J. Pharm. Pharmacol. 1984, 36: 414–415
Communicated August 15, 1983

Nadolol binding to human serum proteins

L. PATEL, A. JOHNSTON*, P. TURNER, *Department of Clinical Pharmacology, St Bartholomew's Hospital, London EC1A 7BE, UK*

Nadolol protein binding was measured in serum from 95 healthy subjects. Binding was found to range between 4 and 27%, with a mean of 14 ± 4% (s.d.). There was a small but significant correlation found between the degree of nadolol binding and the serum α-1-acid glycoprotein concentration. However it was concluded that changes in the serum protein binding of nadolol were unlikely to give rise to any untoward clinical effects.

Nadolol is a non-selective β-adrenoceptor antagonist with a long half-life and possessing no partial agonist activity. In a thorough study we have investigated the extent of binding of nadolol to human serum proteins and the influence of variables such as sex, age, serum albumin concentration and α-1-acid glycoprotein concentration.

Method

Blood was collected by direct venipuncture from healthy subjects (47 male, 48 female), aged between 19–60 years. The blood samples were placed into plain glass tubes and allowed to clot before centrifugation for the removal of serum. The serum protein binding of nadolol was measured by equilibrium dialysis. Two 1 ml aliquots of the serum were added to acrylic dialysis cells and dialysed against an equal volume of isotonic buffered saline at pH 7·4 (Dulbeco A with mineral salts, Oxoid Ltd) to which 500 ng ml^{-1} ^{14}C-labelled nadolol (Squibb, E. R. & Sons Ltd, 99% radiochemically pure by tlc) was added. The cells were separated by 11·5 μm cuprophan membranes and rotated at 45 rev min^{-1}, to ensure constant mixing, for 4 h in an oven at 37 °C. The time to reach equilibrium was established from a preliminary experiment in which nine cells were set up and the serum of one individual was used. Cells were then removed from the oven after 15, 30 and 45 min and 1, 2, 3, 4, 6 and 8 h. After dialysis, two 600 μl aliquots from each side of the cell were added to polythene vials, containing 4 ml Pico Fluor TM 30 scintillant (Packard), and the radioactivity measured using a Packard Prias PLD counter with automatic external standard correction. The quench curve was established using a known concentration of nadolol in the scintillant mixture and varying picric acid concentrations were used to produce colour quenching. The percentage of free drug in serum was calculated as the ratio of the absolute disintegration rates in buffer to those in serum multiplied by 100, and the percentage of bound drug as 100 minus the percentage of free drug. Measurement of serum α-1-acid glycoprotein was using commercial radial immunodiffusion plates (BCL, Lewes, UK). Serum albumin was measured by bromocresol dye binding (Robertson 1981) and total serum protein was determined colourimetrically using Biuret reagent (Gornall et al 1949). Statistical analysis was carried out with Hewlett Packard Basic statistics and data manipulation and Regression Analysis software (Hewlett Packard, Winnersh, UK).

* Correspondence.

Results

Our data shows that the average amount of nadolol bound was 14% but there was considerable interindividual variation as the binding ranged from 3·5 to 27%. The statistical summary of the variables in the 95 volunteers is shown in Table 1. Multiple linear regression of these data yielded the correlation matrix seen in Table 2. Stepwise regression demonstrated a statistically significant relationship between nadolol binding and serum α-1-acid glycoprotein concentration ($P < 0·01$). The serum albumin concentration and the subject's age and sex did not influence nadolol binding significantly.

Table 1. Statistical summary of the data, from the serum of 95 healthy individuals, used in the multiple regression analysis.

Variable	Mean ± s.d.	Range
Fraction nadolol bound	0·14 ± 0·04	0·035– 0·27
α-1-acid glycoprotein (g $litre^{-1}$)	0·88 ± 0·31	0·28 – 1·89
Albumin (g $litre^{-1}$)	41·1 ± 3·0	35·0 –47·9
Age (years)	42·0 (median)	19·4 –62·4
Total protein (g $litre^{-1}$)	64·2 ± 5·5	52·1 –77·4

Table 2. Correlation matrix of nadolol binding with age, sex and total protein. Values are the regression coefficients r for each individual correlation. * Significant at $P < 0·01$.

	Nadolol binding	Total protein	α-AG	Albumin	Sex (M = 0, F = 1)
Age	0·13	−0·43	0·41*	0·59*	−0·04
Sex	−0·18	0·03	0·27	−0·10	
Albumin	−0·01	0·73*	−0·37*		
α-AG	0·30*	−0·15			
Total Protein	0·03				

Discussion

This study has shown that nadolol binding, in common with other basic drugs, is dependent on the circulating levels of serum α-1 acid glycoprotein (Piafsky 1980). However this factor alone explains only a small portion, <10% ($r^2 = 0.09$), of the variance in nadolol binding. It is therefore unlikely that in the variety of diseases associated with increased α-1-acid glycoprotein, nadolol binding would be changed to a clinically relevent extent. In any case the degree to which nadolol is bound to serum proteins is so small that even total displacement of the drug from its binding sites or a doubling of the drugs binding would have minimal clinical effect.

REFERENCES

Gornall, A. G., Bardawill, C. J., David, M. M. (1949) J. Biol. Chem. 177: 751–758
Piafsky, K. M. (1980) Clin. Pharmacokinet. 5: 246–262
Robertson, W. S. (1981) Clin. Chem. 27: 144–146

J. Pharm. Pharmacol. 1984, 36: 415–416
Communicated November 30, 1983

The pharmacokinetic profile of carbidopa in dogs

R. OBACH*, A. MENARGUES, J. M. VALLÈS, *Department of Pharmacology, S.A. Lasa Laboratorios, Barcelona, Spain*

A pharmacokinetic study of carbidopa in beagle dogs has been carried out after intravenous (4 mg kg^{-1}) and oral (75 mg) administration. An open model of three compartments was the best approach for the pharmacokinetic profile of carbidopa administered intravenously. The estimated biological $t_{\frac{1}{2}}$ was 5 h and the plasma clearance 0·0053 (litre kg^{-1}) min^{-1}. The oral absorption of carbidopa was almost complete and the absolute bioavailability (F) was 88%.

Carbidopa (L-(−)-α-hydroazino-3,4-dihydroxy-α-methylhydrocinnamic acid monohydrate) is an aromatic amino acid decarboxylase inhibitor (Porter et al 1962) widely used in association with L-dopa in the therapy of Parkinson's disease (Calne et al 1971). The drug is also used in association with L-5-hydroxytryptophan for the treatment of myoclonus (Chadwick et al 1975) and certain types of depression (Praag 1981). Although the disposition and metabolism of carbidopa in several animal species are known (Vickers et al 1974; 1975) there are not, to our knowledge, published studies on the pharmacokinetic profile of this drug. We report here the pharmacokinetic profile of carbidopa after intravenous and oral administration in the beagle dog.

Materials and methods

Four male beagle dogs (11–16 kg) were used. Carbidopa (Synthesized by LASA Laboratorios. Span. Pat. 486030 and 493201) was administered intravenously at a fixed dose of 4 mg kg^{-1} and, by mouth, using tablets of 75 mg manufactured in our laboratories for the purpose of this study. The time between the i.v. and oral administration was a week. Blood (4 ml) was withdrawn from a cephalic vein immediately before the administration of carbidopa (blank sample) and at different times up to 10 h after the drug administration.

The plasma was immediately separated and stored at −20 °C until analysis. The determination of carbidopa in plasma was by a spectrophotofluorimetric method (Vickers & Stuart 1973). Plasma blank and samples were evaluated using an internal standard calibration curve for each administration. Two and three compartment models were fitted to the plasma concentrations obtained after the intravenous administration of carbidopa with a weighted nonlinear least square regression method that uses Marquardt's algorithm. The data were weighted according to equation 1

$$W_i = \frac{1}{y_i^2} \qquad (1)$$

where W_i is the weight and y_i is the value of the i^{th} observation. In order to determine the simplest exponential equation consistent with the data, the 'F' test proposed by Boxenbaum et al (1974) was used. In the same way a biexponential equation was fitted to the plasma concentrations obtained after the oral administration.

The values of AUC (area under curve) were calculated from the fitted equations and the bioavailability (F) of carbidopa was assessed from equation 2

$$F = \frac{AUC_{oral}}{AUC_{i.v.}} \times \frac{D_{i.v.}}{D_{oral}} \qquad (2)$$

where $D_{i.v.}$ is the i.v. administered dose and D_{oral} the corresponding oral dose.

Results and discussion

An open model of three compartments (lowest F value 19·9 $P < 0.05$) was the best approach for the pharmacokinetic profile of carbidopa administered intravenously (4 mg kg^{-1}) to the beagle dog. According to this model the relevant pharmacokinetic parameters (mean values ± s.e.m., n = 4) are summarized in Table 1.

The biological half-life ($t_{1/2}$, π) of carbidopa was 5 h and the volume of distribution in the central compartment (Vc) was 0·17 (litre kg^{-1}) min^{-1}. The total plasma clearance was 0·0053 litre kg^{-1}. The pharmacokinetic parameters obtained after the oral administration of carbidopa are shown in Table 2. The absorption of

* Correspondence. Departamento de Farmacología, S.A. LASA Laboratorios, c/ Laureà Miró 385, Sant Feliu de Llobregat, Barcelona, Spain.