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Determination of idazoxan in plasma by radioreceptor assay*

A. C. LANE, J. D. NICHOLS, N. D. STEEL, K. SUGDEN† and D. S. WALTER

Reckitt and Colman plc, Pharmaceutical Division, Dansom Lane, Kingston Upon Hull, HU8 7DS, UK

Abstract: A radioreceptor assay to determine the plasma concentration of idazoxan, a potent, highly selective antagonist for the α_2-adrenoreceptor, is described. The assay is based upon a technique in which plasma extracts containing idazoxan compete with radiolabelled ligand for binding sites on receptor-rich tissue prepared from beef brain cortex. Using a logistic data-fit the limit of detection is of the order of 1 ng ml^{-1} and represents a 10-fold increase in sensitivity over that from an established HPLC procedure. Comparison of human plasma data from the two assays indicates a correlation coefficient of 0.92 (N = 27) although the chromatographic method gave consistently higher values than the binding assay. The binding assay requires no sample extraction or pretreatment of plasma and its accuracy, precision and inherent specificity are such that the method represents a useful alternative to HPLC for therapeutic drug monitoring.

Keywords: *Radioreceptor assay, idazoxan, method development, HPLC.*

Introduction

Radioligand binding is a well used technique in molecular pharmacology for studying the distribution and concentration of receptor binding sites in biological tissues. It has also been used recently to screen and select from a series of novel benzodioxan analogues which were shown to contain potent and selective α_2-adrenoreceptor antagonists [1]. Using both pharmacological [2] and radioligand binding screens [3], idazoxan (2-(1,4-benzodioxan-2-yl)-2-imidazoline) was chosen for development.

The technique of radioligand binding is now being adapted to assay blood and tissue concentrations of novel receptor-specific drugs [4] and several methods have recently been published [5–15]. Like immunoassays, the radioreceptor technique offers increased specificity and sensitivity over conventional chromatographic techniques. This report describes the development of a radioreceptor assay for idazoxan and compares it with an established HPLC assay procedure.

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†To whom all correspondence should be addressed.

Experimental

Preparation of receptor tissue

Beef brain was obtained from freshly slaughtered animals at the local abattoir and placed on ice. Within 30 min of receipt the cerebral cortex was dissected from the brain, the surface blood vessels removed and the wet tissue weighed. Each 100 g of cerebral cortex was homogenised in 500 ml of ice cold 50 mM Tris-HCl buffer pH 7.8 using a Silverson homogeniser, duration 15 s. The homogenate was strained through two layers of cheese-cloth to remove large particles and the filtrate centrifuged at 50,000 **g** for 10 min. Pellets were resuspended in 50 mM Tris-HCl buffer pH 7.8 (2.5 ml per g wet weight of original), combined and homogenised for a duration of 5 s and the homogenate centrifuged as before. Pellets were resuspended in 50 mM Tris-HCl buffer pH 7.8 (2.0 ml per g wet weight of original), combined and homogenised for a duration of 5 s. This preparation was dispensed into 4 ml aliquots and stored at −20°C until required.

Binding assay

A 4 ml portion of tissue preparation was thawed, mixed with 20 ml ice-cold 0.1 M sodium tricine buffer, pH 7.8 and homogenised using a Teflon-glass homogeniser with nine strokes of the pestle rotating at 500 rpm. The homogenate was diluted with a further 55 ml of the sodium tricine buffer and equilibrated in a water bath at 25°C for 5 min.

The binding assay was carried out in triplicate in 2 ml plastic tubes to which 20 μl of ^{3}H-idazoxan (4 mM), 10 μl of buffer or standard or saturating ligand, 200 μl of control or test plasma and 970 μl of brain homogenate were successively added. The contents were mixed briefly in a vortex mixer, incubated at 25°C for 15 min and then rapidly filtered under vacuum through 2.5 cm Whatman GF/B glass fibre filters mounted on a Millipore 12-place manifold. The filters were rapidly washed with 3 × 4 ml of ice-cold 50 mM Tris-HCl buffer pH 7.8 and transferred to small vials to which was added 4 ml of ES299 scintillant (United Technologies Packard). Radioactivity on the filters was counted in a Packard 4530 scintillation counter at around 45% counting efficiency.

In each assay the amount of non-specific binding to the brain membrane preparation was determined as that occurring in the presence of a saturating concentration (20 μM) of RX811031 (−)-(2-(2-isopropenyl-1,4-benzodioxan-2-yl)-2-imidazoline), a specific α_2-adrenoceptor antagonist [16].

Calibration of the assay

Routinely, a calibration curve was included comprising eight standards with final concentrations of idazoxan ranging between 0.7 and 25.8 ng ml^{-1}. In addition, quality control samples spiked with 6.3, 12.6 and 22.05 ng ml^{-1} of idazoxan were routinely assayed. The calibration line was calculated from a 4-parameter logistic fit of the data using a standard radioimmunoassay programme (SecuRia, Packard Instruments) run on an IBM Personal Computer.

Saturation binding experiments

The number of binding sites on the beef cortex membrane preparation and the affinity of the radioligand for the receptor population was determined using 10 concentrations of idazoxan over the range 1–20 nM (final concentration). Each concentration was assayed in triplicate in the presence or absence of 20 μM RX811031, the saturating ligand. Binding data were analysed by the Hanes plot to determine the B_{max} and K_D values.

High performance liquid chromatography

The HPLC procedure for the determination of idazoxan in plasma has been published elsewhere [17].

Results and Discussion

The specific binding of ^{3}H-idazoxan to the beef brain receptor preparation was saturable. Hanes analysis showed that the K_D for binding of idazoxan at equilibrium was 2.5 nM and the B_{max} was 189 fmol mg^{-1} protein (13.1 p mol g^{-1} original wet weight) (Fig. 1).

A typical logistic curve (% radioactivity bound versus log drug concentration) showing concentration dependent inhibition of binding is given in Fig. 2. Calibration curves were constructed for each assay and a working linear range of 1–25 ng ml^{-1} was selected for routine use. Interassay accuracy and precision, determined by monitoring spiked control plasma for drug content was satisfactory (Table 1). Plasma was assayed without any pretreatment or extraction, but addition of plasma volumes greater than 200 µl caused some problems with recovery.

To evaluate the utility of the assay in pharmacokinetic studies with idazoxan values obtained from test plasma samples, obtained following single oral administration (60 mg, $N = 5$) to humans, were compared with those found by the established HPLC procedure.

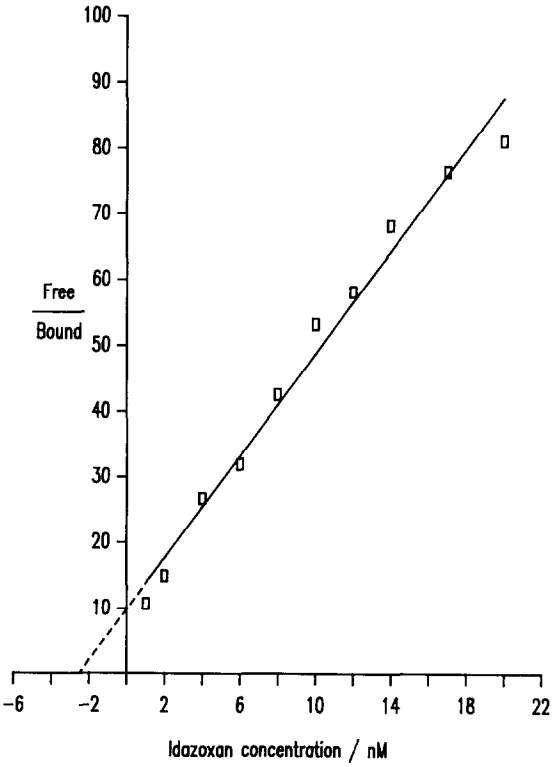

Figure 1
Hanes analysis of idazoxan binding data.

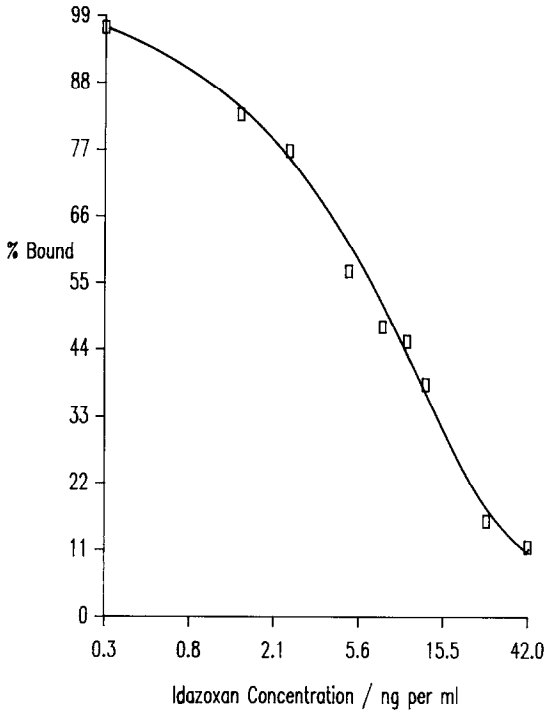

Figure 2
Logistic calibration curve of idazoxan binding to the receptor preparation.

Table 1
Accuracy and precision of the radioreceptor binding assay using control plasma spiked with idazoxan

Nominal concentration ng ml^{-1}	Number of assays	Concentration found ng ml^{-1} ± SEM
6.3	7	6.6 ± 0.5
12.6	6	11.9 ± 0.9
22.1	6	22.2 ± 1.1

Mean plasma profiles from each of the two procedures are illustrated in Fig. 3 and the corresponding pharmacokinetic parameters calculated from the individual data are given in Table 2. The correlation coefficient between results from the binding and HPLC assays was satisfactory ($r = 0.92$, $N = 27$) (Fig. 4), although higher values from the HPLC procedure were consistently observed. This in turn led to significant differences between the two assays in the maximum plasma concentration (C_{max}) found and the areas under the plasma concentration/time profile ($AUC_{0-\infty}$). No significant difference was observed, however, in the time taken to achieve maximum plasma concentration nor in the plasma half-life. The reason for higher plasma assay values from the HPLC procedure remains unclear. The receptor binding assay is inherently more specific than the HPLC procedure.

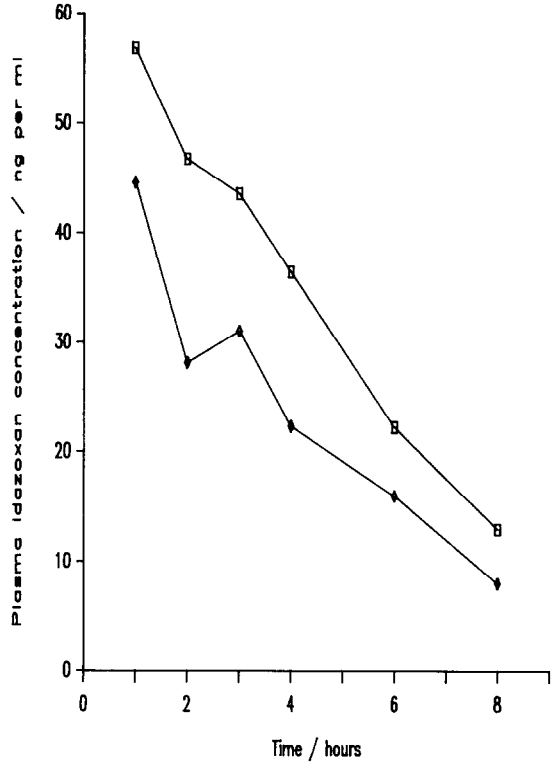

Figure 3
Mean human plasma profiles ($N = 5$) using the receptor binding (◊) and HPLC assays (□).

Table 2
Comparison of idazoxan pharmacokinetic parameters derived from the two assays ($N = 5$)

Parameter	Binding assay	HPLC assay	*P Value
t_{max} (h)	2.2 (±0.5)	1.8 (±0.4)	NS
C_{max} (ng ml^{-1})	38.8 (±5.5)	55.5 (±7.6)	<0.01
$t_{½}$ (h)	2.9 (±0.5)	3.1 (±0.2)	NS
$AUC_{0-\infty}$ (ng ml^{-1} hr)	218 (±23)	319 (±36)	<0.02

*Paired t-test of individual data points.

An advantage of the binding assay is that the detection limit is approximately ten-fold lower than by HPLC and as such it offers the real advantage of allowing monitoring to proceed with much lower plasma levels and hence over a lengthened period of time.

In conclusion, a radioreceptor binding assay for the novel α_2-adrenoreceptor antagonist idazoxan has been compared with an established HPLC assay for the compound. There was good agreement between the levels found in the two assays except that those from HPLC were slightly higher than those from the binding assay. The binding assay has a significantly lower detection limit.

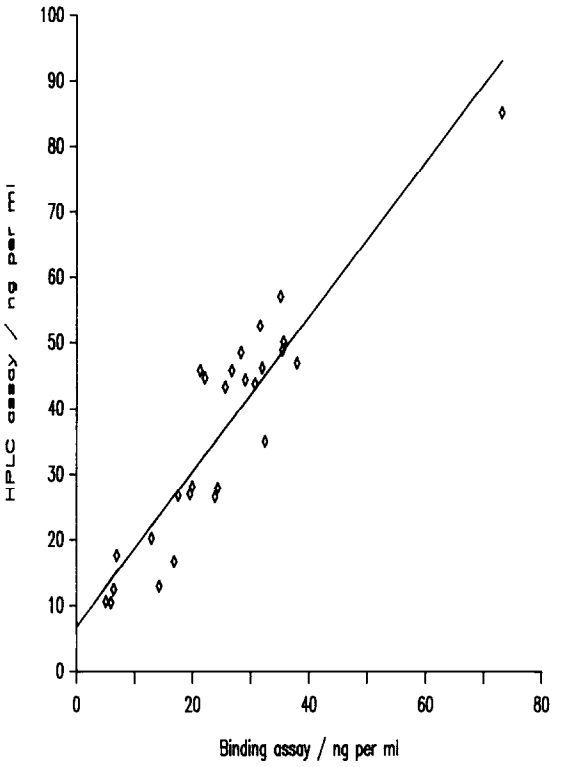

Figure 4
Correlation between the receptor binding and HPLC assays.

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