α_1 -acid glycoprotein concentrations and propranolol binding in elderly patients with acute illness

J. W. PAXTON & R. H. BRIANT

Department of Pharmacology and Clinical Pharmacology, University of Auckland School of Medicine, Auckland, New Zealand

α_1 -acid glycoprotein (AAG) concentrations and propranolol binding were investigated in the serum of elderly hospitalized patients with acute illness, and healthy elderly and young subjects. Significantly greater AAG concentrations and reduced unbound propranolol fraction were observed in the elderly with acute disease compared to the elderly controls. The greatest changes (up to five-fold) occurred with cancer, with lesser changes associated with myocardial infarction and ischaemic heart disease, acute infection, heart failure, chronic obstructive respiratory disease, and cerebrovascular accident. Various miscellaneous conditions were also associated with high AAG concentrations and enhanced propranolol binding. The healthy elderly had higher AAG concentrations and lower unbound propranolol fractions than the healthy young group. Overall there was a highly significant correlation between the propranolol binding ratio (bound/free) and the serum AAG concentration. These results suggest that the elderly population may be particularly susceptible to changes in AAG concentrations, and that during acute illness interpretation of serum concentrations of drugs which bind mainly to AAG, may require knowledge of their free fractions.

Keywords α_1 -acid glycoprotein drug-binding propranolol elderly acute illness

Introduction

There is evidence that the pharmacological activity of a drug is a function of its non-protein bound (free) concentration in plasma (Booker & Darcey, 1973; Kunin *et al.*, 1973; McDevitt *et al.*, 1976; Huang & Oie, 1982). However, laboratory measurements are almost always based on total plasma concentrations. For most drugs, this consists of two fractions, a fraction free in plasma water and a fraction bound to plasma proteins. In most therapeutic situations, the free drug concentration is a relatively constant proportion of the total drug concentration. Thus, total drug concentration is a reasonable index of activity. However, increasingly situations are being recognised where the protein binding of a drug is significantly altered. This can assume major importance in evaluating

plasma drug concentrations. In addition, binding changes may affect the pharmacokinetics of a drug, leading to alterations in the duration and intensity of drug action (Woo & Greenblatt, 1979; Meffin *et al.*, 1979; Lima *et al.*, 1981).

These problems are particularly pertinent to a growing number of lipophilic cationic drugs which bind to α_1 -acid glycoprotein (AAG) in plasma (Paxton, 1983). The concentration of this acute phase reactant protein has been reported to be significantly raised and associated with enhanced drug binding in a number of conditions including inflammatory disease (Piafsky *et al.*, 1978), malignancy (Abramson, 1982) and myocardial infarction (Routledge *et al.*, 1980). Our aim was to investigate AAG concentrations in a hospitalized elderly popula-

Correspondence: Dr J. W. Paxton, Department of Pharmacology & Clinical Pharmacology, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand.

tion with acute disease, and determine the effect on the binding of propranolol.

Methods

The 53 elderly (27 male and 26 female) with acute illness were in-patients, with a mean age of 75 years (range 62–92). Their primary diagnosis is given in Table 1. The 23 healthy elderly (12 male and 11 female) were ambulatory with no obvious signs of a clinical condition, were not taking any drugs, and had a mean age of 68 years (range 60–80). The data for the young healthy group was derived from 45 samples from 29 subjects (19 male and 10 female) with a mean age of 29 years (range 17–46). None had an excessive intake of alcohol, nor were taking any drugs with the exception of six female subjects who were taking contraceptive steroids. In addition AAG concentrations were followed over a 6 month period in two young healthy males.

Blood samples (10 ml) were collected by venepuncture into plain silicone-coated Vacutainers (Becton-Dickinson) which were guaranteed free of the plasticizer, tris(2-butoxyethyl) phosphate, which has been shown to displace propranolol from AAG (Borga *et al.*, 1977; Wiegand *et al.*, 1980). This collection method did not interfere with the binding of propranolol. Serum was separated by centrifugation at 2000 r/min at 4°C, and stored at –20°C until analysed.

Propranolol binding to serum was determined in duplicate by equilibrium dialysis for 4 h at 37°C using a Dianorm Dialyser with 2 × 1 ml teflon half cells separated by a cellulose membrane (Paxton & Calder, 1983). Approximately 250 ng of ¹⁴C-propranolol was added to 1 ml serum, which was dialysed against an equal volume of isotonic phosphate buffer pH 7.4. As no significant loss of the label to the membrane or apparatus was apparent, the unbound fraction (*f*_u) was calculated by the equation suggested by Tozer *et al.* (1983):

$$f_u = \frac{D_B}{D_S - D_B}$$

where *D*_B and *D*_S are the d/min/ml in buffer after dialysis and in serum before dialysis respectively. The equation takes into account volume changes which occur due to osmotic pressure differences between the serum and buffer. The coefficient of variation (c.v.) for eight replicate analyses was 1.9%.

AAG concentrations in serum were determined by a radial immunodiffusion method using commercially available M-Partigen plates

(Behringwerke, AG). (Using a molecular weight of 42,000 for AAG, 1 g/l is equivalent to 23.81 μmol/l.) The intra- and interassay c.v. was 1.8% (*n* = 6) and 3.0% (*n* = 8), respectively.

The results are expressed as mean ± s.d. and were compared by Student's *t*-test for unpaired values.

Results

The results are shown in Table 1. There was a two-fold increase in the mean serum AAG concentration in the elderly group with acute disease compared to the healthy elderly controls. This was accompanied by a two-fold reduction in the unbound propranolol fraction. The greatest increases in AAG concentrations occurred with cancer, with myocardial infarction and ischaemic heart disease, and with acute infection. The greatest reductions in percent

Table 1 AAG serum concentrations and % unbound propranolol

Subjects	n	AAG (g/l)	% unbound
Healthy young	29	0.622 ± 0.114 (0.45–0.86)	12.9 ± 2.5 (7.1–19.0)
Healthy elderly	23	0.731 ± 0.155* (0.49 ± 1.12)	10.8 ± 2.1* (7.6 ± 14.2)
Sick elderly	53	1.500 ± 0.563** (0.54–3.15)	5.5 ± 2.1** (2.2–10.4)
<i>Primary condition</i>			
Cancer	8	2.196 ± 0.624 (1.03–3.15)	4.0 ± 2.3 (2.2–9.1)
MI and IHD	5	1.522 ± 0.708 (0.75–2.32)	5.3 ± 3.1 (2.3–10.4)
Infection	4	1.433 ± 0.646 (0.71–2.27)	5.4 ± 2.7 (3.2–9.7)
Heart failure	13	1.393 ± 0.456 (0.68–2.39)	5.8 ± 1.6 (2.9–9.6)
CORD	6	1.391 ± 0.408 (0.95–2.11)	6.8 ± 2.2 (3.9–9.8)
Cerebrovascular accident	8	1.248 ± 0.378 (1.02–2.15)	6.1 ± 1.9 (4.8–10.4)
Miscellaneous	9	1.363 ± 0.388 (0.54–1.82)	5.0 ± 1.3 (2.8–7.0)

MI and IHD – Myocardial infarction and ischaemic heart disease

CORD – Chronic obstructive respiratory disease

Results expressed as mean ± s.d. (range)

*Significantly different (*P* < 0.005) from healthy young.

**Significantly different (*P* < 0.001) from healthy elderly.

unbound propranolol were also observed in these groups. Of the four patients with acute infection as the primary condition, three had pneumonia and one, viral labyrinthitis. This latter patient had a normal AAG level (0.709 g/l) and unbound propranolol fraction (9.7%). Within the miscellaneous group, the greatest elevations of AAG concentrations and enhanced binding were observed in a male diabetic patient with some dehydration (1.821 g/l AAG concentration, 7.0%, unbound propranolol fraction), a male patient with connective tissue disease (1.650 g/l, 2.8%), a female patient with peripheral gangrene and dehydration (1.615 g/l, 5.7%) a male alcoholic (1.579 g/l, 3.1%), a male patient with vasculitis (1.440 g/l, 5.8%) and a male patient with lithium toxicity and dehydration (1.340 g/l, 5.8%).

A significant difference was observed in AAG concentrations, 1.634 ± 0.541 g/l vs 1.360 ± 0.560 g/l ($P = 0.038$), and percentage unbound propranolol, $5.0 \pm 1.8\%$ vs $6.1 \pm 2.3\%$ ($P = 0.049$) between the males and females in the elderly with acute illness. However these differences may be due to the distribution of the pathological condition rather than genuine sex differences, as no significant differences were observed between males and females in either the elderly or young control groups. However, between the healthy old and

young control groups there was a significant difference between AAG concentration ($P < 0.001$) and percent unbound propranolol ($P = 0.001$).

Over a 6-month sampling period in two young healthy male subjects the c.v. for AAG concentrations was 16.2% ($n = 8$) and 17.9% ($n = 5$) with mean values of 0.568 g/l (range 0.484–0.776) and 0.738 g/l (range 0.535–0.855), respectively.

Combination of all the data from the three groups gave a highly significant ($P < 0.001$) Spearman rank correlation coefficient ($r_s = 0.861$) between the binding ratio (bound/free) for propranolol and the serum AAG concentration (Figure 1).

Discussion

Reports on the effects of age and sex on AAG concentrations are contradictory (Blain *et al.*, 1981; Chapelle *et al.*, 1981; Denko & Gabriel, 1981). Where differences have been reported, they were relatively small compared to the intra-individual ranges which we observed over 6 months in two healthy males. We believe that much of the contradiction on the effects of age and sex on AAG concentrations arises from the relatively large within subject variability in

Figure 1 The relationship between propranolol binding ratio, bound/free (B/F) and serum AAG concentrations in all subjects (○ healthy young, △ healthy elderly, ● sick elderly). The Spearman rank correlation coefficient $r_s = 0.861$ ($P < 0.001$). The solid line represents the best fit straight line.

AAG concentrations, perhaps in relation to environmental stress or subclinical infections. In our study no sex differences in AAG concentrations were observed in the healthy subjects. A small but statistically significant increase in AAG concentrations was found in the healthy elderly compared to the young subjects but it is doubtful whether this difference has any clinical significance as it results in relatively small changes in unbound drug fraction. Whether this increase is due to age per se or perhaps to a greater incidence of subclinical infection in the elderly, is not known.

More importantly our results confirm that very significant elevations of AAG concentrations and enhanced binding of drugs, such as propranolol, likewise occur in the elderly during acute illness, and appear to be associated with a variety of diverse conditions. Altered drug binding can assume major importance in evaluating serum drug concentrations. With a change in the ratio of unbound/total drug concentration, the commonly accepted therapeutic range will not be applicable. Thus, in the sick elderly population use of total serum concentration to guide therapy for a number of basic drugs such

as propranolol, pindolol, disopyramide, erythromycin and many others which are bound by AAG (Paxton, 1983) may be misleading. Determination of the free concentration would be more appropriate in these circumstances.

The therapeutic consequences of changes in AAG concentrations and drug binding remain to be explored for most drugs. The outcome will depend on the relative affinity of the serum binding protein for the drug and the affinity of the various uptake mechanisms which the drug might encounter. Undoubtedly significant alterations in pharmacokinetics and response to some drugs may result from changes in binding (Rowland, 1984). However further research is necessary before one can confidently predict the consequences of altered binding on the bioavailability, elimination half-life, clearance and response of most drugs which bind to AAG.

The study was supported by the Medical Research Council of New Zealand. Thanks are extended to Robyn Calder for excellent technical assistance.

References

- Abramson, F. P. (1982). Methadone plasma binding: Alterations in cancer and displacement from α_1 -acid glycoprotein. *Clin. Pharmac. Ther.*, **32**, 654-658.
- Blain, P. G., Mucklow, J. C., Rawlings, M. D., Roberts, D. F., Routledge, P. A. & Shand, D. G. (1981). Determinants of plasma α_1 -acid glycoprotein concentrations in health. *Br. J. clin. Pharmac.*, **12**, 275p.
- Booker, H. E. & Darcey, B. (1973). Serum concentrations of free diphenylhydantoin and their relationship to clinical intoxication. *Epilepsia*, **14**, 177-184.
- Borga, O., Piafsky, K. M. & Nilsen, O. G. (1977). Plasma protein binding of basic drugs. I. Selective displacement from α_1 -acid glycoprotein by tris(2-butoxyethyl) phosphate. *Clin. Pharmac. Ther.*, **22**, 539-544.
- Chapelle, J. P., Albert, A., Smeets, J. P., Heusghem, G. & Kulbertus, H. E. (1981). The prognostic significance of serum α_1 -acid glycoprotein changes in acute myocardial infarction. *Clin. Chim. Acta*, **115**, 199-209.
- Denko, C. W. & Gabriel, P. (1981). Age and sex related levels of albumin, ceruloplasmin, α_1 -antitrypsin, α_1 -acid glycoprotein, and transferrin. *Ann. clin. lab. Sci.*, **11**, 63-68.
- Huang, J. D. & Oie, S. (1982). Effect of altered disopyramide binding on its pharmacologic response in rabbits. *J. Pharmac. exp. Ther.*, **223**, 469-471.
- Kunin, C. M., Craig, W. A., Kornguth, M. & Monson, R. (1973). Influence of binding on the pharmacological activity of antibiotics. *Ann. N.Y. Acad. Sci.*, **226**, 214-224.
- Lima, J. J., Boudoulas, H. & Blandford, M. (1981). Concentration-dependence of disopyramide binding to plasma protein and its influence on kinetics and dynamics. *J. Pharmac. exp. Ther.*, **219**, 741-747.
- McDevitt, D. G., Frisk-Holmber, M., Hollifield, J. W. & Shand, D. G. (1976). Plasma binding and affinity of propranolol for a beta receptor in man. *Clin. Pharmac. Ther.*, **20**, 152-157.
- Meffin, P. J., Robert, E. W., Winkle, R. A., Harapat, S., Peters, F. A. & Harrison, D. C. (1979). Role of concentration-dependent plasma protein binding in disopyramide disposition. *J. Pharmac. Biopharmac.*, **7**, 29-46.
- Paxton, J. W. (1983). α_1 -acid glycoprotein and binding of basic drugs. *Meth. Find. exp. clin. Pharmac.*, **5**, 635-648.
- Paxton, J. W. & Calder, R. L. (1983). Propranolol binding in serum: Comparison of methods and investigation of effects of drug concentration, pH, and temperature. *J. pharmac. Methods*, **10**, 1-11.
- Piafsky, R. M., Borga, O., Odar-Cederlöf, I., Johansson, C. & Sjöqvist, F. (1978). Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma α_1 -acid glycoprotein. *New Engl. J. Med.*, **299**, 1435-1439.
- Routledge, P. A., Stargel, W. W., Wagner, G. S. & Shand, D. G. (1980). Increased propranolol binding in myocardial infarction. *Br. J. clin. Pharmac.*, **9**, 438-440.

- Rowland, M. (1984). Protein binding and drug clearance. *Clin. Pharmacokin.*, **9**, (Suppl. 1), 1-7.
- Tozer, T. N., Gambertoglio, J. G., Furst, D. E., Avery, D. S. & Holford, N. H. G. (1983). Volume shifts and protein binding estimates using equilibrium dialysis. Application to prednisolone binding in man. *J. pharm. Sci.*, **72**, 1442-1447.
- Wiegand, U. W., Hintze, K. L., Slaterry, J. T. & Levy, G. (1980). Protein binding of several drugs in serum and plasma of healthy subjects. *Clin. Pharmac. Ther.*, **27**, 297-300.
- Woo, E. & Greenblatt, D. J. (1979). Pharmacokinetic and clinical implications of quinidine protein binding. *J. pharm. Sci.*, **68**, 466-470.

(Received January 26, 1984,
accepted July 18, 1984)