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PLASMA CLEARANCE OF NEOSTIGMINE AND PYRIDOSTIGMINE IN THE DOG

P.R. BAKER[1], T.N. CALVEY, K. CHAN[2], C.M. MACNEE & K. TAYLOR

Departments of Pharmacology and Surgery, University of Liverpool, Liverpool, L69 3BX

1 The pharmacokinetics of neostigmine and pyridostigmine was studied in conscious dogs by the use of a cross-over design.

2 Both neostigmine and pyridostigmine were cleared from plasma in a biexponential manner.

3 The apparent volume of distribution of pyridostigmine was invariably greater than that of neostigmine, and its fast disposition half-life was approximately three times longer.

4 The whole body clearance and the urinary elimination of pyridostigmine was approximately twice that of neostigmine.

5 The slow disposition half-life of pyridostigmine was approximately three times longer than that of neostigmine, suggesting that the longer duration of action of pyridostigmine is related to the differential clearance of the two quaternary amines from plasma.

Introduction

Differences in the duration of action of neostigmine and pyridostigmine have been observed in both experimental animals and human subjects. Although equipotent doses of both anticholinesterase drugs produce comparable effects on neuromuscular transmission, the duration of action of pyridostigmine is approximately 30% longer than neostigmine (Smith, Mead & Unna, 1957; Miller, Van Nyhuis, Eger, Vitez & Way, 1974). Since both drugs inhibit acetylcholinesterase in an identical manner, these results may reflect the differential elimination of neostigmine and pyridostigmine from plasma. Indeed in rats with ligated renal pedicles, there are statistically significant differences in the plasma half-lives of [^{14}C]-neostigmine and [^{14}C]-pyridostigmine that may be related to qualitative differences in hepatic metabolism (Burdfield & Calvey, 1973). Comparable studies in other experimental animals with normal renal function have not been reported.

The present experiments are concerned with the pharmacokinetics of intravenous neostigmine and pyridostigmine in the unanaesthetized dog. A cross-over design was used, so that each experimental animal received both quaternary amines on different occasions. The plasma concentration of neostigmine and pyridostigmine was measured by a gas-liquid chromatographic method described in detail in a previous paper (Chan, Williams, Baty & Calvey, 1976).

Methods

Labrador dogs of either sex, obtained from an MRC accredited supplier, were used in the experiments. All studies were carried out in conscious animals at the same time of day, after previous overnight fasting. In a cross-over procedure, four dogs each received neostigmine and pyridostigmine on different occasions (separated by an interval of at least 7 days). In each experiment, the cephalic vein of one foreleg was cannulated with a polypropylene catheter (o.d. = 1.30 mm) attached to a three-way tap. Neostigmine methylsulphate (0.25 mg in 0.9% w/v NaCl solution (saline); approximately 13 μg/kg) or pyridostigmine bromide (1.00 mg in saline; approximately 53 μg/kg) was then injected into the contralateral cephalic vein. Blood samples (approximately 2 ml) were removed from the cannula at 0, 2, 3, 5, 7, 10, 15, 20, 30, 40, 50 and 60 min and placed in tubes containing heparin. Plasma was obtained from each blood sample by centrifugation and stored at −20°C. At the end of each experiment, the animals were placed in a metabolic cage and urine was collected for 24 hours.

[1] Present address: Department of Surgery, Ninewells Hospital, and Medical School, University of Dundee, Dundee.

[2] Present address: School of Pharmacy, Liverpool Polytechnic, Liverpool, L3 3AF.

Samples of plasma (1.0 ml) were diluted (to 3.0 ml) with distilled water. The concentration of neostigmine or pyridostigmine in diluted plasma or urine (3.0 ml) was then determined by a gas-liquid chromatographic procedure (Chan *et al.*, 1976). The method depends on the extraction of the quaternary amines as an iodide complex, followed by pyrolysis of the ion-pair to the corresponding tertiary base. A calibration graph was derived from the analysis of standard solutions of neostigmine (0 to 200 ng/ml) and pyridostigmine (0 to 200 ng/ml), with the other quaternary amine used as an internal standard. The sensitivity limit of the method is approximately 5 ng/ml.

Results

Neostigmine was rapidly eliminated from plasma after intravenous administration (Table 1). The plasma concentration of the quaternary amine rapidly decreased between 2 and 10 min, and none could be detected in plasma after 20 minutes. In contrast, pyridostigmine was eliminated more slowly, and could be detected in plasma for at least 60 min after intravenous administration (Table 1). A semilogarithmic plot relating the plasma concentration of neostigmine (Figure 1) and pyridostigmine (Figure 2) to time was resolved into both two and three components by least squares regression analysis, using a digital computer. In each instance, the residual sums of squares between the experimental points and the computed curves were calculated. When the data for both neostigmine and pyridostigmine were analysed as a triexponential function, there was no significant reduction in the

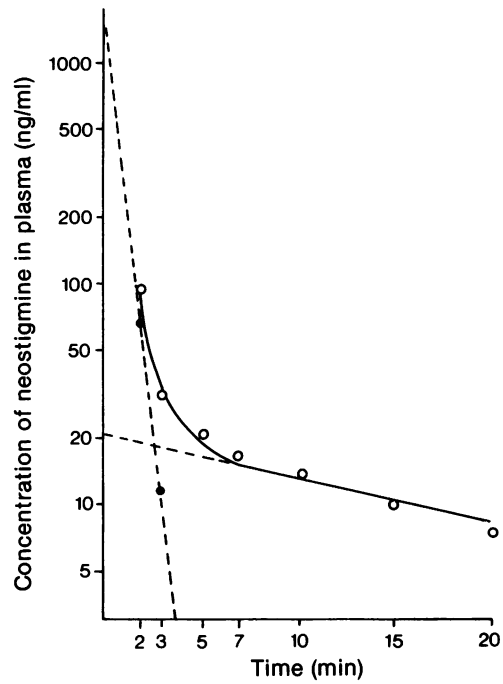

Figure 1 Plasma concentration of neostigmine after intravenous injection in the dog. The figure shows the result of a typical experiment: (○) observed results; complete line, calculated curve from the biexponential function; (●) remaining first term of the function after subtraction of the second exponential term. The dashed lines correspond to the first and second exponential terms.

Table 1 Plasma concentration of neostigmine and pyridostigmine after intravenous administration

	Plasma concentration							
	Neostigmine (ng/ml)				*Pyridostigmine (ng/ml)*			
Time (min)	dog 1	dog 2	dog 3	dog 4	dog 1	dog 2	dog 3	dog 4
0	ND	ND	ND	ND	ND	ND	ND	ND
2	85.7	79.3	64.6	93.5	81.3	138.0	124.0	114.0
3	27.5	28.2	58.0	31.5	54.5	64.3	96.1	66.9
5	17.8	17.8	24.6	20.2	34.9	47.6	62.3	48.8
7	11.5	10.7	18.3	16.1	31.3	26.7	37.2	27.2
10	10.1	9.2	12.5	10.3	22.6	22.7	30.1	26.3
15	7.3	8.4	9.3	8.6	12.3	22.0	24.0	21.2
20	ND	7.1	7.9	7.3	11.9	15.9	18.0	20.2
30	ND	ND	ND	ND	12.5	14.4	14.0	15.1
40	ND	ND	ND	ND	10.1	12.7	15.1	10.6
50	ND	ND	ND	ND	9.2	10.2	9.2	7.4
60	ND	ND	ND	ND		10.1	8.9	

ND = not detectable

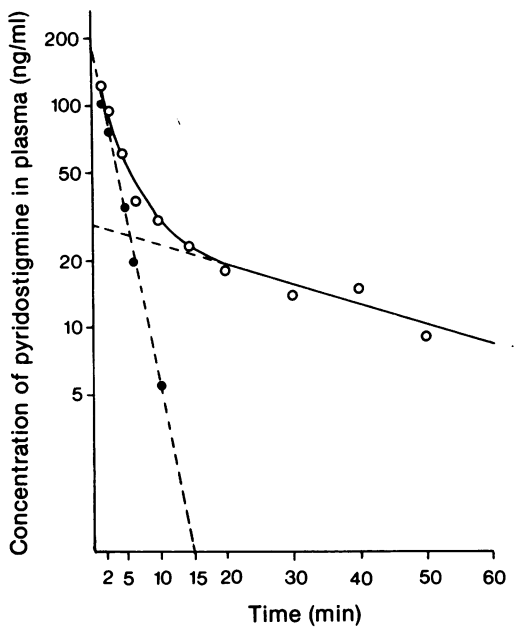

Figure 2 Plasma concentration of pyridostigmine after intravenous injection in the dog. The figure shows the result of a typical experiment: (○) observed results; complete line, calculated curve from the biexponential function; (●) remaining first term of the function after subtraction of the second exponential term. The dashed lines correspond to the first and second exponential terms.

sum of squares (neostigmine: $t = 0.339$, d.f. = 3, $0.80 > P > 0.70$; pyridostigmine: $t = 1{,}084$, d.f. = 3, $0.40 > P > 0.30$). In consequence, the plasma concentration-time curves after a single intravenous injection of neostigmine (Figure 1) and pyridostigmine (Figure 2) were invariably expressed as a biexponential function of the form $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, where C_t is the plasma concentration at time t, and A, B, α and β are constants. Values for the fast disposition half-life ($T_{1/2\alpha}$) and the slow disposition half-life ($T_{1/2\beta}$) were derived from each biexponential function. The apparent volume of distribution ($V_{d(area)}$) and the whole body clearance (C) were calculated from the following equations:

$$V_{d(area)} = \frac{\text{dose}}{\beta(\text{area under the plasma concentration-time curve between } t = 0 \text{ and } t = \infty)}$$

$$C = \frac{\text{dose}}{\text{area under the plasma concentration-time curve between } t = 0 \text{ and } t = \infty}$$

There were considerable differences in these parameters between neostigmine and pyridostigmine (Table 2). Although there was significant individual variability in the results, the apparent volume of distribution and the whole body clearance of pyridostigmine were invariably greater than neostigmine. The slow disposition half-life of pyridostigmine (23.02–82.50 min) was approximately three times longer than that of neostigmine (8.61–21.00 min), and a similar difference in the fast disposition half-life was present (Table 2). The proportion of the unchanged drug recovered in urine in 24 h was greater after administration of pyridostigmine (17.0%) than after neostigmine (9.3%).

Discussion

In the dog, the decrease in the plasma concentration of neostigmine and pyridostigmine after intravenous injection was invariably resolved into two exponential components. Both neostigmine and pyridostigmine were rapidly removed from the circulation, and there was no evidence of a third phase of exponential decline. Nevertheless, the possibility of a subsequent exponential phase that was not detected by the analytical technique cannot be entirely excluded (despite the relatively high sensitivity of the chromatographic method). This possibility clearly imposes certain restrictions on the validity of the pharmacokinetic analysis. In spite of these considerations, the present

Table 2 Pharmacokinetic parameters of neostigmine and pyridostigmine in the dog

	Apparent volume of distribution (V_d) (ml/kg)		*Whole body clearance (C) (ml min^{-1} kg^{-1})*		*Fast disposition half-life ($T_{1/2\alpha}$) (min)*		*Slow disposition half-life ($T_{1/2\beta}$) (min)*	
Dog	Neo-stigmine	Pyrido-stigmine	Neo-stigmine	Pyrido-stigmine	Neo-stigmine	Pyrido-stigmine	Neo-stigmine	Pyrido-stigmine
1	57.3	2183.9	4.6	18.3	0.35	2.99	8.61	82.50
2	141.4	1099.3	7.1	18.7	0.46	1.14	13.89	40.76
3	309.1	788.3	10.2	16.6	1.55	1.93	21.00	33.00
4	121.1	853.1	5.8	24.9	0.41	1.45	14.14	23.02

results suggest that there are important differences in the distribution, clearance and elimination of neostigmine and pyridostigmine in the dog. In the first place, the apparent volume of distribution of pyridostigmine was invariably greater than that of neostigmine, and the fast disposition half-life was approximately three times longer. These results may reflect more extensive distribution and enhanced tissue uptake of pyridostigmine, and could well be related to electrostatic differences between the quaternary amines. Secondly, both the whole body clearance and the urinary elimination of pyridostigmine are approximately twice that of neostigmine. In patients with myasthenia gravis, urinary excretion of unchanged pyridostigmine is also greater than neostigmine; in these conditions only trace amounts of neostigmine can be identified, suggesting that this drug is metabolized to a greater extent (Somani, Roberts & Wilson, 1972). Finally, the slow disposition half-life (elimination half-life) of pyridostigmine was approximately three times longer than neostigmine. Similar differences are present in rats with ligated renal pedicles (Burdfield & Calvey, 1973); indeed, in these conditions both the fast and slow disposition half-lives of the two drugs are closely comparable with the results of the present experiments. The slower elimination of pyridostigmine in both species may be related to differences in hepatic metabolism. Neostigmine is mainly metabolized by liver microsomes, and its metabolism is enhanced by the addition of $NADPH_2$; in contrast, the hydrolysis of pyridostigmine predominantly occurs in the soluble fraction of the liver cell and is not dependent on $NADPH_2$ (Burdfield, Calvey & Roberts, 1973).

The present results suggest that the longer duration of action of pyridostigmine (as compared to neostigmine) is related to the differential elimination of the two quaternary amines from plasma. Both neostigmine and pyridostigmine irreversibly inhibit acetylcholinesterase by combining with the enzyme to form a dimethylcarbamylated derivative that is slowly hydrolysed. Neostigmine has a shorter plasma half-life, and is eliminated from plasma within 20 minutes. Since the half-life of the carbamylated enzyme is approximately 37 min (Wilson, Harrison & Ginsburg, 1960; Kitz, 1964; Wilson, 1966), spontaneous recovery of the inhibited enzyme is unlikely to be followed by any significant recarbamylation. In contrast, the slower decline in the plasma concentration of pyridostigmine may be associated with significant recarbamylation of the regenerated enzyme, and may thus prolong the pharmacological effects of the quaternary amine at the neuromuscular junction.

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