J. Pharm. Pharmac., 1973, **25**, 964–967 *Received August* **1**, 1973

The binding of paracetamol to plasma proteins of man and pig

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The binding of *N*-acetyl-4-aminophenol (paracetamol) to human and porcine plasma at both toxic and therapeutic concentrations was investigated by ultrafiltration and equilibrium dialysis over the range 50–300 μg ml^{-1}. Plasma protein binding occurred at paracetamol concentrations greater than 60 μg ml^{-1}. The extent of protein binding at a plasma concentration of 280 μg ml^{-1} of the drug is between 15 and 21% for both pig and man. There is no appreciable binding to erythrocytes in either species over the whole concentration range studied.

Paracetamol (*N*-acetyl-4-aminophenol) is being increasingly taken in overdosage (Clark, Thompson & others, 1973). The cause of death following the ingestion of a toxic dose is acute hepatic necrosis. Effective treatment may depend on the ability to remove the drug rapidly and efficiently from the body. One method involves the perfusion of the circulating blood through a "polyhaema" coated charcoal column (Willson, Winch & others, 1973). Although this technique enables the drug to be almost completely extracted from the blood stream of the pig, a single passage through the column giving a percentage extraction in excess of 90%, the results in man are less successful, the percentage extraction being 35%. One possible explanation of this lower extraction is a species difference in the binding of the drug to the circulating proteins. The present paper is concerned with an investigation of the binding of paracetamol to human and pig plasma *in vitro.*

MATERIALS AND METHODS

Materials

Three sources of human plasma were used. Pooled citrated plasma was obtained from the Blood Transfusion Centre, Tooting, London and was used within 7 days of collection. Samples of fresh plasma (500 ml) were obtained from patients undergoing venepuncture as a treatment for haemochromatosis, 500 I.U. of heparin being used as an anticoagulant. Heparinized blood samples (10 ml) were obtained from 10 patients who had taken toxic doses of paracetamol, ranging from 10 to 100 g. Aliquots (500 ml) of heparinized blood (1 unit ml^{-1}) were also collected from pigs anaesthetized with halothane. Plasma was separated from red cells by centrifugation at 3000 *g* for 15 min. Powdered paracetamol was a gift from Winthrop Ltd., Surbiton, Surrey and the sodium salicylate was of British Pharmacopoeial grade.

Methods

The protein binding of paracetamol was studied over a concentration range of 50 to 300 μg ml^{-1}. Equilibrium dialysis experiments were performed on plasma and whole

blood (porcine and human) for 24 h at 37° (see McArthur, Dawkins & Smith, 1971 for details). For the *in vitro* ultrafiltration experiments, 20 ml aliquots of plasma were placed in a 60 ml Amicon ultrafiltration cell fitted with an Amicon PM 10 membrane (Amicon, High Wycombe, Bucks), 40 ml of 0·9% (w/v) NaCl were added and the volume reduced by ultrafiltration at 55 lb inch^{-1} (380 mN m^{-2}) and 20° to 20 ml. This process was repeated twice. Appropriate amounts of either paracetamol or sodium salicylate were added in 5 ml of 0·9% (w/v) NaCl, equilibrated for 10 min and the volume reduced by ultrafiltration to 20 ml. The total amount of paracetamol in the ultrafiltrate (5 ml) was measured and the amount of drug remaining in the unfiltered plasma was calculated by subtraction. The concentration of paracetamol in the final ml of the ultrafiltrate was measured separately and taken as being equivalent to the free, i.e. non-protein bound, concentration of the drug in the plasma before ultrafiltration. The process was repeated on the same sample until the required drug concentration was attained in the plasma inside the ultrafiltration cell. After the final ultrafiltration, the drug concentration remaining in the cell was measured to ensure that it corresponded with the predicted value. Plasma samples from patients who had taken an overdose of paracetamol were ultrafiltered in a 10 ml Amicon ultrafiltration cell at 55 lb inch^{-1}. Paracetamol concentrations were determined in the plasma before ultrafiltration and in the ultrafiltrate. Paracetamol was estimated spectrophotometrically at 250 nm (Dordoni, Willson & others, 1973), separate calibration curves being prepared for plasma, saline and plasma ultrafiltrate. Salicylate was determined with an Aminco Bowman spectrophotofluorimeter (activation wavelength 294 nm; detection wavelength, 413 nm).

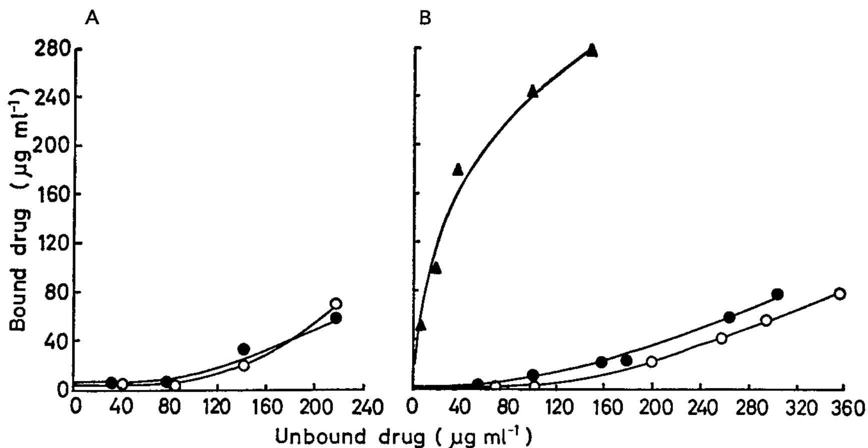

FIG. 1A. Binding curve of paracetamol to plasmas measured by equilibrium dialysis. ● Human ○ Porcine. B. Binding curve of paracetamol and salicylate to plasmas measured by ultrafiltration. ● Paracetamol to human. ○ Paracetamol to porcine. ▲ Salicylate to human.

RESULTS

Binding studies were performed on human and porcine plasma using two different techniques; equilibrium dialysis (Fig. 1A) and ultrafiltration (Fig. 1B). No binding could be detected with human plasma at paracetamol concentration levels less than 80 μg ml^{-1} by equilibrium dialysis and less than 60 μg ml^{-1} by ultrafiltration. At a free paracetamol concentration of 220 μg ml^{-1} equilibrium dialysis showed a bound

concentration of 60 μg ml^{-1} and ultrafiltration a bound concentration of 40 μg ml^{-1}. Similar results were obtained with porcine plasma. Dialysis experiments against saline showed that less than 4% of the added drug was bound to the dialysis membrane. No differences were found between stored pooled citrated human plasma and fresh heparinized human plasma.

The binding of paracetamol to whole fresh human and pig blood was also studied and there was no detectable uptake by red blood cells. To test the validity of the ultrafiltration system used, the binding of sodium salicylate to human plasma was examined and the expected degree of protein binding was observed (Fig. 1B).

The degree of protein binding of the drug in plasma samples from 10 patients who had ingested a toxic dose of paracetamol was measured by ultrafiltration. Total plasma paracetamol concentrations varied from 17 to 280 μg ml^{-1}. Five of the patients with plasma paracetamol levels less than 90 μg ml^{-1} had less than 5% of the total paracetamol bound to protein. In the remaining patients the bound concentrations varied from 8 to 43% with no apparent correlation between degree of binding and either total plasma paracetamol concentration or degree of liver damage as measured by serum bilirubin levels (Clark & others, 1973). We do not consider these data sufficient to allow any further conclusions to be drawn.

DISCUSSION

The results show that at concentrations of paracetamol (up to 60 μg ml^{-1}) equivalent to those found after normal therapeutic doses, no binding of the drug to either human or pig plasma occurs. At a much higher concentration (280 μg ml^{-1}), similar to those observed after ingestion of toxic amounts of the drug in man, the drug was bound to the extent of 15–21% to the plasma proteins of both species. No binding to red cells was observed.

There is little information available about the binding of paracetamol to blood proteins. Brodie & Axelrod (1949) reported that at tissue concentrations of 96 mg kg^{-1}, 25% of the drug was bound in dogs. Farid, Glynn & Kerr (1972), because of this finding, suggested that protein binding of the drug also occurred in man and that it would probably vary inversely with the plasma concentration. The implication is that at high plasma concentrations of paracetamol there would be a larger fraction of the drug present in the free, i.e. non-protein bound form, and hence it would be removable by haemodialysis. Gwilt, Robertson & McChesney (1963) studied the distribution of paracetamol in tissue water and plasma water; the presence of plasma proteins did not appear to affect the distribution, suggesting that any plasma protein-paracetamol interactions are very weak.

The results presented here show no protein binding at the levels used by Brodie & Axelrod (1949) but at higher concentrations a similar degree of binding was observed. The binding curve of paracetamol to human plasma (Fig. 1B) is quite different from that given by salicylate. The latter drug shows a typical saturation curve whereas the protein binding of paracetamol only increases slowly with increasing total plasma concentration. This finding suggests that, unlike salicylate, paracetamol is not strongly bound to specific sites on plasma proteins.

The similarity in the extent of binding of paracetamol to both human and porcine plasma shows that the relative failure to extract paracetamol from the blood of poisoned patients using a charcoal column is not due to a species difference in plasma binding of the drug. Other explanations must therefore be considered. The charcoal

used for haemoperfusion studies has been coated with a biocompatible material, polyhydroxyethylmethacrylate, to reduce the loss of formed elements of the blood and charcoal particle embolization, which hampered early treatment by this technique (Yatzidis, Voudiclari & others, 1965). Clearly the thickness of the membrane covering the charcoal may have an important effect on the rate of diffusion of substances to be adsorbed onto the reactive surface, and the extent of coating (%) of the charcoal was changed by the suppliers during the time when the *in vivo* pig experiments had been completed and the human experiments were in progress. This may be an explanation of the reduced extractions of drug across the column observed in later human experiments.

Acknowledgements

We wish to thank the King's College Hospital and Medical School Research Committee for financial support and the Blood Transfusion Centre, Tooting, London, S.W.17 for the pooled human plasma.

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