Anticholinergic activity in the serum of patients receiving maintenance disopyramide therapy

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1 The steady state serum levels of disopyramide and its metabolite, mono-*N*-dealkylated disopyramide (MND) were measured by gas-liquid chromatography in 40 patients receiving maintenance disopyramide treatment. The levels were mean $3.11 \mu\text{g/ml} \pm 0.28$ (s.e. mean) and $0.9 \mu\text{g/ml} \pm 0.13$ (s.e. mean), respectively.

2 A radioreceptor assay was used for the quantitation of the anticholinergic activity of disopyramide and its metabolite in the same serum samples.

3 The serum levels of anticholinergic activity varied between 0 and 6.7 ng/ml when measured as atropine equivalent (mean 1.88 ± 0.26 , s.e. mean, ng/ml). After a single oral dose of disopyramide the serum anticholinergic activity was, however, not detectable or very low.

4 There was a significant correlation between disopyramide concentrations in serum and the serum anticholinergic activity ($r = 0.655$, $P < 0.001$).

5 No correlation was found between MND concentrations and the anticholinergic activity arguing against the role of MND causing antimuscarinic side effects. The affinity of MND for the muscarinic receptors *in vitro* was also lower than that of disopyramide.

Keywords disopyramide mono-*N*-dealkylated disopyramide steady state serum levels serum anticholinergic activity

Introduction

Disopyramide, γ -diisopropylamino- α -phenyl- α -(2-pyridyl)-butyramide, is an anti-arrhythmic agent with a broad spectrum of efficiency (Nayler, 1976). It is metabolized in man to mono-*N*-dealkylated disopyramide (MND), which represents approximately 25% of the dose given (Aitio *et al.*, 1982a). In animal studies the metabolite has also been shown to have some activity (Grant *et al.*, 1978).

A dry mouth and other antimuscarinic side-effects are frequent problems with disopyramide treatment. They are thought to be due mainly to the metabolite of disopyramide. *In vitro* using guinea pig isolated ileum preparation the relative anticholinergic activities of disopyramide, mono-*N*-dealkyldisopyramide (MND), and

atropine have been reported to be 1, 24 and 1200, respectively (Baines *et al.*, 1976).

The quantitation of serum anticholinergic activity may provide a better means for understanding of the unwanted effects of disopyramide and its metabolite. A sensitive radioreceptor assay for atropine has been developed in our department (Aaltonen *et al.*, 1983). In this assay atropine and other anticholinergic drugs competitively inhibit the binding of the potent tritiated muscarinic antagonist quinuclidinyl benzilate ($[^3\text{H}]\text{-QNB}$) to the muscarinic receptors of rat brain membranes. The anticholinergic activity of the serum of patients treated with any drug possessing anticholinergic effects can be measured as atropine equivalents. Tune & Coyle

(1980) used a corresponding method presented in this study, to measure serum levels of anticholinergic drugs in the treatment of acute extrapyramidal side effects. On the other hand, different radioreceptor assays have been used to measure serum dopamine, 5-HT and α -adrenergic receptor blocking activities in schizophrenic patients receiving long-term chlorpromazine treatment (Nakahara *et al.*, 1983).

The aim of the present study was to find out if there is any anticholinergic activity in the serum of the patients receiving maintenance disopyramide treatment and to correlate the possible activity to serum levels of disopyramide and mono-*N*-dealkyldisopyramide.

Methods

The serum levels of disopyramide and mono-*N*-dealkyl-disopyramide (MND) were measured in 40 adult patients receiving long term oral therapy with disopyramide (300 to 800 mg daily). The blood samples were collected at steady state immediately before the dose of the drug. In addition, four volunteers were given a single oral dose of disopyramide after an overnight fast. Blood and saliva samples were collected up to 24 h after the dose. Mixed saliva was collected by chewing Parafilm®. The volume of saliva in the collection time of 5 min was recorded.

The analysis of disopyramide and MND was carried out by a gas-liquid chromatographic method with alkaline flame ionization detector according to Aitio (1979). Calibration graphs obtained by adding known amounts of disopyramide and MND to human plasma samples were straight lines in the range of 0.5–12.5 $\mu\text{g/ml}$ for disopyramide and 0.5–7.5 $\mu\text{g/ml}$ for MND. The extraction efficiency for disopyramide was $71.6 \pm 7.8\%$ ($n = 22$) at disopyramide levels between 1.5 and 10.0 $\mu\text{g/ml}$. The coefficient of variation within batch of the assay was less than 6% for disopyramide at levels < 2.5 , between, and > 4.0 $\mu\text{g/ml}$, and for MND 13.2% in all determinations (0–5.6 $\mu\text{g/ml}$). The coefficient of variation between batch for disopyramide was 5.5% at 4.0 $\mu\text{g/ml}$ and for MND 17.3% at 1.0 $\mu\text{g/ml}$.

The serum levels of anticholinergic activity in the same samples were determined by a radioreceptor assay according to Aaltonen *et al.* (1983). The incubation medium (300 μl) contained 50 mM Tris-HCl buffer (pH 7.4), 0.95 nM [^3H]-QNB (1-quinuclidinyl (phenyl-4-[^3H])benzilate, specific activity 35 Ci/mmol, Radiochemical Centre, Amersham, England), 25 μl of serum and 200 μl (0.1 mg of protein) of rat brain

membrane suspension. Membrane suspension was prepared as described by Aaltonen & Scheinin (1982). The incubation was performed at room temperature for 45 min and unbound radioactivity was separated by filtration through Whatman GF/B glass fibre filters under vacuum, and by washing the filters, three times, with 5 ml of ice-cold buffer. Specific binding of [^3H]-QNB was defined as the amount displaced by 100 $\mu\text{g/ml}$ atropine. The serum levels of anticholinergic activity were expressed as atropine equivalents (A-E) in ng/ml. The concentration dependent inhibition of specific [^3H]-QNB binding by atropine calibration standards (0.5–100 ng/ml) showed linear regression lines in the logit/log concentration plot. A day-to-day variation in the percentage of inhibition, produced by 1.25 ng/ml standard, was less than 3%. The interassay coefficient of variation for atropine was 2.8% at 5 ng/ml.

The concentrations of atropine, disopyramide and mono-*N*-dealkylated disopyramide producing a 50% inhibition in the specific binding of [^3H]-QNB (IC_{50} -values) were estimated graphically under standard conditions. k_i -values were estimated from the equation $k_i = \text{IC}_{50}/(1 + C/k_D)$, where k_D is the dissociation constant for QNB, and C the concentration of the labelled ligand in the binding assay (Bennett, 1978). k_D for the binding at the equilibrium was 0.48 nM and the maximum number of binding sites 1.42 pmol/mg protein determined by Scatchard analysis of saturation experiments using five concentrations of [^3H]-QNB (between 0.52 nM and 52.0 nM) and a linear regression programme for the assessment of the linearity of the binding data.

Correlations between disopyramide or MND concentrations and anticholinergic activity were tested with linear regression analysis.

Results

The serum levels of disopyramide and its metabolite mono-*N*-dealkylated disopyramide (MND) measured by gas-liquid chromatography (g.l.c.) are shown in Table 1. The steady state disopyramide concentrations varied from 0.2 to 8.3 $\mu\text{g/ml}$ (mean $\pm$ s.e. mean 3.11 ± 0.28 $\mu\text{g/ml}$) and those of MND from 0 to 4.1 $\mu\text{g/ml}$ (mean $\pm$ s.e. mean 0.9 ± 0.13 $\mu\text{g/ml}$).

The anticholinergic activities were measured by a radioreceptor assay (RRA). The IC_{50} - and k_i -values (mean $\pm$ s.e. mean, $n = 5$, final concentrations) of atropine, disopyramide and MND required for 50% inhibition of specific binding of [^3H]-QNB were $1.47 \text{ ng/ml} \pm 0.10$, $0.28 \text{ ng/ml} \pm$

Table 1 The mean steady state concentrations of disopyramide and mono-*N*-dealkylated disopyramide (MND) and the mean anticholinergic activity in serum samples derived from 40 patients receiving continuous disopyramide treatment. The correlation coefficients (*r*) between the individual disopyramide or MND concentrations and the anticholinergic activity are shown below.

	Disopyramide ($\mu\text{g/ml}$)	MND ($\mu\text{g/ml}$)	Anticholinergic activity, A-E (ng/ml)
Mean	3.11	0.90	1.88
s.d.	1.78	0.85	1.62
s.e. mean	0.28	0.13	0.26
	$P < 0.001$	NS	
<i>r</i>	0.66	0.10	

0.02; $6.89 \mu\text{g/ml} \pm 0.19$, $1.29 \mu\text{g/ml} \pm 0.04$ and $9.67 \mu\text{g/ml} \pm 0.46$, $1.82 \mu\text{g/ml} \pm 0.09$, respectively. The serum levels of anticholinergic activity in 40 serum samples varied between 0 and 6.70 ng/ml as atropine equivalents, mean $\pm$ s.e. mean 1.88 ± 0.26 ng/ml (Table 1). The lowest measurable level of atropine was 0.5 ng/ml. Out of the 40 patients, only one had no detectable serum level of anticholinergic activity and four slightly below 0.5 ng/ml (about 0.3–0.4 ng/ml).

The anticholinergic activities measured by RRA were compared to serum levels of diso-

pyramide and MND measured by g.l.c. There was a clear linear correlation between disopyramide concentrations and the anticholinergic activity ($r = 0.655$, $P < 0.001$, Table 1, Figure 1). On the contrary, no significant correlation was found between MND concentrations and the anticholinergic activity in the serum (Table 1).

Serum levels of disopyramide, mono-*N*-dealkylated disopyramide and serum anticholinergic activities with % change in saliva flow in four volunteers after a single oral dose of disopyramide are shown in Table 2.

Discussion

The antimuscarinic activity in the patients receiving the maintenance disopyramide treatment was measured in this study only as atropine equivalents in the plasma. No clinical tests on the state of cholinergic nervous system was carried out on these patients. Because of the late analysis of antimuscarinic activity in the plasma samples of the patients, it was not possible either to ask patients their anticholinergic symptoms.

Tune & Coyle recently (1980) used radio-receptor assay for measuring anticholinergic drug concentrations in plasma. They also used the competitive inhibition of these drugs with the specific binding of tritiated quinuclidinyl benzilate (QNB) to brain muscarinic receptors. Anti-

Figure 1 The relationship of serum anticholinergic activity as atropine equivalents (A-E, ng/ml) to the serum concentration of disopyramide. The serum samples were obtained from 40 patients receiving continuous disopyramide treatment. Anticholinergic activity was assayed by RRA and disopyramide concentration by g.l.c. The correlation coefficient was 0.655 ($P < 0.001$).

Table 2 The serum levels of disopyramide (D) and mono-*N*-dealkylated disopyramide (MND) and the serum anticholinergic activity (A-E) with % change in saliva flow in four volunteers after a single oral dose of disopyramide.

Volunteer	Dose		Time after the dose of disopyramide							
			30 min	60 min	2 h	3 h	4 h	5 h	7 h	24 h
1	200 mg	D ($\mu\text{g/ml}$)	1.7	2.3	2.4	4.3	2.0	1.9	1.6	0.3
		MND ($\mu\text{g/ml}$)	0	0	0	0.4	0.2	0.2	0.2	0.1
		A-E (ng/ml)	<0.5	<0.5	<0.5	<0.5	0.8	0.9	0.8	<0.5
		% change in saliva flow	0	-13.0	-23.0	-28.0	+23.0	-9.0	+40.0	
2	400 mg	D ($\mu\text{g/ml}$)	1.9	3.8	4.4	4.2	3.6	3.1	2.6	0.7
		MND ($\mu\text{g/ml}$)	0.1	0.2	0.2	0.4	0.5	0.3	0.2	0.1
		A-E (ng/ml)	0.9	<0.5	<0.5	<0.5	<0.5	1.0	0.7	<0.5
		% change in saliva flow	-76.0	-85.0	-70.0	-60.0	-50.0	-10.0	-10.0	
3	400 mg	D ($\mu\text{g/ml}$)	3.0	2.9	3.0	3.1	2.5	2.5	2.4	0.4
		MND ($\mu\text{g/ml}$)	0	0.1	0.1	0.1	0.1	0.1	0.1	0
		A-E (ng/ml)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	
		% change in saliva flow	-60.0	-65.0	-53.0	-54.0	-43.0	-22.0	+16.0	+9.5
4	400 mg	D ($\mu\text{g/ml}$)	0	0.6	3.4	3.6	3.3	3.0	2.7	0.6
		MND ($\mu\text{g/ml}$)	0	0	0.1	0.2	0.2	0.3	0.2	0
		A-E (ng/ml)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	
		% change in saliva flow	+33.0	+30.0	-27.0	-58.0	-22.0	-10.0	+8.0	+30.0

cholinergic activity could be detected regardless of drug structure and was quantitated against atropine standards as in our experiments. In the study of Tune & Coyle (1980), there was a highly significant inverse correlation between the presence of acute extrapyramidal side effects, due to neuroleptics, and the serum levels of anticholinergics. In our experiments, a quantitation of anticholinergic activity as a side effect of disopyramide could be done. It seems logical to assume that the method in question could be used in the quantitation of anticholinergic side effects of any drug producing these, regardless of the molecular structure of the drug. In fact, we have preliminary experience of such quantitation of antimuscarinic activity during chronic treatment of several different drugs known to produce anticholinergic side effects.

There are, however, differences between various drugs in the affinity to the receptors, as well as in the speed of manifestation of antimuscarinic effects in the plasma after the administration of the drug treatment. Thus, atropine itself, when injected into the circulation, readily competes with QNB on the occupancy of muscarinic receptors, and the disappearance of the drug from the blood can be followed (Aaltonen *et al.*, 1983). In our experiments, the affinity of atropine *in vitro* for the muscarinic receptors of the brain membrane preparation was over 1000 times higher compared to that of disopyramide or its mono-*N*-dealkylated metabolite (MND). It is possible that a more chronic treatment would be required in order to develop the more stable binding to muscarinic receptors. Thus, the concentrations of disopyramide are correlated to

the antimuscarinic effect only when the 'steady state' between occupation of receptors and the drug in plasma will be reached.

We tried to stimulate acute antimuscarinic effects of disopyramide in four voluntary persons by giving a single oral dose of the drug. In agreement with the findings of Aitio *et al.* (1982a), the concentrations of disopyramide rose up to 2–4 $\mu\text{g/ml}$ in about 2 to 3 h. The peak levels are of the same order of magnitude as the levels found in the course of maintenance treatment. The drug also caused, simultaneously, a rapidly vanishing decrease of salivary flow. The tension during the test situation may have decreased the excretion of saliva before the maximum concentrations of the drug were achieved in two of the volunteers. The antimuscarinic activity of the serum measured as atropine equivalents remained, however, very low. In two out of the four volunteers some degree of antimuscarinic effect was measurable at 4 to 7 h after the oral dose. Further experiments will be required although it seems likely, at the present, that the occupancy of muscarinic receptors is best seen during chronic treatment.

The antimuscarinic side effects of disopyramide are thought to be due mainly to the mono-*N*-dealkylated metabolite of the drug (MND). *In vitro*, MND has an activity which is about 24 times that of the anticholinergic activity of disopyramide (Baines *et al.*, 1976). There are, however, reversed clinical and experimental findings to this concept. Thus, Kumana *et al.* (1982) found only low levels of MND in six patients with urinary retention after disopyramide. Aitio *et al.* (1982b) found an inverse relation between saliv-

ary flow rate and the serum levels of unchanged disopyramide after a single dose in volunteers. In our study, the IC_{50} value was lower for disopyramide in comparison to MND, when the affinity to muscarinic receptors was measured *in vitro*. In agreement with this, we could show with our preliminary findings in 27 patients (Iisalo & Aaltonen, 1983) a clear correlation between antimuscarinic activity measured as atropine equivalents and the concentration of unchanged disopyramide during chronic treatment. There

was no significant correlation between MND and the antimuscarinic effect. It seems obvious that the antimuscarinic effects of disopyramide are not due to the metabolite.

In future, clinical signs of anticholinergic effect should be compared during a long term treatment with the antimuscarinic activity in the plasma. We believe that a quantitation of antimuscarinic side effects of many other drugs will be possible with the method used in this study.

References

- Aaltonen, L., Kanto, J., Iisalo, E. & Pihlajamäki, K. (1983). Comparison of radioreceptor assay and radioimmunoassay for atropine: pharmacokinetic application. Paper read at the Joint Meeting of the Scandinavian and Italian Pharmacological Societies (Taormina).
- Aaltonen, L. & Scheinin, M. (1982). Application of radio-receptor assay of benzodiazepines for toxicology. *Acta Pharmac. Tox.*, **50**, 206–212.
- Aitio, M.-L. (1979). Simultaneous determination of disopyramide and its mono-N-dealkylated metabolite in plasma by gas-liquid chromatography. *J. Chromatogr.*, **164**, 515–520.
- Aitio, M.-L., Allonen, H., Kanto, J. & Mäntylä, R. (1982a). Pharmacokinetics of disopyramide and mono-N-dealkyldisopyramide in man. *Int. J. clin. Pharmac. Ther. Tox.*, **20**, 219–226.
- Aitio, M.-L., Virtanen, R. & Lammintausta, R. (1982b). Disopyramide concentrations in saliva. *Int. J. clin. Pharmac. Ther. Tox.*, **20**, 68–72.
- Baines, M. W., Davies, J. E., Kellett, D. N. & Munt, P. L. (1976). Some pharmacologic effects of disopyramide and a metabolite. *J. int. med. Res.*, **4**, (Suppl.1), 5–7.
- Bennett, J. P. (1978). Methods in binding studies. In *Neurotransmitter receptor binding*, eds. Yamamura, H. I., Enna, S. J. & Kuhar, M. J., pp. 57–90. New York: Raven Press.
- Grant, A. M., Marshall, R. J. & Ankier, S. I. (1978). Some effects of disopyramide and its N-dealkylated metabolite on isolated nerve and cardiac muscle. *Eur. J. Pharmac.*, **49**, 389–394.
- Iisalo, E. & Aaltonen, L. (1983). Antimuscarinic side effects of disopyramide. *Lancet*, **i**, 996–997.
- Kumana, C. R., Rambihar, V. S., Willis, K., Gupta, R. N., Tanser, P. H., Cairns, J. A., Wildeman, R. A., Johnston, M., Johnson, A. L. & Gent, M. (1982). Absorption and antidysrhythmic activity of oral disopyramide phosphate after acute myocardial infarction. *Br. J. clin. Pharmac.*, **14**, 529–537.
- Nakahara, T., Hirano, M., Uchimura, H., Saito, M., Kim, J., Matsumoto, T., Yokoo, H., Shimomura, M. & Mukai, A. (1983). Dopamine, serotonin and alpha-adrenergic receptor blocking activities in serum and their relationships to prolactin level in schizophrenic patients receiving long-term chlorpromazine treatment. *Psychopharmacology*, **79**, 266–270.
- Nayler, W. G. (1976). The pharmacology of disopyramide. *J. int. med. Res.*, **4**, (Suppl.1), 8–12.
- Tune, L. & Coyle, J. T. (1980). Serum levels of anticholinergic drugs in treatment of acute extrapyramidal side effects. *Arch. gen. Psychiat.*, **37**, 293–297.

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