The effect of infinitesimal drug dilutions on the pharmacokinetics of nalidixic acid and atenolol

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- 1 Ten healthy subjects received two treatments: a single 1 g oral dose of nalidixic acid (NA) followed 1 h later by either an infinitesimal dilution of the drug (NA 7CH) or by succussed water which served as placebo. The study was repeated 18 months later in 10 different subjects.
- 2 A further 10 healthy subjects received three treatments: a single 100 mg oral dose of atenolol (AT) followed 3 h later by either placebo or a dilution of AT (AT 7CH) or of bisoprolol (BI 7CH). The homoeopathic preparations were administered by the sublingual route.
- 3 In the first NA experiment NA 7CH significantly shortened the elimination half-life of NA from 8.6 ± 2.2 (placebo) to 6.4 ± 1.6 h (NA 7CH). In the second NA experiment none of the pharmacokinetic parameters was modified significantly by the administration of NA 7CH. Neither AT 7CH nor BI 7CH modified the pharmacokinetics of AT.

Keywords pharmacokinetics homoeopathy nalidixic acid atenolol

Introduction

Homoeopathic therapy is based on the 'similimum' principle (Martiny, 1979), which postulates that infinitesimal dilutions of a drug given to a patient oppose the symptoms induced in healthy subjects by large doses of the same compound. Although many experiments have been carried out *in vitro* (Benveniste, 1981; Boiron *et al.*, 1983; Davenas *et al.*, 1988; Poitevin *et al.*, 1985, 1986) and in animals (Boiron & Cier, 1962; Fisher, 1983; Lapp *et al.*, 1955, 1958) to assess the effectiveness of homoeopathic preparations, few clinical trials have been performed (Aulas, 1985; Ferley *et al.*, 1989; Reilly *et al.*, 1986).

Boiron & Cier (1962) showed that in rats intoxicated with arsenic, the urinary excretion of this compound was increased significantly by the administration of homoeopathic dilutions of arsenic. Following the same principle, the aim of the present study was to determine in humans whether or not, an infinitesimal dilution of a drug affects the elimination of the same drug given at an allopathic dosage. Thus, using double-blind placebo-controlled protocols, two drugs were studied: i) nalidixic acid (NA), which undergoes extensive metabolism to form 7-hydroxynalidixic acid (HNA) and 3,7-dicarbonylnalidixic acid (CNA) (Cuisinaud *et al.*, 1982; Ferry *et al.*, 1981, 1982, 1984; Männistö, 1976; Vree *et al.*, 1988),

ii) atenolol (AT), which is eliminated mostly as unchanged drug in the urine and faeces (McAinsh, 1977; McAinsh *et al.*, 1980; Reeves *et al.*, 1978). To determine the specificity of any effect of infinitesimal dilution the influence on the kinetics of AT of dilutions of both AT and bisoprolol (BI), another cardioselective β -adrenoceptor antagonist, was evaluated.

Methods

Chemicals

Commercially available allopathic formulations were used: nalidixic acid, 1 g tablet (Negram forte®, Winthrop Labs.) and atenolol, 100 mg tablet (Tenormin®, ICI Laboratories).

Homoeopathic dilutions were prepared by Boiron Laboratories (Ste Foy-lès-Lyon, France). Nalidixic acid, atenolol and bisoprolol (Detensiel®, Merck-Clevenot Labs) pure substances were provided by the respective manufacturers. Initial solutions were obtained by dissolving 1 mg of each compound in 1 ml of 0.03 N NaOH (NA) or of ethanol (AT and BI). These solutions were

first diluted 1/10 succussed with double-distilled water to obtain the so-called 2CH (second 'centésimale hahnemannienne') dilution. This last solution was diluted (1/100) successively under the same conditions until the 7CH dilution was obtained. The placebo was prepared according to the same protocol using double-distilled water. Homoeopathic preparations were packaged in 1 ml vials, assigned a code number by Boiron Laboratories and deposited with a lawyer until the experiments were complete.

Study design

The study protocol was approved by the local Ethics Committee, and each subject gave written informed consent. Details of the subjects are given in Table 1. None had clinical or biochemical evidence of hepatic, haematological or renal disease. No concurrent medication was taken for 2 weeks before the study. Each volunteer, received treatments and placebo in a randomized order at a 2 week interval.

Nalidixic acid studies After a light breakfast without fat, 10 subjects received a single 1 g oral dose (one tablet) of NA with 100 ml of tap water. One hour later 5×1 ml (every minute) of infinitesimal dilutions of either NA (NA 7CH) or placebo were given sublingually. The study was repeated in a further 10 subjects 18 months later, according to the same protocol. Sixteen (first study) and 19 (second study) serial heparinized venous blood samples were collected from each subject and stored at 4° C for 2 h when plasma was obtained by centrifugation. The plasma was then stored at -20° C until analysis. Urine was collected before and over the 48 h period after dosing and 5 ml aliquots were stored at -20° C.

Atenolol study Ten subjects received a single oral dose of 100 mg of AT with 100 ml of tap water. Three hours later 5×1 ml of infinitesimal dilutions of either AT (AT 7CH), BI (BI 7CH) or placebo were given sublingually.

Sixteen serial venous blood samples and 11 urine collections were obtained from each subject and stored as described above.

Assay methods

Nalidixic acid Plasma and urine samples were assayed for NA, HNA and CNA by the h.p.l.c. method of Cuisinaud *et al.* (1980). The assay was linear for all compounds up to $50 \mu\text{g ml}^{-1}$ plasma and up to $150 \mu\text{g ml}^{-1}$ urine. The lower limit was 20 ng ml^{-1} for all compounds in urine. This sensitivity was improved to 10 ng ml^{-1} in both plasma and urine in the second study by using a different u.v. detector. The intra- and inter-assay coefficients of variation determined at 1 and $10 \mu\text{g ml}^{-1}$ were less than 6.5% and 9.7%, respectively.

Atenolol Atenolol together with nadolol (Corgar®, Squibb Labs) as internal standard were extracted from alkalised plasma with 8 ml ethyl acetate. Chloroform was used for urine extractions. The separated organic phase was evaporated to dryness under nitrogen and the residue was dissolved and injected onto a C18 Nucleosil (10 μm) reversed-phase column (250 $\times$ 4.6 mm) with a mobile phase (flow-rate = 1.5 ml min^{-1}) of methanol, distilled water and acetic acid (37 : 52 : 1, v/v/v) with 1.2 g l^{-1} of heptane sulphonic acid as counter-ion. A fluorescence detector was used with excitation at 280 nm and emission at 305 nm. The assay was linear up to $5 \mu\text{g ml}^{-1}$ plasma and $50 \mu\text{g ml}^{-1}$ urine. The lower limit was 10 ng ml^{-1} for both plasma and urine. Intra- and interassay coefficients of variation determined at 0.2 and $2 \mu\text{g ml}^{-1}$ in plasma and 2 and $20 \mu\text{g ml}^{-1}$ in urine were less than 4.5% and 6.6%, respectively.

All samples from each subject were analysed in the same run.

Pharmacokinetic analysis

Individual plasma drug concentration-time curves were fitted using a non linear weighted least squares multi-

Table 1 Characteristics (mean $\pm$ s.d.) of subjects included in the studies

Study	Age (years)	Height (cm)	Weight (kg)	Plasma creatinine ($\mu\text{g ml}^{-1}$)	Creatinine clearance* (ml min^{-1})
<i>Nalidixic acid studies</i>					
<i>First study</i>					
Placebo	26 $\pm$ 3	173 $\pm$ 8	64.9 $\pm$ 12.3	11.8 $\pm$ 1.0	107 $\pm$ 22
NA 7CH			64.4 $\pm$ 12.0	11.3 $\pm$ 1.3	111 $\pm$ 22
<i>Second study</i>					
Placebo	28 $\pm$ 4	173 $\pm$ 9	66.5 $\pm$ 11.3	8.9 $\pm$ 1.6	124 $\pm$ 25
NA 7CH			66.7 $\pm$ 11.2	9.0 $\pm$ 1.3	117 $\pm$ 22
<i>Atenolol study</i>					
Placebo	24 $\pm$ 3	174 $\pm$ 7	67.0 $\pm$ 10.1	10.1 $\pm$ 1.9	125 $\pm$ 18
AT 7CH			66.9 $\pm$ 10.3	9.9 $\pm$ 1.9	127 $\pm$ 21
BI 7CH			66.8 $\pm$ 10.0	10.0 $\pm$ 1.7	126 $\pm$ 20

*Creatinine clearance normalized for a body area of 1.73 m^2 .

exponential regression program (SIPHAR®, SIMED, Créteil, France) which used the algorithm of Powell (1964). For NA, HNA and AT all data were described by a triexponential function (Ferry *et al.*, 1981; Männistö 1976; McAinsh, 1977; McChesney *et al.*, 1964). Goodness-of-fit was evaluated using Likelihood, Akaike, Schwartz and Leonard tests. The kinetic analyses were carried out such that the data sets were analysed blind.

The following parameters were derived (Gibaldi & Perrier, 1982): apparent absorption half-life ($t_{1/2, \text{abs}}$), maximum plasma drug concentration (C_{max}), time to C_{max} (t_{max}), terminal elimination half-life ($t_{1/2, \text{z}}$), mean residence time (MRT), area under the curve (AUC(0,48h)) using the linear trapezoidal rule and extrapolated to infinity (AUC), cumulative urinary excretion (Ae(0,48h)) and renal clearance (CL_{R}) as the ratio Ae(0,48h)/AUC(0,48h).

Statistical analysis

The results are expressed as means $\pm$ s.d. A zero value was assigned to plasma or urine drug concentrations

below the minimum assayable level. The influence of the treatment was examined for all pharmacokinetic parameters using three tests:

- the Wilcoxon matched-paired signed-ranks test for non parametric data,
- the Student's *t*-test for paired data
- and the single-factor analysis of variance (ANOVA) including a Bartlett's test to confirm the homogeneity of variance. A value of $P < 0.05$ was taken as being statistically significant.

The power of the study was estimated *a priori* and calculated *a posteriori* from differences between means of groups and variability within groups at a 95% confidence interval.

Results

Nalidixic acid

Figures 1 and 2 show the plasma NA and HNA concentration vs time profiles (mean $\pm$ s.d.) after administration

Figure 1 Mean ($\pm$ s.d.) plasma nalidixic acid concentration-time curves and individual half-lives observed in the first (a,c) and second studies (b,d). (Ten healthy volunteers received a single oral 1 g dose of nalidixic acid followed 1 h later by the administration of a homoeopathic dilution of nalidixic acid (■, NA 7CH) or a placebo (□, placebo)).

Figure 2 Mean ($\pm$ s.d.) plasma hydroxynalidixic acid concentration-time curves and individual half-lives observed in the first (a,c) and second studies (b,d). (Ten healthy volunteers received a single oral 1 g dose of nalidixic acid followed 1 h later by the administration of a homeopathic dilution of nalidixic acid (■, NA 7CH) or a placebo (□, placebo)).

of placebo or NA 7CH. Derived pharmacokinetic parameters are listed in Table 2. As reported previously (Ferry *et al.*, 1981, 1982, 1984), concentrations of CNA were too low to allow kinetic analysis.

In the first study the only significant change induced by NA 7CH compared with placebo was a slight (from 8.6 to 6.4 h) but statistically significant decrease in the terminal elimination half-life of NA. This change was not accompanied by any significant alteration in the kinetics of HNA nor by changes in the renal clearance and the cumulative urinary excretion of NA. The decrease in half-life was not confirmed by the second study in a different set of subjects.

Atenolol

Neither AT 7CH nor BI 7CH altered the kinetics of atenolol significantly (Figure 3, Table 2).

Discussion

The protocols used involved single oral administration of a therapeutic dose of drug followed by either placebo or an infinitesimal dilution given at a time when absorption was expected to be complete. The dilution used was 7CH, which represents a dose of about 10^8 molecules. This choice was based on previous reports of optimal dilution-effect relationships (Boiron *et al.*, 1983). A subject sample size of 10 was used as it was predicted to allow the demonstration of a 50% difference in drug elimination half-life with 95% confidence and 90% power. The first experiment, using NA, gave a positive result as NA7CH induced a significant decrease in NA elimination half-life. However, the results of the replicate study was negative. This discrepancy between the two NA studies may be explained by the small change in NA half-life (-25%) observed in the first study. Thus, according to

Table 2 Pharmacokinetic parameters (mean $\pm$ s.d.) of NA, HNA and AT obtained after a single oral 1 g-dose of NA and 100 mg of AT followed respectively 1 h and 3 h later by the administration of either a 7CH homoeopathic dilution (7CH) or a placebo in 10 healthy volunteers

Compound	Treatment	$t_{1/2,z}$ (h)	$t_{1/2,abs}$ (h)	AUC ($\mu\text{g ml}^{-1}\text{ h}$)	C_{max} ($\mu\text{g ml}^{-1}$)	t_{max} (h)	MRT (h)	Ae(0,48h) (mg)	CL_R (ml min^{-1})
<i>Nalidixic acid</i>									
<i>First study</i>									
NA	Placebo	8.6 ± 2.2	0.51 ± 0.14	103 ± 37	29.1 ± 10.4	2.3 ± 1.3	5.3 ± 2.2	18 ± 6	3.2 ± 1.6
	NA 7CH	$6.4 \pm 1.6^*$	0.53 ± 0.14	100 ± 39	28.0 ± 7.5	2.6 ± 0.9	4.9 ± 0.9	21 ± 6	3.9 ± 1.6
HNA	Placebo	8.4 ± 3.5	0.83 ± 0.15	37 ± 13	5.7 ± 1.9	3.7 ± 0.6	8.1 ± 2.2	93 ± 35	47 ± 23
	NA 7CH	7.8 ± 1.6	0.74 ± 0.20	32 ± 14	5.1 ± 1.9	3.9 ± 1.3	7.5 ± 0.9	99 ± 42	57 ± 22
<i>Second study</i>									
NA	Placebo	7.9 ± 2.2	0.63 ± 0.14	79 ± 27	21.1 ± 5.2	1.9 ± 0.7	4.8 ± 1.7	24 ± 6	6.0 ± 3.2
	NA 7CH	7.7 ± 1.8	0.63 ± 0.19	75 ± 20	21.5 ± 5.6	2.4 ± 0.9	4.3 ± 1.0	21 ± 6	4.9 ± 2.1
HNA	Placebo	8.8 ± 2.3	0.90 ± 0.28	28 ± 18	4.4 ± 2.5	2.9 ± 0.9	7.0 ± 1.3	118 ± 84	82 ± 57
	NA 7CH	7.9 ± 3.0	0.85 ± 0.21	28 ± 13	4.7 ± 2.1	3.7 ± 1.4	7.0 ± 1.0	124 ± 60	73 ± 44
<i>Atenolol</i>									
	Placebo	8.6 ± 3.5	0.64 ± 0.37	4.6 ± 2.1	0.48 ± 0.22	3.1 ± 1.0	11.4 ± 3.0	37 ± 21	129 ± 36
	AT 7CH	10.0 ± 4.4	0.76 ± 0.43	5.2 ± 1.6	0.51 ± 0.21	3.2 ± 1.1	11.6 ± 3.3	37 ± 15	123 ± 34
	BI 7CH	9.3 ± 4.4	0.56 ± 0.31	4.9 ± 2.5	0.48 ± 0.27	2.8 ± 0.9	12.3 ± 4.7	34 ± 13	123 ± 36

*Significantly different from placebo at $P < 0.01$ (Student and Wilcoxon's tests) and at $P < 0.05$ (ANOVA).

Figure 3 Mean ($\pm$ s.d.) plasma atenolol concentration-time curves (a,b) and individual half-lives (c,d) observed in the 10 healthy volunteers after a single oral 100 mg dose of atenolol followed 3 h later by the administration of a homoeopathic dilution of atenolol (■, AT 7CH) bisoprolol (△, BI 7CH) or placebo (□, placebo).

the residual variance in the first NA study the second study should have included 23 subjects in order to demonstrate a comparable change in drug elimination.

Therefore, we conclude that, using the protocols described, it was not possible to demonstrate a significant effect of a 7CH homeopathic dilution on the pharmacokinetics of the two model drugs.

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