# $^{125}$I-labelled Rose Bengal in the quantitative estimation of fazadinium and other quaternary ammonium compounds in biological fluids

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In the original Rose Bengal ion-complexing method of Cohen (1963) for the estimation of bis-quaternary ammonium compounds such as fazadinium, the drug interacts with Rose Bengal to form an ion-pair complex which is readily extractable into an organic solvent and whose fluorescence is measurable. The fluorescent complex is not stable though this can be improved by the use of ethanol in the assay (Watson & McLeod 1977), and difficulties may be encountered when drug concentrations are low, or with small volumes of biological fluids. The instability can be overcome by replacing the dye as the complexing agent by $^{125}$I-labelled Rose Bengal which allows the complex to be determined by scintillation counting.

*Materials.* [$^{125}$I] Rose Bengal (specific activity 0·7mCi mg$^{-1}$) was purchased as a sterile aqueous solution of its sodium salt from Radiochemical Centre, Amersham, U.K. For the assay, this was diluted with carrier Rose Bengal to provide a stock solution of the dye 100 μg ml$^{-1}$. Fazadinium bromide (Fazadon) m.p. 215 °C (uncorr) was a gift from Allen & Hanburys Research Ltd., Ware, Herts., U.K.

*Assay of fazadinium in human plasma.* For calibration, an aqueous solution of fazadinium bromide was added to plasma samples to provide concentrations from 0·02–100 μg ml$^{-1}$. To each sample (0·5 ml in duplicate) in a 15 ml stoppered extraction tube was added 0·2 M phosphate buffer pH 7·8 (1 ml) and [$^{125}$I] Rose Bengal solution (0·5 ml containing approx. $2 \times 10^6$ d min$^{-1}$), then the solutions were mixed by vortexing for 30 s, extracted with chloroform (5 ml) for 15 min on a rotary mixer to avoid emulsions and centrifuged at 2500 rev min$^{-1}$ for 10 min. The upper aqueous layer was removed with a Pasteur pipette attached to a water pump and aliquots ($2 \times 1$ ml) of the chloroform extract transferred by automatic pipette to a 5 ml scintillation counter insert tube. The chloroform was then evaporated off at room temperature with nitrogen, the whole process taking about 10–15 min. The residue was then dissolved in 4 ml of scintillation fluid (Bray 1960) and the $^{125}$I determined using a Packard Tri-Carb liquid Scintillation Spectrometer (Model 3385). The efficiency of counting was about 80%. Blank plasma samples (without added fazadinium) were similarly treated for estimation of background extractable $^{125}$I. The calibration curve is linear over a concentration range of 0·01–10 μg, with a correlation coefficient of 0·99. The reproducibility of the assay over this concentration range was ±5% (n = 5).

Fazadinium in plasma samples from patients, was measured by taking three aliquots (0·5 ml) of each sample through the above procedure and determining the concentration of drug by reference to the calibration curve.

The drug was not appreciably bound to red blood cells, since addition of known concentrations (0·01–50 μg ml$^{-1}$) to whole blood gave a recovery of 89% (range 85–102) from plasma. The assay appears to measure only the unbound fraction of the drug in plasma, since after ultrafiltration of plasma to which fazadinium had been added, the ultrafiltrate contained the same amount of the drug as was measured by assay of the whole plasma.

Table 1 shows that the ability to form a chloroform-soluble ion-pair complex with Rose Bengal is not restricted to fazadinium. Although the simple mono-quaternary ammonium compounds, tetraethylammonium, neostigmine and MIP did not complex, higher

Table 1. Formation by some quaternary ammonium compounds of chloroform-soluble ion-pair complexes with [$^{125}$I] Rose Bengal. 1 μg of each drug was added as an aqueous solution of the appropriate salt to 0·2 M phosphate buffer pH 7·8 (1 ml) then complexed and treated as described in the text.

| Compound | Mol. wt of cation | Chloroform-extractable $^{125}$I (counts min$^{-1}$) formed from interaction of the compounds (1 μg) with [$^{125}$I] Rose Bengal |
|---|---|---|
| Mono-quaternary ammonium | | |
| Tetraethylammonium | 136 | 0 |
| 3-Methyl-3-phenylimidazole (1, 2a)pyridinium (MIP) | 210 | 24 |
| Neostigmine | 223 | 0 |
| Dibenzyldimethyl-ammonium | 226 | 7720 |
| Cetyltrimethylammonium | 285 | 29 180 |
| Tribenzylmethyl-ammonium | 302 | 6900 |
| Bis-quaternary ammonium | | |
| Succinylcholine | 290 | 0 |
| Decamethonium | 258 | 0 |
| Paraquat | 186 | 9020 |
| Diquat | 184 | 3750 |
| Fazadinium | 444 | 78 300 |
| Pancuronium | 572 | 16 098 |
| (+)-Tubocurarine | 625 | 14 800 |

* Correspondence.

molecular weight mono-quaternary ammonium compounds, dibenzyldimethyl-, tribenzylmethyl- and cetyltrimethyl-ammonium did so. All, but succinylcholine and decamethonium, of the bis-quaternary ammonium compounds examined, formed extractable complexes, the most extensive extraction being with the higher molecular weight compounds, (+)-tubocurarine and fazadinium, suggesting that molecular weight may be a factor influencing the extent of formation of lipid-soluble complexes with the dye.

The assay was used to determine the pharmacokinetic parameters of fazadinium in man. Five patients (2M, 3F; ages 52–74 years, wt 53–80 kg), with normal hepatic and renal function, after induction of anaesthesia, had an indwelling cannula placed in a vein in the antecubital fossa. A blood sample (25 ml) was taken immediately before the administration of the drug (i.v.) and this was used as a blank for subsequent analysis and to establish the calibration curve. Further blood samples (5 ml) were taken into heparinized tubes at regular intervals up to 3 h after the drug had been given. The plasma was separated by centrifugation and the concentration of drug present determined. Plasma concentration data were analysed assuming a two-compartment open model (Reigelman et al 1968). Data were processed using a digital computer with the E04FBF program (Nottingham Algorithms Group) which requires initial estimates of the zero-time intercepts (A, B) of the two components of the bi-exponential decay and the slopes of these lines ($\alpha$, $\beta$). These parameters were obtained from log plasma concentration-time plots by feathering. The dimensions of the compartments, the rate constants and other parameters of the model were calculated from A, $\alpha$, B and $\beta$ using the equations of Greenblatt & Koch-Weser (1975). Attempts were also made to fit the data to a three-compartment open model using the above program.

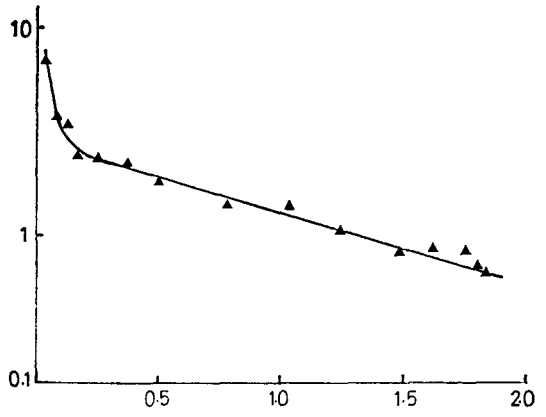


FIG. 1. A representative log plasma concentration-time curve of fazadinium (▲) after i.v. injection of 70 mg. Ordinate: plasma concentration ($\mu$g ml$^{-1}$). Abscissa: time (h).

The disappearance of the drug from plasma exhibited a bi-exponential decay pattern (Fig. 1), and this was analysed assuming a two-compartment open model, the parameters of which (mean ± s.e.) are shown in Table 2.

In all cases studied, plasma concentrations at 2 min post-injection were greater than 10 $\mu$g ml$^{-1}$, and declined rapidly over 10 min. A slower elimination phase followed, and at 1 h after injection the plasma value was 2·2 ± 0·5 $\mu$g ml$^{-1}$ (mean ± s.e., n = 5).

The present results are similar to those of Blogg et al (1973) who used [$^3$H]-labelled fazadinium and measured plasma radioactivity after its intravenous administration.

Comparison of our results with those of other workers for other neuromuscular blocking drugs shows broad similarities between fazadinium and related compounds. Thus, the diquaternary ammonium compound, pancuronium, also shows a biphasic plasma elimination curve which is fitted best by a two-compartment open model (Somogyi et al 1976) the important dimensions of which, i.e. volumes of distribution, microconstants, half lives, were of the same order as those we report.

There has been some controversy over whether the pharmacokinetics of neuromuscular blocking drugs are better described by a three-compartment rather than a two-compartment model. Thus, Agoston et al (1973) claimed that the plasma level-time curve of pancuronium in man is triphasic, but Somogyi et al (1976), who fitted similar data to both two and three-compartment open models, showed that three compartments had no advantages with this drug. However Agoston et al (1978) show a triphasic plasma elimination curve for the triquaternary ammonium compound gallamine, although the only dimensions of the model given are the half lives of the three phases.

When we attempted to fit our data to a three-compartment open model in three cases there was a slight improvement, but in one case, the two-compartment

Table 2. Pharmacokinetic parameters of fazadinium in patients.

| | Mean | s.e. |
|---|---|---|
| A ($\mu$g ml$^{-1}$) | 92·02 | 45·84 |
| $\alpha$ (min$^{-1}$) | 0·509 | 0·11 |
| B ($\mu$g ml$^{-1}$) | 5·30 | 1·74 |
| $\beta$ (min$^{-1}$) | 0·015 | 0·002 |
| $V_1$ (ml kg$^{-1}$) | 41 | 15 |
| $V_2$ (ml kg$^{-1}$) | 143 | 52 |
| $V_d$ area (ml kg$^{-1}$) | 226 | 76 |
| $k_{12}$ (min$^{-1}$) | 0·29 | 0·04 |
| $k_{21}$ (min$^{-1}$) | 0·07 | 0·01 |
| $k_{el}$ (min$^{-1}$) | 0·17 | 0·09 |
| Area under curve (ml min$^{-1}$) | 410 | 113 |
| Plasma clearance ($\mu$g ml$^{-1}$ min$^{-1}$) | 185 | 49 |
| $t_{\frac{1}{2}}\alpha$ (min) | 1·58 | 0·28 |
| $t_{\frac{1}{2}}\beta$ (min) | 50 | 6 |

model was better and in one case the data could not be fitted satisfactorily to a three-compartment model. We therefore conclude that the simplest model consistent with our data is the two-compartment open model.

We have found our adaptation of the method rapid, sensitive and convenient such that large numbers of biological samples can be handled simultaneously.

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REFERENCES

Agoston, S., Vermeer, G. A., Kersten, U. W., Meijer, D. K. F. (1973) Acta Anaesthesiol. Scand. 17: 267

Agoston, S., Vermeer, G. A., Kersten, U. W., Scaf, A. H. J. (1978) Br. J. Anaesth. 50: 345

Blogg, C. E., Simpson, B. R., Martin, L. E., Bell, J. A. (1973) Ibid., 45: 1233–12

Bray, G. A. (1960) Analyt. Biochem. 1: 279–285

Cohen, E. N. (1963) J. Lab. Clin. Med. 61: 338–345

Greenblatt, D. J., Koch-Weser, J. (1975) New Engl. J. Med. 293: 702–705

Reigelman, S., Loo, J. C. K., Rowland, M. (1968) J. Pharm. Sci. 57: 117–123

Somogyi, A. A., Shanks, C. A., Triggs, E. J. (1976) Eur. J. Clin. Pharmacol. 10: 367–372

Watson, M. J., McLeod, K. (1977) Clin. Chim. Acta 79: 511–512

# Steady state plasma concentration of clonidine and its relation to the effects on blood pressure in normotensive and hypertensive rats

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In a previous study it was found that clonidine given intravenously as bolus doses of less than 20 $\mu g\ kg^{-1}$ produced a dose-dependent hypotensive response in conscious normotensive rats. When the intravenous dose was increased, an initial short hypertensive phase became more and more dominant and in doses from 40–500 $\mu g\ kg^{-1}$ a clear dose-dependent increase of blood pressure was obtained. The subsequent fall in blood pressure was delayed up to 2 h in the dose of 500 $\mu g\ kg^{-1}$ (Paalzow & Edlund 1978). Obviously, in a certain dose range clonidine produces a hypotensive response, while in a higher dose-range the hypertensive effects dominate. It has been suggested that the hypotensive response of clonidine is induced by an inhibition of the sympathetic outflow from the brain (Schmitt et al 1968; Klupp et al 1970; Kobinger 1973; Haeusler 1974). The intial rise in blood pressure after i.v. bolus doses which has been observed both in man and in animals, has been suggested to be due to a stimulation of peripheral α-adrenoceptors (Schmitt et al 1971, 1973; Finch 1974; Ozawa et al 1977) as well as due to a central mechanism (Trolin 1975). We have aimed to evaluate the relationship between the steady state plasma concentrations of clonidine and the effects on arterial blood pressure in conscious normotensive and spontaneous hypertensive rats (SHR).

Male Sprague Dawley normotensive rats, 162–315 g, and SHR/Okamoto rats, 191–287 g, were used. Blood pressure was recorded in conscious rats through an indwelling carotid arterial catheter (Silastic o.d. 0·025 in) exteriorized at the back of the neck and connected to a pressure transducer (Statham P23DC) writing on a Grass Polygraph.

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Blood pressure was expressed as mean arterial blood pressure. Clonidine was infused through a catheter in the jugular vein exteriorized together with the arterial catheter. The two consecutive intravenous infusions technique described by Wagner (1975) was used to rapidly attain steady state plasma concentrations of clonidine. Variables needed for a given drug to be used in this technique are plasma clearance and half-life. These data were obtained from a previous study (Paalzow & Edlund 1979). The first more rapid infusion lasted for 30 min; this was then abruptly changed to a lower rate, which was maintained for the 2 h experiment. 15 min after the end of the first infusion, steady state plasma concentrations of clonidine were obtained and to check that these were maintained and in agreement with theoretically calculated values, plasma concentrations were determined from blood samples withdrawn from the jugular vein. Clonidine concentrations were assayed by gas liquid chromatography according to Edlund & Paalzow (1977). The initial infusion rate ($Q_1$) is given by equation 1 and the final rate ($Q_2$) by equation 2.

$$Q_1 = \frac{Q_2}{1 - e^{-\beta \cdot 30}} \quad (\text{ng min}^{-1}\,\text{kg}^{-1}) \qquad (1)$$

$$Q_2 = \frac{\text{Dose} \cdot C_{pss}}{A/\alpha + B/\beta} \quad (\text{ng min}^{-1}\,\text{kg}^{-1}) \qquad (2)$$

A, B, α, β are the coefficients and exponents of the bi-exponential equation describing the disposition of clonidine in plasma after an intravenous bolus dose (Edlund & Paalzow 1977; Paalzow & Edlund 1979).

In normotensive rats, a steady state value above 0·5 ng $ml^{-1}$ was needed to obtain a decrease in blood