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Determination of α_1-adrenoceptor antagonists in plasma by radioreceptor assay

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Abstract

A simple, rapid and sensitive radioreceptor assay (RRA) for the quantification of α_1-adrenoceptor antagonists such as prazosin in plasma is described. The method involves the use of an RRA based on [^{3}H]prazosin displacement in rat cerebral cortical membranes. The method is reliable, with intra-assay and inter-assay RSDs ranging from 5.9 to 9.2%. The limit of detection is 0.2 (prazosin hydrochloride), 0.05 (tamsulosin hydrochloride) and 0.3 (bunazosin hydrochloride) pmol per assay. Using this method the plasma levels of prazosin hydrochloride were determined in beagle dogs administered orally 2.39 μmol kg^{-1} of this drug. The plasma levels of prazosin in beagle dogs are in good agreement with those obtained using a high-performance liquid chromatography (HPLC). This RRA proved to be applicable to the monitoring of plasma prazosin levels in patients with essential hypertension and/or benign prostatic hypertrophy receiving therapy with this drug with the therapeutic dosage schedule. Thus, the concentrations of α_1-adrenoceptor antagonists in plasma can be adequately monitored by RRA as well as by HPLC.

Keywords: α_1-Adrenoceptor antagonists; Plasma; Radioreceptor assay; [^{3}H]Prazosin

1. Introduction

The direct radioreceptor assay (RRA) technique for the determination of receptors for neurotransmitters and hormones is well established in the study of neurotransmitters and their functions, and it is also useful for the determination of drug concentrations in biological fluids by the competitive inhibition of the specific binding of radioligands by administered drugs [1–5]. Thus, RRA has been applied to measurements of β-adrenoceptor antagonists [6,7], neuroleptics [8–10], benzodiazepines [11,12], digoxin [13], cholinergic antagonists [14], calcium channel antagonists [15–17] and α_2-adrenoceptor antagonist [18], but not α_1-adrenoceptor antagonists. RRA possesses the advantages over chemical detection that it measures the total biological activity of a sample and that it is simple, specific and sensitive [1–5]. Depending on the class of compounds under assay, a meaningful correlation can be derived between plasma concentrations and the therapeutic effect elicited. In fact, this assay technique is useful for optimizing the treatment of patients with psychotic disorders who have been treated

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with neuroleptics [19–21] and also to analyse systematic toxicological effects of antihistaminics, anticholinergics and benzodiazepines in humans [3].

Currently, α_1-adrenoceptor antagonists such as prazosin hydrochloride are becoming clinically important agents in the treatment of bladder outlet obstruction in men with symptomatic benign prostatic hypertrophy (BPH) and in the treatment of essential hypertension [22–25]. However, these drugs frequently produce orthostatic hypotension and dizziness as adverse effects. Thus, a simple assay procedure reflecting the biological activities of α_1-adrenoceptor antagonists in human plasma samples may be advantageous for the establishment of appropriate dosage schedules for these patients. The concentrations of these α_1-adrenoceptor antagonists in biological fluids have been determined mainly by high-performance liquid chromatography (HPLC) [26–29]. This paper reports a simple and highly sensitive RRA procedure using [^{3}H]prazosin with high specific activity for the determination of prazosin and other α_1-adrenoceptor antagonists in plasma.

2. Materials and Methods

2.1. Materials

[^{3}H]Prazosin (87.0 Ci mmol^{-1}; radiochemical purity 99%) was purchased from Dupont–NEN (Boston, MA, USA). Prazosin hydrochloride, bunazosin hydrochloride and tamsulosin hydrochloride were kindly donated by Pfizer Pharmaceutical (Tokyo, Japan), Eizai (Tokyo, Japan) and Yamanouchi Pharmaceutical (Tokyo, Japan), respectively. All other chemicals were obtained from commercial sources. For the determination of blood levels, adult beagle dogs weighing 9–11 kg were fasted overnight and given prazosin hydrochloride (2.39 μmol kg^{-1}) orally as a solution. After 0.5–8 h, blood samples were taken from the forearm (antecubital) vein with a heparinized syringe. Blood samples were also taken from 12 patients with BPH receiving 1.79–3.59 μmol day^{-1} of prazosin hydrochloride. The plasma was separated by centrifugation.

2.2. Extraction

Prazosin was extracted from plasma by a modification of the method described by Twomey and Hobbs [28]. Two volumes of methanol were added to 1 volume of the plasma sample (200 μl). After stirring, the mixture was centrifuged at 15 000g for 15 min. The supernatant was transferred to a glass centrifuge tube by decantation, and 0.01 N NaOH (1 ml) and diethyl ether (2 ml) were added and vortex mixed for 1 min. After a brief centrifugation, the diethyl ether was transferred to a glass assay tube and evaporated under a stream of nitrogen. The residue was dissolved in 50 μl of methanol and used as a radioreceptor sample. To obtain a standard curve, 50 μl of drug solution were added to the drug-free plasma. The deproteinization and extraction procedures were performed as described above. The determination of bunazosin hydrochloride and tamsulosin hydrochloride was performed as described for prazosin hydrochloride.

2.3. Receptor preparation

Rat cerebral cortical membranes, which are rich in α_1-adrenoceptors [30], were prepared and utilized as a source of α_1-adrenoceptors. The cerebral cortex from male Wistar rats weighing 250–300 g was homogenized in 10 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 40 000g for 15 min at 4°C and the pellet was suspended in the original volume of Tris–HCl buffer. It was recentrifuged at 40 000g for 15 min at 4°C, and the resulting pellet was finally resuspended in the original volume buffer. Aliquots were kept at −60°C until RRA for no more than 10 days. After thawing, the homogenate suspension was diluted with Tris–HCl buffer and used in the [^{3}H]prazosin binding assay.

2.4. Binding assay

The binding assay for [^{3}H]prazosin was performed according to the method of Yamada et al. [31]. The assay was performed in a total volume of 1 ml, adding rat cerebral cortical membranes

(100–200 μg of protein per assay) to test-tubes containing [^{3}H]prazosin (final concentration 0.2 nM) and "plasma extract" (dissolved in methanol) in 50 mM Tris–HCl buffer (pH 7.4). After 60 min of incubation at 25°C, the reaction was terminated by rapid filtration (Cell Harvester; Brandel, Gaithersburg, MD, USA) through Whatman GF/B glass-fiber filters, and the filters were rinsed three times with 3 ml of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid (2 l of toluene, 1 l of Triton X-100, 15 g of 2,5-diphenyloxazole, 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene) and the radioactivity was determined in a liquid scintillation counter. Specific [^{3}H]prazosin binding was determined experimentally from the difference between counts in the absence and counts in the presence of 3 μM phentolamine. The counts of [^{3}H]prazosin used, total binding and non-specific binding in rat cerebral cortical membranes under these analytical conditions were 37776 ± 3230, 5271 ± 202 and 348 ± 24 dpm ($n = 3$), respectively.

2.5. Determination of prazosin by HPLC

The plasma concentration of prazosin was determined by HPLC as previously described [28]. Two volumes of methanol were added to plasma samples containing 25 ng of trimazosin hydrochloride as internal standard. After being stirred, the mixture was centrifuged at 15 000*g* for 5 min. The supernatant was transferred to a glass tube by decantation, 0.01 N NaOH and diethyl ether were added and the mixture was vortex mixed vigorously. After brief centrifugation, the diethyl ether was transferred to a glass tube and evaporated under a stream of nitrogen. The dry residue was dissolved in 100 μl mobile phase and used as the HPLC sample.

The HPLC system was constructed with a pump (CCPP; Tosoh, Tokyo, Japan) and a fluorescence detector (FP-210; Japan Spectroscopic, Jasco, Tokyo, Japan). The analysis was performed on an ODS analytical column (25 cm × 4.6 mm i.d.) of 5 μm particle size (Finepak SIL C18-5; Jasco). The ODS guard column had dimensions 5 cm × 4.6 mm i.d. (Finepak SIL C18-5; Jasco). The mobile phase for assay consisted of methanol–water–PIC B-5 (Waters, Milford, MA, USA) (456:24:1, v/v) at a flow rate of 1.0 ml min^{-1}. The column eluate was monitored fluorimetrically at excitation and emission wavelengths of 340 and 405 nm, respectively. The limit of detection was 0.5 pmol ml^{-1} of prazosin hydrochloride in plasma.

2.6. Data analysis

The plasma concentration of prazosin after oral administration was determined in beagle dogs according to the one-compartment open model with a first-order absorption process. Data were fitted by the non-linear least-squares regression program MULTI [32]. The area under the plasma concentration–time curve ($AUC_{0\rightarrow\infty}$) after oral administration was calculated by the trapezoidal rule for the observed values and extrapolation to infinity. The total plasma clearance (Cl) was calculated using the equation $Cl = \text{dose}/AUC_{0\rightarrow\infty}$.

3. Results and discussion

The direct addition of dog plasma (30–500 μl) to the assay tube was shown to reduce specific [^{3}H]prazosin binding to rat cerebral cortical membranes by 20–70% in proportion to the amount of plasma. The methanol deproteinization of plasma (500 μl) and the diethyl ether extraction under alkaline conditions markedly reduced the inhibitory effect of plasma itself on specific [^{3}H]prazosin binding, yielding 95.5 ± 8.5% ($n = 5$) of the specific binding in the absence of plasma extract.

The reliability of RRA for prazosin was evaluated by testing its linearity over a range of concentrations from 0.1 to 30 pmol of prazosin hydrochloride per assay in the presence of dog blank plasma (100 μl). As shown in Fig. 1, a linear correlation between the logit of the percentage inhibition of specific [^{3}H]prazosin binding and the logarithm of the dog plasma prazosin concentration was observed. Similar linear graphs on a logit–log scale were obtained in the presence of human plasma (data not shown). The lower limit

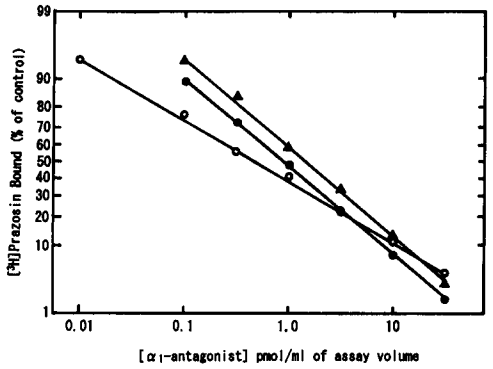

Fig. 1. Standard curves (logit–log scale) in the radioreceptor assay of tamsulosin hydrochloride (○), prazosin hydrochloride (●) and bunazosin hydrochloride (▲). Differential concentrations of tamsulosin hydrochloride, prazosin hydrochloride and bunazosin hydrochloride were added to beagle dog blank plasma and the extraction and radioreceptor assay of these drugs were performed as described under Materials and Methods. The points represent a typical standard curve for each drug, and each curve fits a linear equation. Similar standard curves were obtained in three to four experiments.

of detection by this method, defined as the amount of unlabelled prazosin hydrochloride displacing approximately 15% of the control [^{3}H]prazosin binding, was about 0.2 pmol (84 pg) per assay (0.4 pmol ml^{-1} plasma). When highly concentrated samples were used, the amount of plasma could be decreased. The standard curves for other α_1-adrenoceptor antagonists, bunazosin hydrochloride (0.1–30 pmol per assay) and tamsulosin hydrochloride (0.01–30 pmol per assay), which are now used clinically, were linear in the presence of dog and human plasma. The detection limits of bunazosin hydrochloride and tamsulosin hydrochloride were approximately 0.3 pmol per assay (0.6 pmol ml^{-1} plasma) and 0.05 pmol per assay (0.1 pmol ml^{-1} plasma), respectively.

To examine the reproducibility and reliability of the RRA procedure, two different concentrations of prazosin hydrochloride (1.0 and 10.0 pmol ml^{-1}) were added to the dog plasma. The relative standard deviations (RSDs) for these concentrations of prazosin hydrochloride were intra-assay 9.1% and 9.2%, respectively, and inter-assay 5.9% and 6.3%, respectively (Table 1).

Following the oral administration of prazosin hydrochloride at a dose of 2.39 μmol kg^{-1} to beagle dogs, it was rapidly absorbed; the plasma concentration attained the maximum level at 1 h, and thereafter it gradually disappeared. The pharmacokinetic parameters derived from the non-linear least-squares regression program MULTI are shown in Table 2. The plasma samples (0.5–8 h) from beagle dogs administered orally prazosin hydrochloride (2.39 μmol kg^{-1}) were also analysed by HPLC, and the values were compared with those measured by the RRA method for the same samples. As shown in Fig. 2, there was good agreement between the values for prazosin given by the two assay procedures (correlation coefficient $r = 0.93$). This finding suggests that active metabolites of prazosin either are not produced or do not accumulate to significant levels in beagle dogs.

In the present study, the sensitivity of the RRA procedure for prazosin has been shown to be significantly enhanced by the extraction and concentration steps in the plasma. The direct inhibitory effect of plasma on specific [^{3}H]prazosin binding was almost excluded by the methanol deproteinization and subsequent diethyl ether extraction under alkaline conditions as a pretreatment of plasma. Also, the sensitivity of

Table 1
Precision of the radioreceptor assay for prazosin hydrochloride

Added (pmol ml^{-1})	Intra-assay		Inter-assay	
	Found (pmol ml^{-1}) [a]	RSD (%) [b]	Found (pmol ml^{-1}) [a]	RSD (%) [b]
1.00	1.00 ± 0.09	9.1	0.95 ± 0.06	5.9
10.0	9.50 ± 0.87	9.2	9.12 ± 0.57	6.3

[a] Mean ± SD of five determinations.
[b] RSD = relative standard deviation.

Table 2
Pharmacokinetic parameters calculated from plasma concentration of prazosin hydrochloride after oral administration to beagle dogs

K_a (h^{-1}) [a]	K_{el} (h^{-1}) [a]	Cl ($L\ h^{-1}\ kg^{-1}$) [a]	$AUC_{0\rightarrow\infty}$ ($pmol\ h\ ml^{-1}$) [a]
5.89 ± 3.27	0.19 ± 0.06	1.62 ± 0.22	1494 ± 292

[a] Mean ± SD for three beagle dogs. K_a = absorption rate constant; K_{el} = elimination rate constant; Cl = total plasma clearance; $AUC_{0\rightarrow\infty}$ = area under the plasma concentration–time curve.

measurement by RRA depends on the binding affinity of radioligands and drugs for the receptor. A higher sensitivity and accuracy of measurements of drugs by RRA could be obtained by using selective radioligands with a higher affinity. [^{3}H]Prazosin has been utilized as a selective radioligand to measure the affinity and density of α_1-adrenoceptors in a number of human and experimental animal tissues by RRA. The present study has demonstrated that this radioligand is also useful for the determination of prazosin and possibly other α_1-adrenoceptor antagonists in plasma. The determination of prazosin by the RRA procedure is considerably sensitive and reproducible. It was reported previously that the detection limit of prazosin hydrochloride by HPLC was 0.2–0.5 ng ml^{-1} in plasma [28,29]. In fact, the sensitivity of the present RRA procedure for prazosin is at least 2–3 times greater than that of HPLC procedures, and it might be further enhanced by the use of larger volumes of plasma samples. In addition, the intra- and inter-assay relative standard deviations were low (<10%). Consequently, the RRA developed in the present study is a simple and sensitive procedure for the determination of α_1-antagonists in biological fluids. In particular, this method is faster than HPLC. The assay technique can be applied to the measurement of prazosin, bunazosin and tamsulosin in human plasma. In fact, this RRA proved to be useful in monitoring plasma prazosin levels 0.5–8 h later in 12 patients with BPH receiving therapy with this drug at oral doses of 1.79–3.59 μmol day^{-1}.

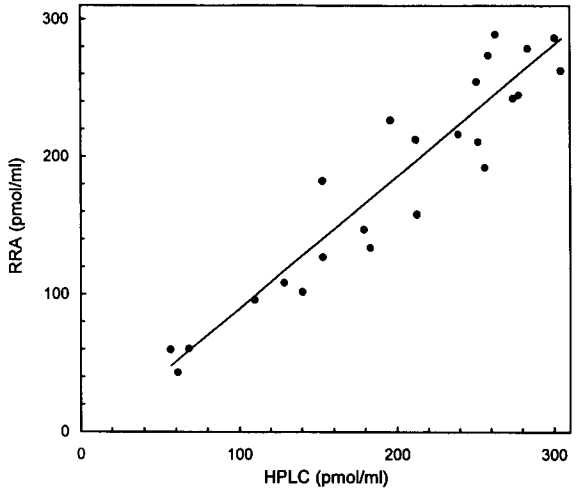

Fig. 2. Correlation between plasma concentrations of prazosin determined by radioreceptor assay (RRA) and high-performance liquid chromatography (HPLC) in beagle dogs. Plasma samples taken from the forearm vein 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after oral administration of prazosin hydrochloride (2.39 μmol kg^{-1}) to beagle dogs were analysed for prazosin by both procedures. Each point represents a single data pair from three dogs. The values fit the linear equation $y = 0.96x - 6.49$ ($r = 0.93$).

The major advantage of RRA in drug estimation is that this assay is essentially a "bioassay", sensitive to all active drug species, both metabolites and the parent compound, in proportion to their affinities at the specific receptor. Accordingly, it is expected that the plasma concentration of drugs determined by this method reflect well their pharmacological or clinical effects in vivo. A gradual increase in dosage is clinically required for α_1-antagonists until tolerance to these drugs occurs. Thus, the most appropriate dosage schedules could be determined, based on the plasma concentrations correlating with pharmacological or clinical effects. Hence, the assay procedure of α_1-adrenoceptor antagonists in plasma by RRA may represent a useful alternative to HPLC for therapeutic drug monitoring of these drugs in patients with essential hypertension and/or BPH.

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