PLASMA PROTEIN BINDING OF AZAPROPAZONE IN PATIENTS WITH KIDNEY AND LIVER DISEASE

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1 The free fraction of azapropazone in the plasma of 37 healthy volunteers ranged from 0.0027 to 0.0070 (0.0044 ± 0.0009 , mean $\pm$ s.d.). The principal binding protein was found to be albumin.

2 In 27 patients with various degrees of renal failure the free fraction values of azapropazone were markedly enhanced (0.0260 ± 0.0239 , mean $\pm$ s.d.) and increased more than tenfold in some patients. There was a weak correlation ($r = 0.46$, $P < 0.05$) between the free fraction and the clearance of endogenous creatinine. Such correlation was not found for serum creatinine, serum albumin, serum uric acid and serum urea nitrogen.

3 In 32 patients with chronic liver disease the free fraction values of azapropazone were also markedly higher (0.0210 ± 0.0242 , mean $\pm$ s.d.) than in healthy subjects. There were statistical significant correlations between the free fraction values and the prothrombin complex activity in the plasma ($r = 0.40$, $P < 0.05$) and the total bilirubin concentration in the plasma ($r = 0.90$, $P < 0.001$), respectively. Such correlation was not found for serum albumin, serum glutamic oxalacetic transaminase, serum γ -glutamyl transpeptidase and serum alkaline phosphatase.

4 In patients with kidney and liver disease the free fraction values of azapropazone correlated well with those of the anticoagulant drug phenprocoumon ($r = 0.93$, $P < 0.001$). However, the binding of the latter drug was less impaired. Bilirubin, when added *in vitro*, displaced both drugs from plasma proteins but this displacing effect was much smaller than the binding changes observed in patients with liver disease.

5 Kidney and liver disease caused a marked impairment of the plasma protein binding of azapropazone. In patients with kidney disease the degree of impairment of azapropazone binding cannot or only poorly (creatinine clearance) be predicted from the biochemical parameters of kidney function whereas in patients with chronic liver disease the total bilirubin concentration in the plasma may serve as an index of the binding defect.

Introduction

Impaired plasma protein binding of drugs in patients with kidney and liver disease is well recognized (reviewed by Reidenberg, 1976; Jusko & Gretch, 1976; Blaschke, 1977; Tillement, Lhoste & Giudicelli, 1978). No such information is available for the antirheumatic drug azapropazone which is strongly bound to plasma proteins (Breuing, Gilfrich, Meinertz & Jähnchen, 1979). This might be of interest, since patients to be treated with that drug may suffer from kidney and liver disease. Changes in plasma protein binding could alter its disposition and efficacy per dose or total drug concentration in the plasma.

Although there is general agreement that it is primarily the protein binding of acidic drugs which decreases in kidney disease (Reidenberg, Odar-Cederlöf, von Bahr, Borgå & Sjöqvist, 1971; Andreassen, 1973; Reidenberg & Affrime, 1973) the quantitative changes in the protein binding of a particular drug of interest cannot be predicted (Yacobi & Levy, 1977). The situation seems to be even more complicated in patients with liver disease (Affrime & Reidenberg, 1975; Klotz, 1976; Perez-Mateo & Erill, 1977; Blaschke, 1977; Tillement *et al.*, 1978). Thus, it was considered necessary to study the protein binding of azapropazone in patients with impaired kidney and liver function.

Methods

Normal volunteers

Plasma was obtained from 37 healthy volunteers. Their ages ranged from 18 to 62 years. Twelve were female.

Patients with kidney disease

Twenty-seven patients were studied. Their age was between 17 and 76 years. Eight were female and 19 male. Most of these patients suffered from chronic renal failure of different aetiology. Two of them had acute renal failure due to interstitial nephritis and five of them were on haemodialysis. From the latter five patients blood was drawn a short time before dialysis. Three patients had end-stage renal failure and were considered for treatment by haemodialysis. Six patients also suffered from cardiac failure which was treated with digoxin or digitoxin. Other medications which were taken by the patients included diuretics, vitamins, antibiotics, sedatives, non-systemic antiacids and iron salts.

Patients with liver disease

Thirty-two patients were included in the study if the diagnosis of chronic liver disease was defined by biochemical parameters and by liver biopsy. Their age was between 26 and 73 years, 12 were female and 20 male. All of these patients, but two of them with chronic active hepatitis, suffered from liver cirrhosis of different aetiology. All patients had normal serum creatinine values. Drugs prescribed frequently included laevulose, lactulose, spironolactone, butizide, paromomycin, vitamins and potassium chloride.

Measurement of plasma protein binding

Plasma protein binding was determined in freshly drawn heparinized blood samples by equilibrium dialysis using custom made plexiglas cells of 1.0 ml, separated by a cellophane dialysis membrane (Union Carbide Corp., Chicago, Ill.). [14 C]-azapropazone (specific activity of 0.5 μ Ci/mg) dissolved in methanol was added to a phosphate buffer solution (0.15 M, pH 7.4) to obtain a final concentration of 75 μ g/ml. Portions of this buffer solution (0.9 ml) were dialyzed for 16 h at 37°C against the same volume of plasma. After dialysis 0.1 ml of the protein solution and 0.5 ml of the buffer solution were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer Modell 3380. All determinations were performed in duplicate.

To investigate whether a degradation product of [14 C]-azapropazone was formed during the dialysis procedure some samples of the buffer and the protein

solution were also determined by thin-layer chromatography. For this purpose the samples were extracted with methanol and the extracts were subjected to chromatogram sheets (Polygram Sil G/UV₂₅₄; Macherey-Nagel & Co., Düren, Germany). The sheets were developed in chloroform : methanol : water (75 : 24 : 1). Thereafter, the sheets were cut in strips of 1 cm and counted. Only radioactivity associated with unchanged azapropazone (R_f value of 0.77) could be detected. Moreover, the binding values determined after chromatography agreed with those determined directly.

The plasma protein binding of [3 H]-phenprocoumon was determined as described earlier (Schmidt & Jähnchen, 1979). The binding of azapropazone to albumin was determined using a commercially available human serum albumin preparation (dried, purified, electrophoretic purity 100%; Behringwerke AG, Marburg, Germany).

Results

Plasma protein binding in healthy volunteers

Plasma was prepared from heparinized blood samples. Heparin when added *in vitro* did not affect the protein binding of azapropazone (the highest concentration of heparin investigated was 75 U/ml). The frequency-distribution of azapropazone binding to plasma obtained from 37 volunteers is shown in Figure 1. The free fraction values ranged from 0.0027

Figure 1 Frequency-distribution diagram of azapropazone's free fraction values in the plasma of 37 volunteers.

Figure 2 Free fraction of azapropazone in the plasma of 27 patients with liver disease and 32 patients with kidney disease. Shaded area gives the range of the free fraction values found in 37 healthy volunteers (compare Figure 1).

Figure 3 Effect of dilution of pooled plasma with phosphate buffer solution on the free fraction values of azapropazone. Plasma obtained from three healthy volunteers was pooled.

to 0.0070. The distribution of the free fraction values seems to be unimodal with the maximum frequency between 0.0035 and 0.0045. There was no correlation between the free fraction values and either total protein concentration ($r = 0.045$, $P > 0.05$) or albumin concentration ($r = 0.050$, $P > 0.05$) in the plasma of the subjects.

When the binding of azapropazone to purified human serum albumin was investigated, free fraction values of the same order of magnitude were found as those in plasma (0.0037 and 0.0049, mean of two determinations, albumin concentrations of 5 g/100 ml and 3 g/100 ml, respectively).

The relationship between the free fraction of azapropazone and the degree of dilution of pooled plasma (obtained from three healthy subjects) is shown in Figure 3. In the plasma containing 75% of the original concentration the free fraction increased by about 20% whereas in a 50% diluted plasma the free fraction was doubled. A higher degree of dilution produced a sharp rise of the free fraction.

No significant concentration dependency of the plasma protein binding was found in the concentration range between 50 and 100 $\mu\text{g/ml}$ azapropazone. An increase of the total azapropazone concentration to 200 $\mu\text{g/ml}$ was followed by an about 50% increase of the free fraction.

Patients with kidney disease

The free fraction values of azapropazone obtained in patients with various degrees of renal failure are shown in Figure 2. There was an up to 12-fold increase in the free fraction values in these patients when compared with the upper normal range found in healthy volunteers. The degree of impairment of azapropazone binding was not statistically significant correlated to the concentration of serum albumin, serum creatinine, serum uric acid or serum urea nitrogen (Table 1). There was, however, a weak but significant correlation ($P < 0.05$) between the free fraction values of azapropazone and the clearance of endogenous creatinine (Table 1).

Patients with liver disease

In most of the patients with liver disease the binding of azapropazone was also drastically reduced (Figure 2). There was no significant correlation between the free fraction values of azapropazone and either albumin concentration, serum glutamic oxalacetic transaminase, serum alkaline phosphatase, or serum γ -glutamyl transpeptidase (Table 2). There was, however, a weak correlation between the free fraction values and the prothrombin complex activity in the plasma and a strong correlation between the free fraction values and the total bilirubin concentration (Table 2).

Table 1 Correlations between several biochemical parameters and the free fraction of azapropazone in 27 patients with different degrees of renal failure.

<i>Parameter</i>	<i>Mean</i>	<i>Range</i>	<i>Correlation coefficient</i>	<i>Statistical significance</i>	<i>Normal values</i>
Serum albumin (g/100 ml)	3.4	2.5–4.8	0.35	NS	3.5–5.0
Serum creatinine (mg/100 ml)	7.4	1.5–14.8	0.33	NS	0.7–1.4
Creatinine clearance (ml/min)	11.8	1.0–52.0	0.46	$P < 0.05$	90–140
Serum uric acid (mg/100 ml)	6.3	3.5–10.4	0.22	NS	2.6–7.8
Serum urea nitrogen (mg/100 ml)	121	58–240	0.36	NS	9–28

Comparison of the plasma protein binding of azapropazone and phenprocoumon in patients with kidney and liver diseases

In 18 patients with liver disease and 20 patients with kidney disease the plasma protein binding of azapropazone was compared with that of the anti-coagulant drug phenprocoumon (Figure 4). Both drugs are bound in the plasma of healthy volunteers to about the same extent. A linear correlation was found between the free fraction values of both drugs in patients with kidney and liver disease. However, the increase in the free fraction of azapropazone was much more pronounced than that of phenprocoumon (Figure 4). As found for azapropazone in patients with liver disease the free fraction values of phenpro-

coumon were positively correlated to the total bilirubin concentration in the plasma ($r = 0.82$; $P < 0.001$).

Effect of bilirubin on the plasma protein binding of azapropazone and phenprocoumon

When pooled human plasma was spiked with therapeutic concentrations of azapropazone (75 µg/ml) or phenprocoumon (1 µg/ml) a free fraction of about 0.003 was found for both drugs (Figure 5). The addition of bilirubin in concentrations up to 20 mg/100 ml produced an approximately linear increase of the free fraction values of both drugs (about 1.6-fold of phenprocoumon and 1.8-fold for azapropazone when 20 mg/100 ml bilirubin was added). Bilirubin

Table 2 Correlations between several biochemical parameters and the free fraction of azapropazone in 32 patients with different stages of chronic liver disease.

<i>Parameter</i>	<i>Mean</i>	<i>Range</i>	<i>Correlation coefficient</i>	<i>Statistical significance</i>	<i>Normal values</i>
Serum albumin (g/100 ml)	3.2	1.8–4.3	0.05	NS	3.5–5.0
Serum glutamic oxalo-acetic transaminase (U/l)	58	15–300	0.01	NS	5–21
Serum γ -glutamyl-transpeptidase (U/l)	91	14–227	0.15	NS	4–28
Serum alkaline phosphatase (U/l)	296	90–1692	0.03	NS	<200
Prothrombin complex activity (% of normal)	53	29–90	0.40	$P < 0.05$	85–100
Total serum bilirubin (mg/100 ml)	6.6	0.2–32.2	0.90	$P < 0.001$	<1.2

Figure 4 Correlation of the free fraction values of azapropazone and phenprocoumon in 18 patients with liver disease (●) and 20 patients with kidney disease (○). Correlation coefficient $r = 0.92$; $P < 0.001$. One patient with liver cirrhosis also had a markedly reduced kidney function (▲).

concentrations between 20 and 40 mg/100 ml produced a sharp increase of azapropazone's free fraction but a still proportional increase of phenprocoumon's free fraction.

Discussion

Azapropazone is strongly bound to plasma proteins. The free fraction values obtained in the plasma of healthy volunteers ranged from 0.0027 to 0.0070 (Figure 1). The principal binding protein seems to be albumin and analysis of the Scatchard plot revealed one high and two low affinity binding sites of the albumin molecule for azapropazone (Fehske, Jähnchen, Müller & Stillbauer, 1980). In the therapeutic plasma concentration range between 50 and 100 $\mu\text{g/ml}$ (Faust-Tinnefeldt & Geissler, 1977) there seems to be no distinct concentration-dependency of the plasma protein binding of azapropazone.

In kidney as well as in liver disease the binding of azapropazone is markedly reduced resulting in a more than tenfold increase of the free fraction values in some patients (Figure 2). The binding of the anticoagulant drug phenprocoumon, which is also strongly bound to plasma proteins, was considerably less impaired. The free fraction values of both drugs, however, were positive correlated in patients with kidney and liver disease (Figure 4). For the anticoagulant drug warfarin a similar degree of impairment of plasma protein binding (an up to 3-fold increase in the free fraction values) as found for

phenprocoumon was seen in patients with kidney disease (Sjöholm, Kober, Odar-Cederlöf & Borgå, 1976; Bachmann, Shapiro & Mackiewicz, 1976).

These quantitative differences between azapropazone and phenprocoumon in the disease-induced binding defect is surprising, since there is evidence that both drugs bind to the same binding site (i.e. warfarin site) of human serum albumin (Fehske *et al.*, 1980).

The reduced binding of azapropazone in liver and kidney disease cannot be explained only by a reduced albumin content of the plasma, since there was no significant correlation between the albumin concentration and the free fraction values (Tables 1, 2). Hypoalbuminaemia could only be one of the contributing factors, since a 1 : 2 dilution of plasma e.g. produced a twofold increase of the free fraction values (Figure 3).

In patients with kidney disease the free fraction values were not statistically significant correlated to serum creatinine, serum albumin, serum uric acid and serum urea nitrogen although there was a tendency towards a greater reduction in binding with increasing values of each of these parameters. There was, however, a significant correlation ($P < 0.05$) between

Figure 5 Free fraction values of azapropazone (○) and phenprocoumon (●) in pooled plasma before and after addition of different amounts of bilirubin. Values were determined at an azapropazone concentration of 75 $\mu\text{g/ml}$ and a phenprocoumon concentration of 1 $\mu\text{g/ml}$.

the free fraction values and the clearance of endogenous creatinine (Table 1). Thus each of the measured biochemical parameters in itself is not or only a poor indicator of the disease-induced binding effect. A lack of correlation between drug binding and creatinine serum concentrations was also seen for example in the case of thiopentone (Ghonheim & Pandya, 1975) and sulfadiazine (Boobis, 1977) while for several other drugs such correlation was apparent (Reidenberg *et al.*, 1971; Campion, 1973; Dromgoole, 1974; O'Malley, Velasco, Pruitt & McNay, 1975; Bachmann *et al.*, 1976; Gugler & Mueller, 1978). Several authors have suggested that the decrease in the plasma protein binding of acidic drugs in kidney disease is due to the accumulation of endogenous binding inhibitors (Craig, Evenson, Sarver & Wagnild, 1976; Sjöholm *et al.*, 1976; Odar-Cederlöf, 1977) which can be removed by charcoal treatment of plasma or might disappear following renal transplantation (Levy, Baliah & Procknal, 1976; Odar-Cederlöf, 1977). These binding inhibitors may also reduce the binding of azapropazone and phenprocoumon but in a quantitative different manner. There is some evidence that both drugs bind to different areas of the same binding site (Fehske *et al.*, 1980) which could explain the observed quantitative differences in the effect of disease on the binding of both drugs.

In patients with liver disease the free fraction values of azapropazone were negative correlated to the prothrombin complex activity and positive to the total bilirubin concentration in the plasma while such correlation was not found for other biochemical parameters measured (Table 2). The same was true for phenprocoumon. Binding measurements after addition of bilirubin to normal plasma indicate that

bilirubin is able to displace azapropazone and phenprocoumon from their binding sites (Figure 5). In the case of azapropazone this displacing effect was most apparent when the bilirubin concentration exceeded 20 mg/100 ml. Nevertheless, the binding changes observed *in vitro* were much smaller than those seen in patients with liver disease (Figure 2, 5). Thus the decreased binding of azapropazone and phenprocoumon in liver disease could only partially be due to displacement by bilirubin. On the other hand, bilirubin concentration in the plasma could merely serve as an index of the severity of the disease stage and may parallel other factors responsible for the binding defect. The latter assumption finds also support by the significant correlation observed between the free fraction values and the prothrombin complex activity in the plasma (Table 2).

In conclusion, azapropazone's plasma protein binding is markedly impaired in patients with kidney and liver disease. Since azapropazone is eliminated from the body partially by renal excretion of unchanged drug and partially by metabolism in the liver (Schatz, Adrian, Mixich, Molnarova, Reller & Jahn, 1970) this finding could be of clinical importance especially in such cases where in addition to the decreased protein binding a reduction of the intrinsic hepatic or renal clearance occurs. Thus it may be favourable to obtain an estimate of free drug clearance when azapropazone is used in patients with kidney and/or liver disease.

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References

- AFFRIME, M. & REIDENBERG, M.M. (1975). The protein binding of some drugs in plasma from patients with alcoholic liver disease. *Eur. J. clin. Pharmac.*, **8**, 267–269.
- ANDREASEN, F. (1973). Protein binding of drugs in plasma from patients with acute renal failure. *Acta Pharmac. Tox.*, **32**, 417–429.
- BACHMANN, K., SHAPIRO, R. & MACKIEWICZ, J. (1976). Influence of renal dysfunction on warfarin plasma protein binding. *J. clin. Pharmac.*, **16**, 468–472.
- BLASCHKE, T.F. (1977). Protein binding and kinetics of drugs in liver diseases. *Clin. Pharmacokin.*, **2**, 32–44.
- BOOBIS, S.W. (1977). Alteration of plasma albumin in relation to decreased drug binding in uremia. *Clin. Pharmac. Ther.*, **22**, 147–153.
- BREUING, K.H., GILFRICH, H.J., MEINERTZ, T. & JÄHNCHEN, E. (1979). Pharmacokinetics of azapropazone following single oral and intravenous doses. *Arzneimittel-Forsch. Drug Res.*, **29**, 971–972.
- CAMPION, D.S. (1973). Decreased drug binding by serum albumin during renal failure. *Tox. appl. Pharmac.*, **25**, 391–397.
- CRAIG, W.A., EVENSON, M.A., SARVER, K.P. & WAGNILD, Y.P. (1976). Correction of protein binding defect in uremic sera by charcoal treatment. *J. lab. clin. Med.*, **87**, 637–647.
- DROMGOOLE, S.H. (1974). The binding capacity of albumin and renal disease. *J. Pharmac. exp. Ther.*, **191**, 318–323.
- FAUST-TINNEFELDT, G. & GEISLER, H.E. (1977). Azapropazon und rheumatologische Basistherapie mit Chloroquin unter dem Aspekt der Arzneimittel-Interaktion. *Arzneimittel-Forsch. Drug Res.*, **27**, 2170–2174.
- FEHSKE, K.J., JÄHNCHEN, E., MÜLLER, W.E. & STILLBAUER, A. (1980). Azapropazone binding to human serum albumin. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **313**, 159–163.
- FENNER, H. & MIXICH, G. (1973). NMR-Untersuchungen zur Molekülstruktur von Azapropazon und Deutung

- seiner Pharmakokinetik und Biotransformation. *Arzneimittel-Forsch. Drug. Res.*, **23**, 667-669.
- GHONHEIM, M.M. & PANDYA, H. (1975). Plasma protein binding of thiopental in patients with impaired renal or hepatic function. *Anesthesiology*, **42**, 545-549.
- GUGLER, R. & MUELLER, G. (1978). Plasma protein binding of valproic acid in healthy subjects and in patients with renal disease. *Br. J. clin. Pharmacol.*, **5**, 441-446.
- JUSKO, W. & GRETCH, M. (1976). Plasma and tissue binding of drugs in pharmacokinetics. *Drug Metab. Rev.*, **5**, 43-140.
- KLOTZ, U. (1976). Pathophysiological and disease-induced changes in drug distribution volume. *Clin. Pharmacokin.*, **1**, 204-218.
- LEVY, G., BALIAH, T. & PROCKNAL, J.A. (1976). Effect of renal transplantation on protein binding of drugs in serum of donor and recipient. *Clin. Pharmacol. Ther.*, **20**, 512-516.
- ODAR-CEDERLÖF, J. (1977). Plasma protein binding of phenytoin and warfarin in patients undergoing renal transplantation. *Clin. Pharmacokin.*, **2**, 147-153.
- O'MALLEY, K., VELASCO, M., PRUITT, A.W. & MCNAY, J.L. (1975). Decreased plasma protein binding in uremia. *Clin. Pharmacol. Ther.*, **18**, 53-58.
- PEREZ-MATEO, M. & ERILL, S. (1977). Protein binding of salicylate and quinidine in plasma from patients with renal failure, chronic liver disease and chronic respiratory insufficiency. *Eur. J. clin. Pharmacol.*, **11**, 225-231.
- REIDENBERG, M.M. (1976). The binding of drugs to plasma proteins from patients with poor renal function. *Clin. Pharmacokin.*, **1**, 121-125.
- REIDENBERG, M.M. AFFRIME, M. (1973). Influence of disease on binding of drugs to plasma proteins. *Ann. N.Y. Acad. Sci.*, **226**, 115-126.
- REIDENBERG, M.M., ODAR-CEDERLÖF, J., VON BAHR, C., BORGÅ, O. & SJÖQVIST, F. (1971). Protein binding of diphenylhydantoin and desmethylinipramine in plasma from patients with poor renal function. *New Engl. J. Med.*, **285**, 264-267.
- SCHATZ, F., ADRIAN, R.W., MIXICH, G., MOLNAROVA, M., RELLER, J. & JAHN, U. (1970). Pharmakokinetische Untersuchungen mit dem Antiphlogistikum Azapropazon (Prolixan® 300) am Menschen. *Therapiewoche*, **20**, 2327-2333.
- SCHMIDT, W. & JÄHNCHEN, E. (1979). Interaction of phenylbutazone with racemic phenprocoumon and its enantiomers in rats. *J. Pharmacokin. Biopharm.*, **7**, 643-663.
- SJÖHOLM, J., KOBER, A., ODAR-CEDERLÖF, J. & BORGÅ, O. (1976). Protein binding of drugs in uremic and normal serum: The role of endogenous binding inhibitors. *Biochem. Pharmacol.*, **25**, 1205-1213.
- TILLEMENT, J.P., LHOSTE, F. & GIUDICELLI, J.F. (1978). Diseases and drug protein binding. *Clin. Pharmacokin.*, **3**, 144-154.
- YACOBI, A. & LEVY, G. (1977). Interindividual relationships between serum protein binding of drugs in normal human subjects, patients with impaired renal function, and rats. *J. pharm. Sci.*, **66**, 1285-1288.

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