J. Pharm. Pharmac., 1975, **27**, 322–328 *Received October* 28, 1974

Pharmacokinetics of haloalkylamines: cyclization and distribution in blood *in vitro* and *in vivo*

SVANTE B. ROSS

Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden

The cyclization of *N*-(5-chloropentyl)-*N*-methylaminoaceto-2,6-xylidide hydrochloride (RAD 150) and *N*-(5-bromopentyl)-*N*-methylaminoaceto-2,6-xylidide hydrobromide (RAD 154) in red blood cells of rats and rabbits was examined under *in vitro* and *in vivo* conditions. The rate of cyclization was much slower in plasma and blood than in a buffer solution, probably due to influence of protein binding of the compounds. The tertiary amines disappeared rapidly from the blood cells *in vitro* and *in vivo* and the piperidinium derivative (RAD 179) formed from the haloalkylamines disappeared almost as rapidly as the tertiary amines from the plasma *in vivo*. In contrast, the efflux of RAD 179 formed in the blood cells was slow ($T_{\frac{1}{2}}$ for rabbit erythrocytes: 9 h *in vitro*; 8 h *in vivo*) following a 1st order reaction. RAD 179 itself was only to a small extent taken up in the blood cells.

Tertiary haloalkylamine derivatives are able to cyclize *in vitro* to the corresponding cyclic ammonium derivatives (Levine, Weinstock & others 1961; Ross, Johansson & others 1973). This reaction occurs also under *in vivo* conditions (Levine & others, 1961; Ross & Frödén 1970) and has potential pharmacological interest since it may be an aid in overcoming the membrane barrier for quaternary ammonium derivatives (Levine & others, 1961; Ross & Frödén, 1970; Ross & Åkerman, 1972) or in giving a drug a new pharmacokinetic profile. In previous studies, the cyclization of RAD 150 (*N*-(5-chloropentyl)-*N*-methylaminoaceto-2,6-xylidide hydrochloride) and RAD 154 (*N*-(5-bromopentyl)-*N*-methylaminoaceto-2,6-xylidide hydrobromide) were examined under various *in vitro* and *in vivo* conditions (Ross & Frödén 1970; Ross & Åkerman, 1971; 1972). The present report deals with the cyclization and distribution of these two compounds, and the piperidinium derivative (RAD 179) formed on cyclization, in the blood after intravenous injection and under *in vitro* conditions.

MATERIALS AND METHODS

White rabbits of either sex, 2·5–4·5 kg, and male Sprague-Dawley rats, 140–180 g were used. Tritium labelled RAD 150 (specific activity 12 mCi mmole^{-1}), RAD 154 (specific activity 10 mCi mmole^{-1}) and RAD 179 (specific activity 4·6 mCi mmole^{-1}) with the tritium atom in 1-position of the haloalkylamine chain or the piperidinium ring (RAD 179) were used. Solutions of the compounds were made immediately before use and the pH of the solutions was between 3 and 4 to minimize the cyclization before the experiment.

In vivo *experiments*. The compounds were dissolved in 0·85% sodium chloride

solution and were injected in the cannulated jugular vein in pentobarbitone-anaesthetized rabbits. The dose was 2·5 mg kg^{-1} and the volume injected was 5–8 ml. In the experiments when the blood samples were collected the day after the administration of the compounds, the injections were made in a marginal vein of the ear. The blood samples were collected from the cannulated carotid artery in heparinized centrifuge tubes (Vitrum) different times after the injection, the first one being collected after 1 min. In some experiments the whole blood was analysed for the amounts of the tertiary amine and the piperidinium derivative formed, in other experiments, the red blood cells were separated from the plasma by centrifugation and analysed separately.

In vitro *experiments.* Heparinized blood of rabbits or rats was incubated at 37° with 0·5 mM of RAD 150 for 50 min or RAD 154 for 5 min. The red blood cells were separated from the plasma by centrifugation and washed twice with 0·85% sodium chloride containing 0·05 M sodium phosphate buffer, pH 7·4. The blood cells were suspended in this solution to the original blood volume, transferred to a washed dialysing membrane closed at the bottom and tied to a filtration funnel at the top. The blood cell suspension was "dialysed" against 1 litre of the saline-phosphate buffer warmed to 37° and stirred with a magnetic stirer. The blood cells were kept in suspension by mixing at regular intervals. Samples were collected after different times, centrifuged in small plastic centrifuge tubes and aliquots of 50 μl of the plasma and the blood cells were analysed for the amount of the tertiary amine and RAD 179.

The rate of cyclization of RAD 150 and RAD 154 was determined in Krebs-Henseleit buffer, pH 7·4, in a 5% solution of bovine albumin in Krebs-Henseleit buffer, in rat plasma and in heparinized rat blood at 37° by analysing the amounts of the unreacted tertiary amine and the amounts of RAD 179 formed after different times of incubation. The cyclization rate was expressed as the half life time ($T_{\frac{1}{2}}$) for the tertiary amine.

Ion pair extraction. The tertiary amines and RAD 179 were extracted as ion complexes with β-naphthalene sulphonate into cyclohexane-n-pentanol (80:20) as described previously (Ross & Frödén, 1970). The samples were added to 2·0 ml of 0·1 M β-naphthalene sulphonate, pH 3·0, containing 10 μg of inactive tertiary amine and 10 μg of RAD 179 as carriers in glass-stoppered centrifuge tubes and the ion pairs were extracted into 10 ml of the cyclohexane-pentanol solution by vigorous mixing for 20 s. After centrifugation, 3·0 ml of the organic layer was transferred to counting vials containing 1·0 ml of ethanol and 10 ml of the scintillation liquid (Permablend III, Packard, in toluene), while 5·0 ml was transferred to a glass-stoppered centrifuge tube and immediately washed with 2·0 ml of 0·1 M sodium phosphate buffer, pH 6·5, by vigorous mixing for 10 s. An aliquot of 3·0 ml of the organic layer was taken for radioactive determination. The first extract contains ion pairs of the tertiary amine and RAD 179 with β-naphthalene sulphonate, whereas the washed extract contains the main part of the tertiary amine, RAD 179 being extracted into the buffer solution. Standards of labelled tertiary amine and RAD 179 containing the same amounts of the blood components examined were extracted in the same way in each experiment. The recoveries of the extracted compounds and the distributions between the organic phase and the water phase in the washing procedure were determined in each experiment and the calculation of the amount of the compounds was based on these figures.

RESULTS

In vitro *experiments*

The rate of cyclization. The rates of cyclization of RAD 150 and RAD 154 in blood plasma and heparinized blood of the rat were compared with that in a buffer at pH 7·4. RAD 150 cyclized much more slowly in plasma (half life: $T_{\frac{1}{2}} = 48$ min) and heparinized blood ($T_{\frac{1}{2}} = 50$ min) than in the buffer solution ($T_{\frac{1}{2}} = 13$ min) (Fig. 1). Since a possible reason for the slower reaction in plasma and blood is protein binding of RAD 150, the rate of the cyclization in a buffer solution containing 5% of bovine albumin was examined. The reaction in this solution was even slower than in the plasma ($T_{\frac{1}{2}} = 98$ min). If it is assumed that protein binding is the only factor reducing the cyclization, this binding for RAD 150 can be calculated to be 68% in rat plasma and 84% in 5% of bovine albumin solution.

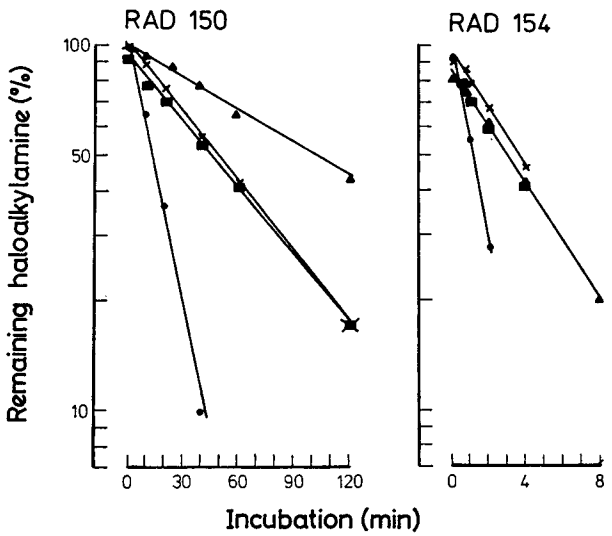

FIG. 1. Cyclization of ^{3}H-RAD 150 and ^{3}H-RAD 154 *in vitro* in Krebs-Henseleit buffer pH 7·4 (●), rat plasma (×), rat blood (■), Krebs-Henseleit buffer, pH 7·4 containing 5% bovine albumin (▲). The starting concentration of RAD 150 and RAD 154 was 0·5 mM.

RAD 154 cyclizes much more rapidly than RAD 150 (Fig. 1). The reaction for RAD 150 was also slower in plasma ($T_{\frac{1}{2}} = 3{\cdot}8$ min), in heparinized blood ($T_{\frac{1}{2}} = 4{\cdot}0$ min) and in 5% bovine albumin solution, ($T_{\frac{1}{2}} = 4{\cdot}1$ min) than in the buffer solution ($T_{\frac{1}{2}} = 1{\cdot}1$ min). From these values the protein binding of RAD 154 was calculated to be 64% in rat plasma and 70% in 5% of bovine albumin solution.

Rate of the efflux of RAD 179 from red blood cells. Tertiary haloalkylamines are able to diffuse into cells and there cyclize to the quaternary ammonium derivatives (Ross & Frödén, 1970; Ross & Åkerman, 1971, 1972). Red blood cells are suitable for the examination of this principle. The rate of the efflux of RAD 179 formed from RAD 150 in erythrocytes from rats and rabbits was determined as described in "methods". This efflux had the nature of a first order reaction and the rate was rather slow (Fig. 2). The half life for the efflux of RAD 179 from rabbit erythrocytes was about 9 h and that from rat blood cells 4·4 h. To examine if depolarization of the cell membranes with potassium chloride influenced the rate of the efflux of RAD 179 from rat erythrocytes, the efflux was assessed in isotonic potassium chloride

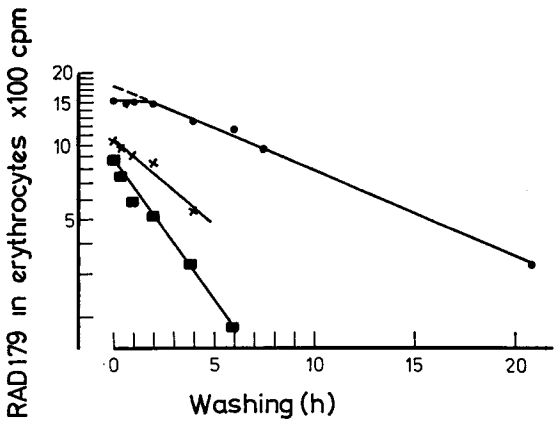

FIG. 2. Efflux of ^{3}H-RAD 179 from red blood cells of rabbit (●) and rat in 0·9% of NaCl + 0·05 M phosphate buffer (×) and rat in 1·14% of KCl + 0·05 M phosphate buffer (■). The blood cells were incubated with ^{3}H-RAD 150 (0·1 mM) for 1 h and washed with saline twice before the blood cells were "dialysed" against 1 litre of the saline solutions.

solution instead of the sodium chloride solution. The half life in this experiment was 2·7 h, *i.e.* the efflux was more rapid than in sodium chloride solution.

Accumulation of RAD 179 in red blood cells on repeated incubation with RAD 154

Because of the disappearance of the diffusion barrier upon cyclization of the haloalkylamine inside the cells, the quaternary ammonium derivative formed is accumulated in the cells on repeated incubation with the same concentration of the haloalkylamine. The accumulation is unlimited from a theoretical point of view. This principle was tested by an experiment in which washed rat blood cells suspended in saline phosphate buffer were incubated with 0·1 mM RAD 154 for 5 min periods between each of which the blood cells were centrifuged and fresh saline-phosphate buffer solution with 0·1 mM RAD 154 was added. The result of this experiment clearly shows a linear accumulation of RAD 179 in the blood cells (Fig. 3). When the erythrocytes were repeatedly incubated with 0·1 mM of RAD 179 the level of RAD 179 in the cells did not reach the concentration in the incubation fluid. Repeated incubation with lignocaine resulted in rapid equilibrium between the concentration in the erythrocytes and the incubation medium.

Pharmacokinetic experiments in vivo

Cyclization of RAD 150 in the blood circulation in vivo. The cyclization of RAD 150 after intravenous injection of 2·5 mg kg^{-1} in rabbits was examined. The heparinized blood was analysed for the concentrations of RAD 150 and RAD 179 at different times after the injection. During the first 30 min the concentration of RAD 150 was higher than that of RAD 179, which at this phase was increasing whereas RAD 150 concentration was rapidly decreasing (Fig. 4). After 1 h RAD 179 concentration exceeded that of RAD 150, which after 4 h was near the limit of the detection. RAD 179 disappeared from the blood according to a first order reaction with a $T_{\frac{1}{2}}$ of 8 h, *i.e.* similar to the rate of the efflux of RAD 179 from rabbit erythocytes *in vitro*.

Distribution of RAD 179 formed from RAD 150 or RAD 154 in the blood in vivo. Erythrocytes and plasma from rabbits injected intravenously with RAD 150 or

RAD 154 were analysed separately for the concentrations of the tertiary amine and RAD 179. In the experiments with RAD 150 the plasma level of RAD 150 was higher than that of RAD 179 (Fig. 5). The rate of disappearance of RAD 150 from plasma

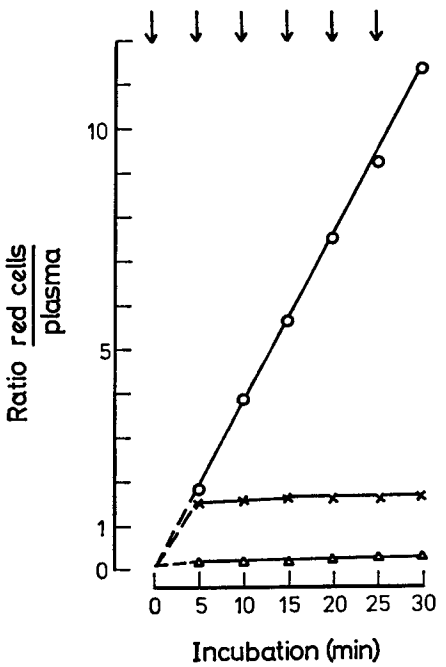

FIG. 3. Accumulation of ^{3}H-RAD 179 in red blood cells upon repeated incubation with ^{3}H-RAD 154 (⊙) (0·1 mM). Rat blood cells were washed twice with 0·9% of NaCl + 0·05 mM phosphate buffer and incubated with 0·1 mM ^{3}H-RAD 154 for 5 min. After 5 min centrifugation the blood cell were resuspended in fresh saline solution and incubated for 5 min with 0·1 mM ^{3}H-RAD 154. This procedure was repeated 5 times. Samples were taken each time from the blood cells and the "plasma" and analysed. The same type of experiments was performed with ^{3}H-RAD 179 (△) and [^{3}H]lignocaine (×).

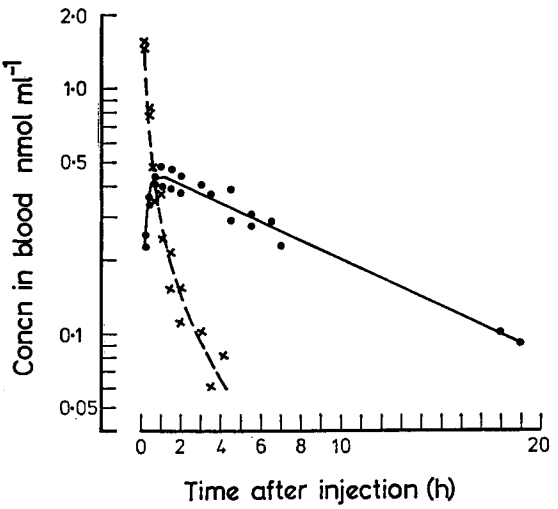

FIG. 4. Cylization of ^{3}H-RAD 150 in the rabbit blood *in vivo*: ^{3}H-RAD 150 (×) and ^{3}H-RAD 179 formed (●). ^{3}H-RAD 150, 2·5 mg kg^{-1}, was injected i.v.

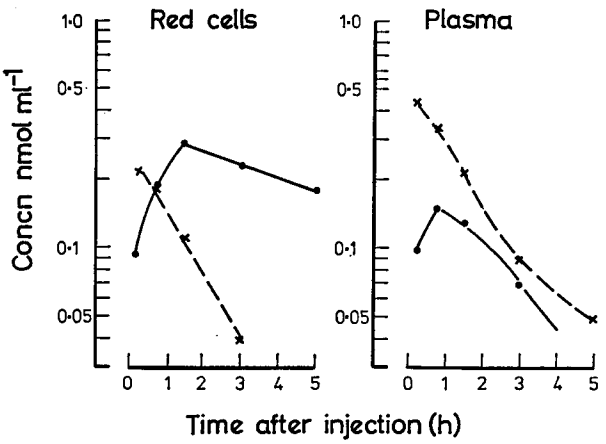

FIG. 5. Distribution of ^{3}H-RAD 150 (×) and ^{3}H-RAD 179 (●) formed from RAD 150 in the rabbit blood *in vivo*.

was $T_{\frac{1}{2}} = 1{\cdot}2$ h. During the initial phase, the concentration of RAD 179 in plasma was increased followed by a phase of disappearance with about the same rate as for RAD 150. In the erythrocytes RAD 150 disappeared with the same rate as in the plasma. Due to the formation of RAD 179 in the erythrocytes the concentration was increased during the first one and a half hours followed by a slow disappearence with about the same rate as observed in the heparinized blood.

In the experiments with RAD 154 the plasma level of RAD 154 disappeared very rapidly and only small amounts were found in the erythrocytes (Fig. 6). RAD 179 formed from RAD 154 disappeared rapidly from the plasma whereas the efflux from the erythrocytes was slow with a $T_{\frac{1}{2}}$ of about 7 h.

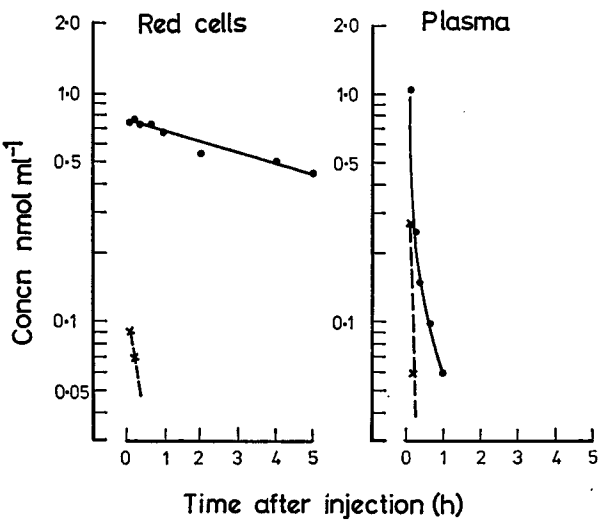

FIG. 6. Cyclization and the distribution of ^{3}H-RAD 154 in the rabbit blood *in vivo*. ^{3}H-RAD 154 (×) and ^{3}H-RAD 179 (●) formed. The dose of ^{3}H-RAD 154 was 2·5 mg kg^{-1} i.v.

DISCUSSION

The rate of the cyclization of tertiary haloalkylamine is determined by several factors such as the length of the haloalkyl chain, substituents on this chain and the halogen atom (Ross, Johansson & others, 1973). When measured at physiological pH the basicity of the amine is also important since only the fraction of the amine in basic form takes part in the cyclization (Johansson, Lindborg & others, 1973). The nature of the environment is another factor, the cyclization being slower in hydrophobic than in hydrophilic solvents (Ross & Frödén, 1970). The much higher rate of the cyclization of RAD 150 found in this study ($T_{\frac{1}{2}} = 13$ min) compared to that in an previous report (Ross, Sandberg & others, 1973) ($T_{\frac{1}{2}} = 50$ min) is an example of the influence of the solvent on the cyclization, since in the earlier determination the cyclization was performed in 50% ethanol but in the present study the solvent was water. The results in the present study demonstrate that protein binding in an additional important factor for the cyclization *in vivo* by reducing the concentration of the free amine. This observation may explain the strong decrease of the cyclization of RAD 150 in a brain homogenate observed previously (Ross & Frödén, 1970), although lipids in the brain homogenate may have an additional effect.

The slow rate of efflux of RAD 179 from the erythrocytes under *in vitro* and *in vivo* conditions supports previous observations (Ross & Frödén, 1970, Ross & Åkerman, 1971, 1972) that a similar membrane barrier hinders the efflux as it does the influx of quaternary ammonium compounds. This barrier seems to be changed by depolarization of the erythrocytes with potassium chloride, since the efflux of RAD 179 was increased.

The unique capacity of the cyclizing compounds to accumulate in cells as demonstrated by the experiment with repeated incubations of erythrocytes with RAD 154 may have important pharmacological applications. For instance, if a high level of an active compound is required in blood cells, substitution of the compound with a tertiary haloalkylamine in such a way that the pharmacological effects are not destroyed can give the new compound the capability of accumulating as the quaternary derivative in the cells upon slow intravenous infusion. In order to get optimal effect the cyclization rate must be rather high since the diffusion barrier consists of the concentration of the tertiary amine in the cell but not of the cyclized quaternary derivative. The principle may also be applied in other tissues which are richly vasculated, *e.g.* the heart and the lungs.

Acknowledgements

The technical assistance of Mrs Lisbeth Sörensen is gratefully acknowledged. This work was supported by the Swedish Medical Research Council (Project No. 04X-4339).

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