METHODS

The subjects for the study were five patients undergoing diagnostic cardiac catheterization, who routinely receive heparin during the procedure. All subjects received diazepam, 10 mg orally, just prior to catheterization. Two 20 ml blood samples were obtained from each subject. One sample was collected at the time of venipuncture, before the subject had received heparin. The second sample was collected 5 min after the intravenous administration of heparin (The Upjohn Co., Kalamazoo, MI), 46 units/kg. This is the time after which a bolus of heparin purportedly exerts a maximum effect on NEFAs and drug binding to plasma proteins (5,10). Both the pre- and post-heparin samples were collected before radiographic contrast agents were given. Each sample was divided into two tubes containing disodium EDTA (1.5 mg/ml blood) as an anticoagulant. One tube contained 3 μl of paraoxon (diethyl-p-nitrophenyl phosphate, Sigma Chemical Co., St. Louis, MO), a potent inhibitor of lipase activity, whereas the other did not (11). Blood was centrifuged at room temperature within 5 min after collection and the plasma stored at -20°.

The binding of [^{14}C]-phenytoin (sp. act. 0.185 mCi/mg, New England Nuclear Corp., Boston, MA) and [^{14}C]-prazosin (sp. act. 0.168 mCi/mg, Pfizer Inc., New York, NY) was determined separately in each plasma sample by equilibrium dialysis. Radiochemical purity of both phenytoin and prazosin was established by thin-layer chromatography (12). One ml of each plasma sample (with and without paraoxon) was dialyzed in duplicate at 37° against 1 ml of isotonic Krebs-Ringer bicarbonate buffer (pH 7.4). The buffer contained either 6.76 nCi [^{14}C]-phenytoin and 15 μg of unlabeled phenytoin or 5 nCi [^{14}C]-prazosin (30 ng). Dialysis

was carried out for 4 hr in teflon cells (Dianorm, Spectrum, Los Angeles, CA) with a cellophane membrane (Spectrapor 2, Spectrum) separating the plasma from the buffer. In preliminary experiments, the 4 hr dialysis time had been determined as the time necessary to attain equilibrium. After dialysis, aliquots of plasma and buffer were determined by liquid scintillation counting and the dpm/ml of buffer or plasma calculated using external standardization. The unbound fraction (α) was calculated by the equation, $\alpha = C_{dpm(b)}/C_{dpm(p)}$, where $C_{dpm(b)}$ and $C_{dpm(p)}$ represent the concentration of radioactivity in the buffer and plasma respectively.

After equilibrium dialysis was complete, the concentration of NEFAs was determined in each plasma sample using the method of Laurell and Tibbling (13). Statistical analysis was carried out by Student's paired t-test.

RESULTS

The unbound fractions of both drugs in plasma obtained before and after heparin administration are shown in Table 1 (phenytoin) and Table 2 (prazosin). In four of the five subjects, the unbound fractions of both prazosin and phenytoin increased after heparin administration in plasma that did not contain the inhibitor (paraoxon). In the fifth subject, the unbound fraction of both drugs did not change after administration of heparin, even when the inhibitor was not present.

TABLE 1. Unbound fraction of phenytoin in plasma obtained from five subjects before and after administration of heparin*

	Unbound fraction (α)			
	Pre-heparin		Post-heparin	
Subject	Control	Paraoxon	Control	Paraoxon
1	0.13+ (0.80)‡	0.16 (0.79)	0.24 (1.1)	0.13 (0.80)
2	0.12 (0.57)	0.12 (0.45)	0.19 (0.98)	0.12 (0.42)
3	0.14 (0.49)	0.16 (0.51)	0.23 (1.0)	0.15 (0.56)
4	0.12 (0.59)	0.14 (0.60)	0.19 (1.2)	0.13 (0.60)
5	0.13 (0.34)	0.14 (0.32)	0.14 (0.68)	0.13 (0.40)
Mean ± S.D.	0.13 ± 0.0084 (0.56 ± 0.17)	0.14 ± 0.017§ (0.53 ± 0.18)	0.20 ± 0.040‖ (0.99 ± 0.20)¶	0.13 ± 0.011 (0.56 ± 0.16)

* Subjects received 46 units/kg intravenously.

\+ Each value is the mean of duplicate determinations.

‡ Values in parentheses are concentrations of NEFAs, m-equiv./l, after equilibrium dialysis.

§ Value is significantly different from pre-heparin control ($P < 0.05$).

‖ Value is significantly different from pre-heparin control, pre-heparin paraoxon, and post-heparin paraoxon values ($P < 0.02$).

¶ Value is significantly different from pre-heparin control, pre-heparin paraoxon, and post-heparin paraoxon values ($P < 0.001$).

As is seen in Table 1, paraoxon alone produced a small but statistically significant increase in the unbound fraction of phenytoin in the plasma. The plasma which contained paraoxon turned a brighter yellow by the end of dialysis and the corresponding buffer solutions were tinged yellow. In the samples collected after heparin administration, there was no significant change in the unbound fraction of either prazosin or phenytoin compared with pre-heparin values if continued lipase activity was inhibited. In contrast, if continued in vitro hydrolysis was not inhibited, the unbound fraction of both drugs increased in four of the five patients. The average increase in the unbound fraction of phenytoin in these four patients was 62 percent when comparing pre- and post-heparin plasma without inhibitor ($P < 0.005$). For prazosin, the corresponding increase was 128 percent in plasma without paraoxon.

Corresponding concentrations of NEFAs in the dialyzed plasma samples are shown in parentheses in Tables 1 and 2. Paraoxon did not interfere with the analytical procedure for NEFAs as evidenced by comparing the concentrations of NEFAs in the pre-heparin control and the pre-heparin paraoxon-containing plasma samples. There was no significant rise in NEFA concentrations in the post-heparin samples when continued in vitro hydrolysis was inhibited. However, concentrations of NEFAs rose by an average of 99 percent in the post-heparin samples which did not contain paraoxon, and in which continued hydrolysis occurred ($P < 0.01$).

TABLE 2. Unbound fraction of prazosin in plasma obtained from five subjects before and after the administration of heparin*

	Unbound fraction (α)			
	Pre-heparin		Post-heparin	
Subject	Control	Paraoxon	Control	Paraoxon
1	0.086+ (0.66)‡	§	0.25 (1.6)	0.084 (0.64)
2	0.060 (0.66)	0.074 (0.60)	0.14 (1.1)	0.081 (0.85)
3	0.090 (0.49)	0.089 (0.52)	0.18 (1.5)	0.083 (0.54)
4	0.051 (0.59)	0.055 (0.58)	0.089 (1.3)	0.050 (0.66)
5	0.085 (0.34)	0.091 (0.34)	0.082 (0.64)	0.089 (0.42)
Mean ± S.D.	0.074 ± 0.018 (0.55 ± 0.14)	0.077 ± 0.017 (0.51 ± 0.12)	0.15 ± 0.070 (1.2 ± 0.39)‖	0.077 ± 0.016 (0.62 ± 0.16)

* Subjects received 46 units/kg heparin intravenously.

\+ Each value is the mean of duplicate determinations except for those values obtained in Subject 1.

‡ Values in parentheses are the concentrations of NEFAs, m-equiv./l, after equilibrium dialysis.

§ Sample lost.

‖ Value is significantly different from pre-heparin control ($P < 0.01$).

DISCUSSION

In contrast to a number of reports in the literature (1-9), the results of this study suggest that administration of heparin does not produce significant changes in the binding of drugs to plasma proteins *in vivo*. When continued hydrolysis of plasma triglycerides was inhibited, there was no change in the unbound fraction of either phenytoin, a weak acid, or prazosin, a weak base, after administration of heparin. If, however, no precautions were taken to stop the *in vitro* formation of NEFAs, the unbound fraction of both compounds increased concomitantly with the concentration of NEFAs. Because none of the prior investigators of heparin effects on the binding of drugs has employed an inhibitor of *in vitro* lipase activity (1-9), the results of these studies have been misinterpreted, and an *in vitro* phenomenon has been mistaken for an *in vivo* event.

This study and a previous study from this laboratory (10) confirm that the reported effect of heparin on the concentration of NEFAs is largely a result of *in vitro* hydrolysis. *In vivo*, NEFAs that are formed are rapidly removed from the plasma by tissues, whereas *in vitro* there is no rapid removal process. This study confirms the hypothesis that NEFAs can be responsible for the displacement of drugs from binding sites on plasma proteins. When continued *in vitro* lipase activity after heparin administration was inhibited, there were no significant changes over pre-heparin values in either the binding of drugs to plasma proteins or the concentration of NEFAs. Without inhibitor, both concentrations of NEFAs and unbound fractions increased. In view of the known effects of NEFAs alone on the protein binding of drugs (14,15), these findings further support a cause and effect relationship between increases in concentrations of NEFAs and decreases in the fraction of drug bound to plasma proteins.

In conclusion, this study demonstrates that, even after a substantial (46 units/kg, i.v.) dose of heparin, the binding of both prazosin and phenytoin to plasma proteins *in vivo* was not altered 5 min after heparin administration, the time at which heparin reportedly acts maximally on NEFAs and drug binding (5,10). Furthermore, the published studies which report that administration of heparin decreases the binding of many drugs to plasma proteins are probably incorrect due to substantial *in vitro* effects which have been interpreted as having occurred *in vivo*. Finally, if heparin has been administered, precautions must be taken when unbound concentrations of drugs are measured to avoid possible *in vitro* artifacts (10).

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