Clinical pharmacology of pyridostigmine and neostigmine in patients with myasthenia gravis

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SUMMARY Determination of plasma concentration of pyridostigmine in 20 myasthenic patients on maintenance therapy revealed rather small intraindividual variations within a dose interval. The predose concentration varied considerably between different patients and up to seven fold in patients on the same daily dose. No pharmacokinetic interaction between pyridostigmine and neostigmine was found in five patients studied. In six patients the decrement in the deltoid muscle was studied in parallel with determination of the plasma concentrations following administration of pyridostigmine or neostigmine. In these patients the existence of a "bell-shaped" dose response curve is suggested with the maximal effect at a concentration of 30–60 ng/ml for pyridostigmine and 5–15 ng/ml for neostigmine.

The introduction of steroids and immunosuppressive drugs in the treatment of myasthenia gravis has not changed the rôle of cholinesterase inhibitors as the basic medical therapy of the disease. Among these drugs pyridostigmine and neostigmine are those commonly prescribed in Sweden. Therapy is in most instances pursued with pyridostigmine but sometimes a combination of the two drugs is used.

The first study on the pharmacokinetics of pyridostigmine using a gas-chromatographic method was published in 1976.¹ A more selective analytical procedure was developed by Chan *et al*² and used in the study of the pharmacokinetics of pyridostigmine³ and neostigmine.^{4,5} With this method, however, it is not possible to measure the two drugs simultaneously. The development of a more sensitive and selective analytical procedure using gas chromatography-mass spectrometry with deuterated internal standards enabled us to study the basic pharmacokinetics of pyridostigmine and neostigmine.^{6,7} The pharmacokinetic profiles of the two drugs were found to be fundamentally similar with a terminal half-life in plasma of about 1.4 and 0.9 hours for pyridostigmine and neostigmine, respec-

tively. The oral bioavailability was higher for pyridostigmine (7.6%) than for neostigmine (2%), which in combination with the longer terminal half-life might offer some pharmacokinetic advantages in maintenance therapy.

The aim of the present study was to elucidate the clinical pharmacology of pyridostigmine and neostigmine in patients with myasthenia gravis. The main objectives have been: (1) to establish the relation between daily dose and steady-state plasma concentration, (2) to investigate if combination therapy with neostigmine and pyridostigmine results in pharmacokinetic interactions, (3) to relate the pharmacokinetics of pyridostigmine and neostigmine to the effect as measured by improvement of decrement of muscle response.

Material and methods

Blood sampling and analysis of pyridostigmine and neostigmine

Blood samples were collected at regular intervals and immediately cooled on ice and plasma was separated. Two ml of plasma were transferred to a glass tube and in the case of neostigmine deuterated internal standard (d_6 -neo) was added before the samples were stored in the freezer (-18°C) to compensate for the *in vitro* degradation earlier demonstrated.⁶

Pyridostigmine and neostigmine were quantitated after ion-pair extraction and determination by gas chromatography-mass spectrometry with chemical ioniza-

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Table 1 Plasma concentrations of cholinesterase inhibitors during maintenance therapy

No	Age (yr)	Sex	Pyridostigmine daily dose mg	Interdose pyridostigmine concentration range ng/ml	Neostigmine daily dose mg	Interdose neostigmine concentration range ng/ml	Atropine daily dose mg	Prednisone alternate day dose mg
A. Patients with ocular and mild generalised myasthenia (1 and 2A)								
1	73	M	60 × 6	63–110	15 × 6	3–5	0.5 × 4	
2	72	F	120 × 6	98–115			0.5 × 4	
3	71	M	60 × 6	32–45			0.5 × 3	
4	63	F	180 × 5	210–280			0.5 × 5	
5	62	M	60 × 6	59–83			0.5 × 4	
6	58	M	60 × 6	51–74			0.5 × 4	
7	51	F	60 × 7	57	15 × 6	4		
8	50	F	60 × 6	26–44				
9	39	F	60 × 6	17–35				
10	36	M	60 × 6	39–54				
11	32	F	120 × 6	11–40			0.5 × 3	
12	28	F	60 × 6	45–74			1.0 × 2	
13	27	F	60 × 6	40–68				
14	25	F	60 × 8	64				
B. Patients with severe generalised myasthenia (2B, 3 and 4)								
15	83	M	120 × 6	190–260	15 × 3	not measured	1.0 × 1	125
16	71	M	120 × 6	102–136			1.0 × 3	125
17	71	M	60 × 8	250–318			0.5 × 3	50
18	68	F	120 × 6	220–242				
19	67	F	60 × 8	30–68	30 × 5	4–6		
20	36	M	120 × 6	54–73				

tion detection and selected ion monitoring.^{6,7} The relative standard deviations in the analysis of 20 ng/ml of pyridostigmine and neostigmine were 8 and 4%, respectively. The lower detection limits were 5 and 1 ng/ml, respectively.

Patients on maintenance therapy

Twenty patients with myasthenia gravis were studied during their ordinary oral medication with pyridostigmine. Details of the patients are presented in table 1. Four patients were on parallel therapy with neostigmine and all except three (Nos 7, 8, 14) were inpatients. The patients were classified by the criteria of Osserman and Oosterhuis⁸ in: ocular and mild generalised (1, 2A) and moderately severe and severe generalised (2B, 3, 4) forms of myasthenia. The latter group comprised six patients but at the time of the investigation three of these (15, 17, 20, table 1) had mild symptoms only, due to treatment. Three patients were treated with prednisone on alternate days and 11 used atropine. Eleven patients had additional medication such as antacids (n = 4), thiazide diuretics (n = 4), digitalis (n = 3), sulphonylureas (n = 3) and phenytoin (n = 2).

Interaction between neostigmine and pyridostigmine

In five patients a possible pharmacokinetic interaction between the two drugs was investigated. The patients were given oral neostigmine on several occasions for at least one day and on the next day five blood samples were collected regularly during a dosage interval. Thereafter the two drugs were administered together for three days and blood samples were taken as above. Neostigmine was then withdrawn and the patients were treated with pyridostigmine only. After one to three days a new series of blood samples was taken during a dosage interval. In the investigation period no other medications were given.

Decrement of muscle response at repetitive nerve stimulation

In six patients the improvement of decrement was studied after administration of pyridostigmine 5–6 mg iv (n = 3), neostigmine 0.5–1.5 mg iv (n = 5) or neostigmine 30 mg orally (n = 1). The patients had more than 25% decrement in the deltoid muscle and had not been treated with cholinesterase inhibitors previously.

The investigation was performed in the morning before the patients had left the bed or with the patients seated in a comfortable chair after minimal preceeding exercise. The patients rested completely for 60 min before the drug was given. Throughout the investigation a thermostatically controlled heating lamp was used to keep a constant skin temperature of 32°C.

Recording of the muscle response was made with surface electrodes, one over the midpoint of the deltoid muscle, the other over the acromion. Stimulation was made with surface electrodes over Erb's point with a stimulation strength set to 25% above that giving maximal amplitude, with a duration of 0.1 ms and a frequency of 2 Hz. By means of a computer (LSI 11/23) the negative amplitude and area under the whole signal was measured. The relative difference between the first and the fourth response was measured for these two parameters. The area measurement was added as a test of the recording quality. Normally the two parameters show a good correlation but in cases of artifacts they usually differ and the test has to be repeated. In the following only the amplitude results will be given. Measurements were made every tenth minute during 30–60 minutes before the administration of the drugs and then every fifth minute after intravenous and every tenth minute after oral administration for another 3 to 5 hours.

Pharmacokinetic analysis

A polyexponential equation was used to fit the plasma concentration-time data by using the computer program ESTRIP.⁹ The area under the curve was calculated by the trapezoidal rule and extrapolated to infinite time. The absolute oral biological availability was estimated approximately by comparison of the dose corrected areas under the curves with the iv data.

Results

PLASMA CONCENTRATIONS DURING MAINTENANCE THERAPY

Pyridostigmine The plasma concentration of pyridostigmine within a dose interval did not show large intraindividual variations (table 1). However, on the same daily dose the interindividual variations were 4-7 fold (tables 1 and 2). The mean daily dose was somewhat higher in the older patients (> 60 years) than in the younger group (table 3). The predose plasma concentration of pyridostigmine was also higher in the older patients ($p < 0.01$). In this group severe generalised myasthenia was more frequent (tables 1 and 3).

In the 11 patients concomitantly treated with atropine the mean maximal plasma concentration was significantly higher (139 ± 30 , $n = 11$) than in the patients not treated with atropine (78 ± 26 , $n = 9$).

In the three patients on prednisone the plasma concentration of pyridostigmine did not seem to differ from that in other patients on the same daily dose of pyridostigmine. Influences of the other medications are difficult to assess but there were no apparent systematic variations.

Neostigmine Four patients were treated simultaneously with neostigmine and pyridostigmine and the plasma concentration of neostigmine ranged from 3-6 ng/ml.

Table 2 Plasma concentrations of pyridostigmine after different daily doses

Mean daily dose, mg	No of patients	Mean interdose range of pyridostigmine, plasma concentration ng/ml ($\pm$ SE)
360	9	41 $\pm$ 5.1 - 65 $\pm$ 7.8
480	3	115 $\pm$ 68.3 - 193 $\pm$ 125
720	6	112 $\pm$ 32.4 - 144 $\pm$ 36.4
900	1	210 - 280

Table 3 Plasma concentrations of pyridostigmine in different age groups

Age group no of patients	Daily dose of pyridostigmine mg (mean $\pm$ SE)	Predose plasma concentration ng/ml (mean $\pm$ SE)	No of patients with severe myasthenia
< 60 $n = 10$	450 $\pm$ 146	49.3 $\pm$ 14.5	1
> 60 $n = 10$	582 $\pm$ 196	142.3 $\pm$ 95.4*	5

* = significantly higher ($p < 0.01$) than in the age group < 60 years of age

INTERACTION BETWEEN NEOSTIGMINE AND PYRIDOSTIGMINE

The mean interdose plasma concentration of pyridostigmine and neostigmine during a dosage interval in five patients given each drug alone and in combination can be seen in table 4. The mean pyridostigmine plasma concentrations were almost identical on the two occasions. Similarly, the mean neostigmine plasma concentration was not significantly changed when the drug was given together with pyridostigmine.

PHARMACOKINETIC ANALYSES OF SINGLE-DOSE DATA

The results are shown in table 5. For the iv data the best fit was a biexponential equation. The total plasma clearance was slightly higher for pyridostigmine (1.0 l/(hours.kg)) than for neostigmine (0.91 l/(hours.kg)) and was similar to the values observed in our earlier studies.^{6,7} The mean terminal half-life for pyridostigmine was 1.05 hours and for neostigmine 0.77 hours. However, there was a rather large interindividual variation for neostigmine. In the three patients where the pharmacokinetics of both drugs were studied the half-life for pyridostigmine was significantly longer (1.05 hours) as compared to neostigmine (0.48 hours). In the patient studied the bioavailability after oral administration of neostigmine was estimated to 2%, a figure earlier found.⁶

RELATIONSHIP BETWEEN PLASMA CONCENTRATIONS AND MUSCLE DECREMENT

Following iv injection of pyridostigmine and neostigmine a rapid decrease in muscular decrement occurred with a maximal effect after about 15 minutes. Typical experiments are shown in fig 1 (a-d). In most cases the maximal effect on decremental response remained relatively unchanged for 1-2 hours. Only one patient (GA) could manage to take part during the six hours experimental time needed to await a complete restoration of the decrement to the preinjection level.

In fig 3 (a-c) the log plasma concentrations versus the effect on the decrement (moving averages) are shown. As can be seen there was a direct linear relation between log plasma concentration and effect only for the oral data (figs 2, 3c) while the iv data are more complex. A positive linear correlation (nega-

Table 4 Interaction between neostigmine and pyridostigmine

Drug	Mean interdose plasma concentration (mean $\pm$ SE, ng/ml, n = 5)	
	Monotherapy	Combination therapy
Neostigmine	2.5 $\pm$ 1.5	3.6 $\pm$ 1.3
Pyridostigmine	51.8 $\pm$ 21.0	50.4 $\pm$ 27.5

Table 5 Pharmacokinetic analyses

Patient	Drug	Dose mg/case	r ²	t 1/2 (hr)	K _A	AUC (hr.ng . ml ⁻¹)	Vd (l . kg ⁻¹)	Cl (l . hr ⁻¹ . kg ⁻¹)
GA	pyr	5 /iv	0.999	0.76	—	74	1.23	0.97
KL	pyr	5 /iv	0.995	0.70	—	61	1.20	0.87
MB	pyr	6 /iv	0.988	1.7	—	80	2.85	1.15
MEAN $\pm$ SE	pyr	—	—	1.05 $\pm$ 0.32	—	—	1.76 $\pm$ 0.54	1.0 $\pm$ 0.08
GA	neo	1.5/iv	0.999	0.42	—	49	0.26	0.43
KL	neo	1.5/iv	0.998	0.40	—	29	0.41	0.72
MB	neo	1.5/iv	0.998	0.62	—	17	1.35	1.50
MÖ*	neo	1.0/iv	0.993	0.78	—	12	1.51	1.33
ME	neo	1.5/iv	0.929	1.95	—	64	1.0	0.36
MW	neo	0.5/iv	0.993	0.42	—	7	1.0	1.1
MEAN $\pm$ SE	neo	—	—	0.77 $\pm$ 0.24	—	—	0.92 $\pm$ 0.2	0.91 $\pm$ 0.19
UN	neo	30/orally	0.930	1.7	1.57	24	0.9†	0.89†

*In this patient muscle decrement was not determined

†Compensated for the oral bioavailability

Fig 1 a-d Plasma concentrations (Δ --- Δ) of cholinesterase inhibitor and muscular decrement ($\square$ — $\square$) following pyridostigmine or neostigmine iv to two myasthenic patients (GA and KL).

Fig 2 Neostigmine plasma concentrations (Δ --- Δ) and muscular decrement ($\square$ — $\square$) following 30 mg neostigmine orally.

tive slope) between log plasma concentration following iv injection and effect is seen when the plasma concentration is lower than 30–60 ng/ml for pyridostigmine and about 5–15 ng/ml for neostigmine. Higher concentrations give a negative correlation (positive slope) between plasma concentration and effect on muscle decrement. In one patient (ME, fig 3b) with a low clearance of neostigmine there was a negative correlation still after 3.5 hours; at this time the plasma concentration was above 6 ng/ml. In one patient (GA, fig 3b) maximal effect on decrement was seen at a very high plasma concentration of neostigmine (100 ng/ml), whereas following pyridostigmine the corresponding effect was seen at a plasma concentration similar to what was found in the other patients.

Fig 3 a-c The relation between log-plasma concentrations and effect on muscular decrement in patients with myasthenia gravis. Δ — Δ GA; $\times$ — $\times$ KL; $\circ$ — $\circ$ MB; $\blacksquare$ — $\blacksquare$ ME; $\times$ — $\times$ MW; $+$ — $+$ UN.

Discussion

PLASMA CONCENTRATION DURING MAINTENANCE THERAPY

On maintenance therapy with pyridostigmine the intraindividual variations in plasma concentration of the drug were rather small during a 3 hour dose interval. This is in agreement with results earlier obtained.⁷ The limited variation in steady-state concentration of pyridostigmine in spite of the short half-life is probably explained by the slow absorption from the gastrointestinal tract earlier shown.⁷ Much larger variations in pyridostigmine concentration during a dose interval have been reported¹⁰ and an enterohepatic circulation has been postulated due to an observed second peak in the plasma concentration curve. In the present study no such phenomenon was seen. In view of the lag-time together with food intake⁷ the "second peak" may be the plasma concentration peak of the tablet before.

The interindividual differences in steady-state plasma concentration of pyridostigmine between myasthenic patients on maintenance therapy were considerable (table 1) which is in agreement with earlier reports.^{7,10} The interindividual variations were larger and the predose plasma concentrations higher in the older patients who had a higher mean daily dose (table 3). The relation between dose and plasma concentration is, however, complex and the possible influence of a number of factors as, for example, age, severity of disease, impaired kidney function and other medications cannot be determined in any detail due to the relatively small number of patients. It is known that pyridostigmine is predominantly eliminated in the urine and a decreased renal function could lower the total clearance of the drug.¹¹⁻¹³ In the older age group more severe forms of the disease were found (tables 1 and 2) which may explain the use of higher doses of cholinesterase inhibitors. This is in accordance with the findings of White *et al*¹⁰ who observed higher plasma concentrations of pyridostigmine in "poorly controlled patients" than in "well controlled".

EFFECT OF OTHER DRUGS

It has earlier been shown that the oral bioavailability of pyridostigmine is decreased in patients on steroids¹⁰ but in the present investigation no influence of steroid therapy could be seen. Chan and Calvey¹² suggested that a competition for the renal excretion could exist between pyridostigmine and basic drugs. In our patients treated with atropine the mean maximal plasma concentration of pyridostigmine was much higher than in patients not taking atropine. This is probably not a result of phar-

macokinetic interaction but of increased need for anticholinergic therapy in patients having higher plasma concentration of pyridostigmine and thus more side-effects.

The plasma concentrations of neostigmine are in the same range (3-6 ng/ml) as earlier reported following 90-150 mg orally.⁶ Theoretically the simultaneous therapy with neostigmine and pyridostigmine could give rise to drug-drug interactions during absorption, or by competition for enzymatic degradation or renal secretion as has been postulated.^{12,14} In the present study no pharmacokinetic interaction could be detected.

PHARMACOKINETIC DATA

There were rather large interindividual variations in clearance and terminal half-lives of pyridostigmine and neostigmine. The mean half-lives of the two drugs did not differ significantly. In the crossover experiment (patients GA, KL and MB), however, there was a 2:1 relation between the half-life of pyridostigmine (1.05 hours) compared with neostigmine (0.48 hours) while the total clearance was similar. This is explained by the much lower volume of distribution for neostigmine (0.67 l/kg) compared with pyridostigmine (1.76 l/kg). The results are similar to those earlier found.^{6,7}

The rationale of parallel therapy with pyridostigmine and neostigmine with very much the same pharmacodynamic and pharmacokinetic profile could be questionable. The higher oral bioavailability and the slightly longer terminal half-life favour the use of pyridostigmine as monotherapy.

DOSE-EFFECT STUDIES

A previous study of nine myasthenic patients who were examined neurologically at 1/2 hour intervals after administration of oral pyridostigmine showed improved motor performance which in most patients seemed to be positively related to the time-course of pyridostigmine plasma concentration.¹ However, clinical evaluation of the effect involves many problems; for example standardised tests of power and fatigue in several muscles are time consuming and necessitate rather long rest between each examination. Furthermore, myasthenic weakness typically varies considerably between different muscle groups in the same patient and may be restricted to a few muscles. A certain dose of anticholinesterase may improve function of some muscles but not others. The difficulties encountered in these types of studies were recently illustrated by Davison *et al*¹⁵ who found that only two out of nine myasthenic patients on their normal drug regime showed a significant correlation between plasma levels of pyridostigmine and a global evaluation of muscle functions.

Determinations of muscle decrement only assess the function in a single muscle and can only be performed in selected patients with a pathological decrement in this muscle. A positive correlation between the plasma concentrations of pyridostigmine and the decremental response in the adductor pollicis has previously been reported in four myasthenic patients on their normal drug regime.¹⁶

In the present study there was a linear relation between log-plasma concentration and effect on muscle decrement in the range 5–30 ng/ml after iv administration of pyridostigmine and 1 to about 10 ng/ml after oral and iv administration of neostigmine (fig 3 a–c). At higher concentration the effect declined with increasing plasma concentrations suggesting “bell-shaped” dose response curves. This interpretation is in agreement with the findings by Stålberg *et al.*¹⁷ In single muscle fibre recordings some fibres responded with an increased blocking of the action potential when higher doses of edrophonium were administered, while lower doses had the opposite effect. On the other hand, it must be realised that high plasma concentrations were measured (figs 1–3) mainly in the distribution phase of the cholinesterase inhibitors implying that an equilibrium between plasma and effector compartment was not attained. Some support for the existence of a “bell-shaped” dose response curve is obtained from the “low clearance patient”, ME. In this patient there was still a negative correlation between plasma concentration of neostigmine and effect after 3–5 hours.

The interindividual differences in steady-state plasma concentration of neostigmine and pyridostigmine in patients on “optimal” therapy are considerable and our results do not at present support the existence of a generally useful “therapeutic interval”. However, there is a striking difference between the rather low plasma concentrations of pyridostigmine which give maximal effect on the decrement in previously untreated patients and the steady-state concentrations in some patients on their ordinary daily dose. Although the effect on decrement may not equal the global clinical effect, it seems important to analyse further dose-effect relationships by examining the muscle decremental response during infusion experiments, permitting the establishment of different steady-state levels of the cholinesterase inhibiting drug in the same myasthenic patient.

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