Comparative Pharmacokinetics of Four Cholinesterase Inhibitors in Rats

Koujiro YAMAMOTO,* Yasufumi SAWADA, and Tatsuji IGA

Department of Pharmacy, The University of Tokyo Hospital, Faculty of Medicine, The University of Tokyo, 3-1, Hongo 7-chome, Bunkyo-ku, Tokyo 113, Japan. Received April 11, 1995; accepted June 5, 1995

Pharmacokinetics of a very short-acting, a short-acting and two long-acting cholinesterase (ChE) inhibitors, edrophonium, neostigmine, pyridostigmine and ambenonium, respectively, were compared to elucidate the major determinant of their pharmacokinetics. No dose-dependency in pharmacokinetic behavior was observed within the range of 2–10 $\mu\text{mol/kg}$ for edrophonium, 0.5–2 $\mu\text{mol/kg}$ for pyridostigmine, 0.1–0.5 $\mu\text{mol/kg}$ for neostigmine and 0.3–3 $\mu\text{mol/kg}$ for ambenonium, respectively. Neostigmine has the shortest elimination half-life, and edrophonium, pyridostigmine and ambenonium follow in that. Four ChE inhibitors have similar $V_{d_{ss}}$ values within the range of 0.3–0.7 l/kg, which is similar to the muscle/plasma concentration ratio of these drugs. The liver or kidney to plasma concentration ratio of all ChE inhibitors at 20 min after i.v. administration ranged from 5 to 15. Small distribution volumes estimated from the plasma concentration profiles may reflect the distribution to muscle and to the extracellular space of other organs/tissues, while the rapid disappearance of ChE inhibitors from plasma may reflect the concentrative uptake to the liver and kidney.

Key words cholinesterase inhibitor; pharmacokinetics; rat

Reversible cholinesterase (ChE) inhibitors are used as the first choice of therapy for myasthenia gravis. ChE inhibitors elevate the acetylcholine (ACh) concentration at the synaptic cleft of the neuromuscular junction by acetylcholinesterase (AChE) inhibition, and intensify contractile muscle tension. However, the relationship between the concentration of ChE inhibitors in plasma and the enhancement of contractile muscle tension have not been clearly explained.

Several pharmacokinetic studies have been reported for pyridostigmine, a frequently used ChE inhibitor. The pharmacokinetics following i.v., i.m. and p.o. administration of ^{14}C -pyridostigmine to rats and dogs were studied,^{1–3)} and further, plasma concentration,^{4–7)} bioavailability,^{8–10)} metabolism¹¹⁾, and excretion to breast milk¹²⁾ in myasthenic patients were reported. Pharmacokinetic studies of ^{14}C -neostigmine were also carried out in rats.^{13–16)} Plasma concentrations of neostigmine in myasthenic patients were determined in some cases.^{17,18)} A few investigators have reported the plasma concentration–time profiles of edrophonium in rats^{19–21)} and in human.²²⁾ The pharmacokinetics of ambenonium is almost completely unknown.²³⁾ Tharasse-Bloch *et al.*²⁴⁾ and Ohtsubo *et al.*²⁵⁾ reported HPLC methods for the determination of ambenonium and its pharmacokinetics in dogs was investigated.²⁶⁾ We developed HPLC²⁷⁾ and enzymatic assay methods²⁸⁾ for ambenonium and previously investigated its pharmacokinetic²⁹⁾ and pharmacodynamic³⁰⁾ behavior in rats. However, it remains unknown whether the difference among ChE inhibitors in pharmacokinetic behavior reflects their pharmacological character.

In this study, the pharmacokinetics of a very short-acting, a short-acting and two long-acting ChE inhibitors, edrophonium, neostigmine, pyridostigmine and ambenonium, respectively, were compared to elucidate the major determinant of their pharmacokinetics, such as total body clearance and the distribution volume at steady state.

METHODS

Chemicals and Reagents Edrophonium chloride, neostigmine bromide and pyridostigmine bromide were purchased from Sigma (U.S.A.). All other reagents were of analytical grade and used without further purification.

Animal Experiments Male Wistar rats weighing 250–330 g were used in all the experiments. Under light ether anesthesia, polyethylene cannulas were inserted into the left femoral artery and vein, bile duct and urinary bladder. The rats were left for 2 h after the surgery to recover from anesthesia. At 1, 3, 5, 10, 15, 20 and 30 min after i.v. administration of edrophonium (2–10 $\mu\text{mol/kg}$) and neostigmine (0.1–0.5 $\mu\text{mol/kg}$), and at 2, 5, 15, 30, 45 and 60 min for pyridostigmine (0.5–2 $\mu\text{mol/kg}$), 0.5 ml of blood was taken and the plasma was obtained immediately by centrifugation at 1620 g for 5 min. Bile and urine were collected at appropriate intervals until 6 h after administration. In tissue distribution experiments, rats were killed by exsanguination at 20 min after the administration of drugs to collect the tissues. The tissue samples were gently rinsed with saline and blotted with filter paper to remove excessive moisture. The sample was stored at -20°C until analysis. The concentrations of ChE inhibitors in biological samples were determined by HPLC method.³¹⁾

To estimate the pharmacokinetic parameters, A , B , α and β , the concentration of ChE inhibitors in plasma obtained in all single bolus dose experiments were simultaneously fitted to Equation 1 by the non-linear least squares method. The total body clearance (CL_{tot}), the steady state volume of distribution ($V_{d_{ss}}$) and the elimination half-life ($t_{1/2}$) were calculated according to Eqs. 2, 3 and 4, respectively.

$$C_p = \text{dose} \cdot (A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t)) \quad (1)$$

$$CL_{\text{tot}} = \frac{\alpha \cdot \beta}{A \cdot \beta + B \cdot \alpha} \quad (2)$$

* To whom correspondence should be addressed.

$$Vd_{ss} = \frac{A \cdot \beta^2 + B \cdot \alpha^2}{(A \cdot \beta + B \cdot \alpha)^2} \quad (3)$$

$$t_{1/2} = \frac{\ln 2}{\beta} \quad (4)$$

where C_p is the concentration of ChE inhibitors in plasma, dose is the i.v. dose administered and t is the time after administration. Excretion ratios in urine and bile were estimated with extrapolation to infinite time using the terminal slope of the semi-logarithmic plots of excretion rate in the urine and bile *versus* time curve.

RESULTS AND DISCUSSION

Plasma concentration profiles after i.v. bolus administration of edrophonium (2–10 $\mu\text{mol/kg}$), pyridostigmine (0.5–2 $\mu\text{mol/kg}$) and neostigmine (0.1–0.5 $\mu\text{mol/kg}$) to rats are shown in Fig. 1. The plasma concentration profile of ambenonium (0.3–3 $\mu\text{mol/kg}$) reported previously²⁹ was also shown. The disappearance of these drugs in plasma was described by biexponential curves in all cases. The estimated pharmacokinetic parameters are listed in Table 1. No dose-dependency in pharmacokinetic parameters was observed within the dose range in this study. Four ChE inhibitors have similar Vd_{ss} values within the range of 0.3–0.7 l/kg. Neostigmine has the shortest $t_{1/2}$ and edrophonium, pyridostigmine and ambenonium followed in that order. The plasma elimination half-lives of ChE inhibitors in rats have been reported as 6.3–13.5 min and 81–175 min for edrophonium^{20,22} 19 min for pyridostigmine¹ and 147 min for neostigmine.¹⁴ The elimination half-life of ambenonium from plasma was determined in rats²⁹ and dogs²⁶ as 21–24 min and 36 min, respectively. However, they cannot be

compared, simply because the experimental conditions such as dose and sampling schedule are different. The elimination half-lives of ChE inhibitors estimated in the present study are rather short (Table 1), resulting from the short experimental period of less than 90 min.

The elimination half-life of edrophonium is longer than neostigmine in the present study, while pharmacological effects of the former disappear more rapidly than those of the latter in human.²³ We have reported the time course of pharmacological effect after the i.v. administration of ChE inhibitors in rats, and found that the duration of muscle contractile potentiation or bradycardia induced by edrophonium diminished more rapidly than that by neostigmine.³² Therefore, a pharmacologically effective duration of ChE inhibitors may not be determined by their pharmacokinetics, but by their affinity to a muscarinic receptor or to the desensitization rate of nicotinic receptors.

All ChE inhibitors are readily excreted in urine, and

Table 1. Pharmacokinetic Parameters of ChE Inhibitors After i.v. Administration to Rats

Parameters	Edrophonium	Pyridostigmine	Neostigmine	Ambenonium
CL_{tot} (ml/min/kg)	47.8 $\pm$ 1.0	15.0 $\pm$ 0.2	44.4 $\pm$ 3.7	9.46 $\pm$ 1.1
Vd_{ss} (l/kg)	0.651 $\pm$ 0.290	0.353 $\pm$ 0.050	0.289 $\pm$ 0.067	0.265 $\pm$ 0.095
$t_{1/2}$ (min)	17.9 $\pm$ 8.1	24.2 $\pm$ 4.2	7.3 $\pm$ 1.3	26.7 $\pm$ 9.6
Urinary excretion (%) ^a	38.0 $\pm$ 10.6	59.5 $\pm$ 11.2	38.0 $\pm$ 10.6	36.7 $\pm$ 4.7
Biliary excretion (%) ^a	0.93 $\pm$ 0.42	0.23 $\pm$ 0.22	0.56 $\pm$ 0.24	24.1 $\pm$ 7.0

Edrophonium: 10, 5, 2 $\mu\text{mol/kg}$, pyridostigmine: 2, 1, 0.5 $\mu\text{mol/kg}$, neostigmine: 0.5, 0.2, 0.1 $\mu\text{mol/kg}$, ambenonium: 3 ($n=4$), 1, 0.3 ($n=4$) $\mu\text{mol/kg}$. Data for ambenonium was obtained from the previous report.²⁹ a) Biliary and urinary excretion were determined after the i.v. administration of: edrophonium: 5 $\mu\text{mol/kg}$, pyridostigmine: 1 $\mu\text{mol/kg}$, neostigmine: 0.5 $\mu\text{mol/kg}$, ambenonium: 1 $\mu\text{mol/kg}$. ($n=3$ if not specified, optimal estimates $\pm$ S.E.)

Fig. 1. Plasma Concentration-Time Profiles of ChE Inhibitors after i.v. Administration to Rats

Data for ambenonium was obtained from the previous report.²⁹ Edrophonium: 10 ($\blacktriangle$), 5 ($\circ$), 2 $\mu\text{mol/kg}$ ($\bullet$), pyridostigmine: 2 ($\blacktriangle$), 1 ($\circ$), 0.5 $\mu\text{mol/kg}$ ($\bullet$), neostigmine: 0.5 ($\blacktriangle$), 0.2 ($\circ$), 0.1 $\mu\text{mol/kg}$ ($\bullet$), ambenonium: 3 ($\blacktriangle$; $n=4$), 1 ($\circ$), 0.3 $\mu\text{mol/kg}$ ($\bullet$; $n=4$). ($n=3$ if not specified, mean $\pm$ S.E.)

40–60% of the dose were found as parent drugs in the present study. About 25% of the dose was excreted in bile for ambenonium, while biliary excretion of the other drugs was negligible. This difference in hepatobiliary disposition may be due to the large molecular weight (608.5) and bisquaternary structure of ambenonium.³³⁾ From these results, 40–60% of these drugs may be excreted as the metabolites. In previous reports, Back and Calvey¹⁹⁾ reported that 5% of radioactivity was excreted in bile as glucuronide after the i.v. administration of ¹⁴C-edrophonium to rats. Urinary excretion of edrophonium in rats had not been reported previously, but 90% of the dose was excreted in urine after the i.v. administration of ¹⁴C-pyridostigmine, and about one-third of radioactivity was related to a metabolite, 3-hydroxy-*N*-methylpyridinium.¹⁾ For ¹⁴C-neostigmine, 50% of the dose was excreted in urine within 2 h after i.m. administration, and half the radioactivity was related to a hydrolyzed metabolite.¹⁵⁾ It is well known that various quaternary ammonium compounds, including ChE inhibitors, are readily excreted in urine by renal tubular secretion.^{13,34-36)}

Contrary to the rapid elimination of ChE inhibitors, high accumulations are found in the liver and kidney.^{1,15,19,27)} As shown in Fig. 2, the liver or kidney to plasma concentration ratio of all ChE inhibitors at 20 min ranged from 5 to 15, suggesting that ChE inhibitors

Fig. 2. Apparent Tissue/Plasma Partition Coefficients (K_p) of ChE Inhibitors

Data for ambenonium was obtained from the previous report.²⁹⁾ Drug concentration at 20 min after i.v. administration of edrophonium 10 μ mol/kg, pyridostigmine 1 μ mol/kg, neostigmine 0.2 μ mol/kg or ambenonium 1 μ mol/kg were determined ($n=3$, mean $\pm$ S.E.).

Fig. 3. Steady-State Distribution Volume and Apparent Muscle/Plasma Partition Coefficients of ChE Inhibitors

Data for ambenonium was obtained from the previous report.²⁹⁾ Drug concentration at 20 min after i.v. administration of edrophonium 10 μ mol/kg, pyridostigmine 1 μ mol/kg, neostigmine 0.2 μ mol/kg or ambenonium 1 μ mol/kg were determined ($n=3$, mean $\pm$ S.E.).

may be concentratively uphill-transported into these tissues similarly to other quaternary ammonium compounds.^{32,33)} Though approximately 50% of the dose was distributed into the liver and kidney at 20 min after i.v. administration, the distribution volumes at steady state are a relatively small value of 0.3–0.7 l/kg, which was somewhat larger than the extracellular space and similar to the muscle/plasma concentration ratio (Fig. 3). It is possible that, since the ChE inhibitors transported into the liver and kidney may hardly be able to return to the plasma compartment, the uptake to these tissue may be regarded as an elimination process. Therefore, the uptake process into the liver and kidney may be the major determinant of the total body clearance of ChE inhibitors.

In conclusion, the rapid disappearance of ChE inhibitors from plasma may reflect the concentrative uptake into the liver and kidney. However, their steady state volumes of distribution are the relatively small value of 0.3–0.7 l/kg, suggesting that a main determinant of the distribution of ChE inhibitors may be distribution to the muscle and extracellular space of other organs/tissues. The primary determinant of the pharmacologically effective duration of ChE inhibitors may not be a pharmacokinetic character, but rather the pharmacological/toxicological potencies and the rate of sensitivity changes by the drugs. Further investigation about the pharmacodynamics and toxicodynamics of ChE inhibitors will appear elsewhere.

REFERENCES

- 1) Birtley R. D. N., Roberts J. B., Thomas B. H., Wilson A., *Br. J. Pharmacol.*, **26**, 393 (1966).
- 2) Husain M. A., Roberts J. B., Thomas B. H., Wilson A., *Br. J. Pharmacol.*, **34**, 445 (1968).
- 3) Taylor T., Hawkins D. R., Forrest T. J., Chung H., *J. Pharm. Sci.*, **80**, 353 (1991).
- 4) Calvey T. N., Chan K., *Clin. Pharmacol. Ther.*, **21**, 187 (1977).
- 5) White M. C., Silva P. D., Havard C. W. H., *Neurology*, **31**, 145 (1981).
- 6) Sørensen P. S., Flachs H., Friis M. L., Hvidberg E. F., Paulson O. B., *Neurology*, **34**, 1020 (1984).
- 7) Cohan S. L., Pohlmann J. L. W., Mikszewski J., O'Doherty D. S., *Neurology*, **26**, 536 (1976).
- 8) Breyer-Pfaff U., Maier U., Brinkmann A. M., Schumm F., *Clin. Pharmacol. Ther.*, **37**, 495 (1985).
- 9) Aquilonius S.-M., Eckernäs S.-Å., Hartvig P., Lindström B., Osterman P. O., *Eur. J. Clin. Pharmacol.*, **18**, 423 (1980).
- 10) Cohan S. L., Dretchen K. L., Neal A., *Neurology*, **27**, 299 (1977).
- 11) Somani S. M., Roberts J. B., Wilson A., *Clin. Pharmacol. Ther.*, **13**, 393 (1972).
- 12) Hardell L.-I., Lindström B., Lönnerholm G., Osterman P. O., *Br. J. Clin. Pharmacol.*, **14**, 565 (1982).
- 13) Roberts J. B., Thomas B. H., Wilson A., *Nature (London)*, **200**, 1330 (1963).
- 14) Roberts J. B., Thomas B. H., Wilson A., *Br. J. Pharmacol.*, **25**, 234 (1965).
- 15) Roberts J. B., Thomas B. H., Wilson A., *Br. J. Pharmacol.*, **25**, 763 (1965).
- 16) Roberts J. B., Thomas B. H., Wilson A., *Biochem. Pharmacol.*, **15**, 71 (1966).
- 17) Nowell P. T., Scott C. A., Wilson A., *Br. J. Pharmacol.*, **18**, 617 (1962).
- 18) Aquilonius S.-M., Eckernäs S.-Å., Hartvig P., Hultman J., Lindström B., Osterman P. O., *Eur. J. Clin. Pharmacol.*, **15**, 367 (1979).
- 19) Back D. J., Calvey T. N., *Br. J. Pharmacol.*, **44**, 534 (1972).
- 20) Back D. J., Calvey T. N., *Br. J. Pharmacol.*, **46**, 355 (1972).

- 21) Barber H. E., Calvey T. N., Muir K. T., Taylor K., *Br. J. Pharmacol.*, **56**, 93 (1976).
- 22) Calvey T. N., Williams N. E., Muir K. T., Barber H. E., *Clin. Pharmacol. Ther.*, **19**, 813 (1976).
- 23) Aquilonius S.-M., Hartvig P., *Clin. Pharmacokin.*, **11**, 236 (1986).
- 24) Tharasse-Bloch C., Chabenat C., Boucly P., Marchand J., *J. Chromatogr.*, **421**, 407 (1987).
- 25) Ohtsubo K., Higuchi S., Aoyama T., *J. Chromatogr.*, **496**, 397 (1989).
- 26) Tharasse-Bloch C., Chabenat C., Boucly P., Boismare F., Marchand J., *Arzneim.-Forsch./Drug Res.*, **39**, 257 (1989).
- 27) Yamamoto K., Kohda Y., Saitoh Y., Nakagawa F., *J. Chromatogr.*, **433**, 167 (1988).
- 28) Yamamoto K., Sawada Y., Iga T., *Biol. Pharm. Bull.*, **16**, 280 (1993).
- 29) Yamamoto K., Kohda Y., Sawada Y., Iga T., *Biopharm. Drug Dispos.*, **12**, 613 (1991).
- 30) Yamamoto K., Sawada Y., Iga T., *Biol. Pharm. Bull.*, **17**, 944 (1994).
- 31) De Ruyter M. G. M., Cronnelly R., *J. Chromatogr.*, **183**, 193 (1980).
- 32) Yamamoto K., Shimizu M., Ohtani H., Hayashi M., Sawada Y., Iga T., *Xenobio. Metabol. Dispos.*, **9** (suppl.), S289 (1994).
- 33) Meijer D. K. F., Mol W. E. M., Müller M., Kurz G., *J. Pharmacokin. Biopharm.*, **18**, 35 (1990).
- 34) Mintun M., Himmelstein K. J., Schroder R. L., Gibaldi M., Shen D. D., *J. Pharmacokin. Biopharm.*, **8**, 373 (1980).
- 35) Neef C., Meijer D. K. F., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **328**, 103 (1984).
- 36) Neef C., Meijer D. K. F., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **328**, 111 (1984).