

Dramatic inhibition of amiodarone metabolism induced by grapefruit juice

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Aims Grapefruit juice increases blood concentrations of many drugs metabolized by CYP3A. Amiodarone is metabolized by CYP3A to *N*-desethylamiodarone (N-DEA). The aim of this study was to determine amiodarone kinetics when administered with and without grapefruit juice.

Methods Eleven healthy adult volunteers took part in a single sequence, repeated-measures design study. Each subject, who had been evaluated 6 months previously for amiodarone pharmacokinetics, was given a single oral dose of amiodarone (17 mg kg⁻¹) with three glasses of 300 ml of grapefruit juice on the same day.

Results Grapefruit juice completely inhibited the production of N-DEA, the major metabolite of amiodarone, in all subjects and increased the area-under-the-curve (AUC) and maximum concentration of amiodarone (C_{\max}) by 50% and 84%, respectively, as compared with the control period during which water had been administered instead of grapefruit juice (AUC: 35.9 ± 14.3 vs 23.9 ± 11.2 $\mu\text{g ml}^{-1}$ h, $P < 0.005$ and C_{\max} : 3.45 ± 1.7 vs 1.87 ± 0.6 $\mu\text{g ml}^{-1}$, $P < 0.02$, respectively) (means \pm s.d.). This inhibition of N-DEA production led to a decrease in the alterations caused by amiodarone on PR and QTc intervals.

Conclusions Grapefruit juice dramatically alters the metabolism of amiodarone with complete inhibition of N-DEA production. These results are in agreement with *in vitro* data pointing to the involvement of CYP3A in the metabolism of amiodarone and suggests that this interaction should be taken into account when prescribing this antiarrhythmic drug.

Keywords: amiodarone, grapefruit juice, interaction, metabolism, pharmacokinetics

Introduction

Amiodarone is an antiarrhythmic with predominantly class III (Vaughan – William's classification) effects [1]. The major metabolite of amiodarone, *N*-desethylamiodarone (N-DEA), has been identified in humans as a consequence of hepatic and possibly intestinal mucosa *N*-dealkylation [2–4]. Both amiodarone and N-DEA are characterized by long elimination half lives with an average of 50 days for

the parent drug and 60 days for the metabolite [5–7]. Formation of N-DEA is mediated by cytochrome P450 3A activity in both rodent and human microsomes [8–10].

There is marked intersubject variation in amiodarone elimination which can be explained, at least in part, by differences in CYP3A4 and formation of N-DEA. Since grapefruit juice has been shown to inhibit CYP3A4 activity significantly, in both the intestine and the liver, thus reducing first-pass metabolism, the bioavailability of various orally administered drugs is markedly increased. Examples are dihydropyridine calcium-channel blockers, cyclosporin, the benzodiazepines midazolam and triazolam and the antihistamine terfenadine [11–17]. A better

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understanding of the origin of the variations of N-DEA production is important and might lead to some guidelines for limiting the use of concurrently prescribed treatments or beverage intake.

It has been suggested in experimental protocols that N-DEA could have clinically relevant activities: for example, N-DEA has been shown to produce larger increases than amiodarone in QRS duration, and in atrial and ventricular refractory periods in anaesthetized dogs as a consequence of its relatively greater effect on the fast sodium channel [18]. Zhou *et al.* [19] have also recently demonstrated that N-DEA increases ventricular defibrillation threshold to a greater extent than its parent drug in a randomized single blind study in anaesthetized pigs when using clinically relevant dosages of either amiodarone or N-DEA.

Given the putative therapeutic consequences [18–22] of variations of N-DEA production, we decided to study the effects of grapefruit juice on the pharmacokinetic properties of amiodarone.

Methods

Study population

Eleven nonsmoking, non obese healthy male volunteers with a mean age of 24 years (19–40 years) and an average weight of 72.6 kg (61–81 kg) were included in the study after local ethics committee approval and informed consent were obtained. All subjects had been thoroughly evaluated 6–8 months earlier for their ability to metabolize amiodarone. This first evaluation of amiodarone pharmacokinetics was performed with the same study protocol as that used in the present evaluation (see study protocol). The long washout period following the first evaluation was justified by the long half-life of the parent drug and its main metabolite [4–7, 23]. The subjects were required to have no history of iodine intolerance, thyroid disorder or any other clinical, biological or electrocardiographic contraindications to amiodarone prescription.

Subjects excluded from the study included those currently using medications (including over-the-counter treatments), those who drank alcoholic, cola or caffeine-containing beverages, and those who took any food or drink known to contain significant amount of flavonoids within 48 h before the beginning of the pharmacokinetic evaluation. In addition, subjects were required to fast for 14 h preceding the evaluation with a view to avoiding any xenobiotic interaction. A clinical examination was carried out and blood samples taken for thyroid hormone and amiodarone concentration measurements were carried out before inclusion in the study.

Study protocol

Each subject was given one oral dose of amiodarone in 300 ml of grapefruit juice (as opposed to the water used in the first evaluation). The grapefruit juice was commercially prepared (REA jus de fruits, Sarre-Union, France) and was all from the same preparation batch with a calibrated amount of flavonoids (narirutin: 135 mg l⁻¹; hesperidine: 5.5 mg l⁻¹; naringine: 419.1 mg l⁻¹; neoheperidine: 8.2 mg l⁻¹; dydimine: 7.1 mg l⁻¹ and poncirine: 19.8 mg l⁻¹). The dose of amiodarone was about 17 mg kg⁻¹. This loading dose (5–7 × 200 mg tablets according to the body weights of each individual of the studied population; Sanofi Pharmaceutical Inc.) is in accordance with the mean loading dose recommended by the U.S. and European Drug Regulatory Agencies [24]. In contrast with the first evaluation, subjects were also given 300 ml grapefruit juice 3 h and 9 h following amiodarone administration. ECG monitoring was then done over a 24 h period. No other oral intake was permitted during this period, save for a light calibrated lunch and supper lacking in flavonoids which were given, respectively, 5 and 12 h later. After 24 h of monitoring, subjects were allowed to leave the clinical unit and return home. Five follow up visits were scheduled 48 h, 1 week, 1 month, 3 months and 6 months after drug intake. Blood pressure

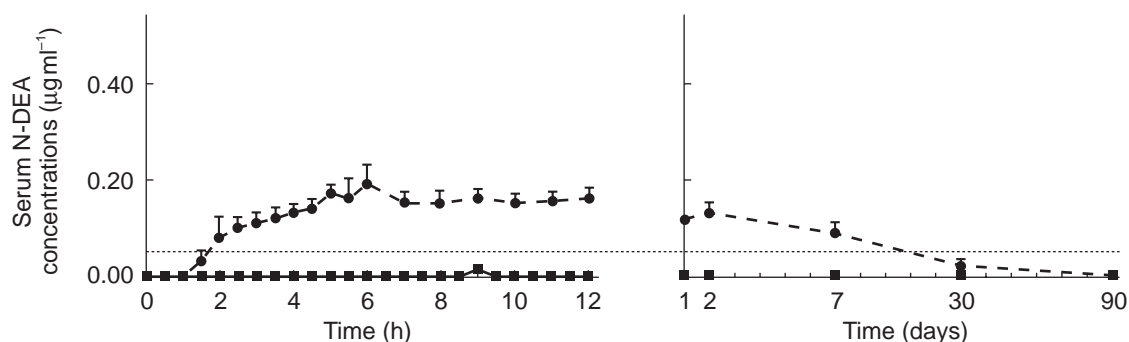


Figure 1 Mean serum concentration *vs* time graph for N-DEA in 11 subjects receiving amiodarone with grapefruit juice (solid line and full squares) or with water (broken line and full circles). Each point is mean \pm s.e.mean Throughout grapefruit juice administration, N-DEA concentrations remained below 0.05 $\mu\text{g ml}^{-1}$ (limit of detection: dotted line).

and ECG data were recorded every 3 h during the first 24 h, at 48th hour, and at days 7, 30, and 90. The study design was justified by the risk of an unforeseeable occurrence of major electrocardiographic alterations which could happen in individuals after an amiodarone loading dose given with grapefruit juice and was decided upon ethics committee advice which suggested the non inclusion of subjects who had major ECG alteration after the first amiodarone intake. This design was also justified given the very long wash-out period needed by the long elimination half-lives of amiodarone and its main metabolite: if a randomized cross-over design had been used, this period (at least 6 months) could not have allowed the use of the same preparation batch of grapefruit juice. On the contrary, by performing the entire grapefruit juice period in less than 2 months, our design allowed the use of the same batch of grapefruit juice, thus avoiding disparities in the various components of this fruit juice such as flavonoids which could be season dependent.

Analysis of electrocardiograms

All 12-lead electrocardiograms were obtained by means of a paper ECG recorder (EK53 Helige cardioteest recorder) and were performed by the same research nurse throughout the study. ECG intervals were coded and then measured randomly by a single blinded observer without knowledge of the randomization data. The QTc was calculated from the Bazett formula [25] after R-R and QT intervals were measured on V2 lead.

Measurement of drug and metabolite concentrations

A total of 24 blood samples (at each evaluation) were taken to measure serum amiodarone and N-DEA levels. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 11, 12, 24, 48 h and 7, 30, and 90 days after the loading dose.

The concentrations of amiodarone and its principal metabolite N-DEA were measured by high-pressure liquid chromatography (h.p.l.c.) with u.v. detection at 242 nm, using the method described by Trivier *et al.* [26] with a slight modification to analyse serum amiodarone and N-DEA. The samples were analysed after acetonitrile deproteinization on a C18 reversed-phase 5 μm ODS Hypersil column (150 \times 4.6 mm I.D. – Touzart & Matignon, Vitry-sur-Seine, France) using a mobile phase consisting of methanol-water-58% ammonium hydroxide (88 : 10 : 2, v/v/v) delivered at a flow-rate of 1.8 ml min^{-1} . All assays were performed at room temperature.

All manipulations were carried out in amber glass-capped tubes following our observation that, in the presence of light, deiodinated compounds were formed

(monoiodoamiodarone and bis-desiodoamiodarone), the retention times of which could interfere with those of N-DEA and bis-desethylamiodarone, respectively.

The limit of quantification was 0.1 $\mu\text{g ml}^{-1}$ and the limit of detection was 0.05 $\mu\text{g ml}^{-1}$ for both amiodarone and N-DEA in serum. When the concentrations were below the quantification limit, the samples were concentrated by a factor 2 or 3. From the analysis of 10 serum samples using concentrations of 0.2 $\mu\text{g ml}^{-1}$ for amiodarone and N-DEA, the intra-assay precision was 7.43 and 7.71%, respectively, for amiodarone and N-DEA and the interassay precision for these two compounds were 9.5 and 10.39%, respectively.

All analytical procedures were blindly done by the same laboratory.

Statistical analysis

Pharmacokinetic parameters were determined by using a standard monocompartmental method. Area-under-the-curve (AUC) was computed by the linear trapezoidal rule for the measured concentrations during the dosing interval [27]. Other pharmacokinetic parameters determined were: observed maximum concentration (C_{max}), time at which maximum concentration was observed (t_{max}), and initial elimination half lives ($t_{1/2}$) for both amiodarone and N-DEA which were estimated from the elimination rate constant calculated as the negative slope of the initial log-linear regression of the elimination using a monocompartmental model. The choice of the initial elimination half-life instead of the terminal half-life was made because the terminal phases of amiodarone and its metabolite are very long and follow an asymptotic curve leading to failure in precise evaluation of these terminal half-lives.

ECG data recorded 6 h after amiodarone intake were selected for statistical analysis. This time of recording was chosen because it was the nearest to the t_{max} and showed the greatest changes in ECG.

Analysis of amiodarone and N-DEA concentrations was first done using an ANOVA for repeated measures to test the main effect and the interactions between both studied periods (control and grapefruit juice) and time. The ANOVA was performed on ranks, according to the method of Conover & Iman [28] as a result of the limited number of subjects included ($n=11$) and because the studied parameters could not be assumed to have a Gaussian distribution.

Comparison of AUC, C_{max} , t_{max} and $t_{1/2}$ for amiodarone and N-DEA was by the Wilcoxon paired test [29] to test the two periods (control and grapefruit juice periods) (Figures 1 and 2). Electrocardiographic data at 6 h were also compared using the Wilcoxon paired test [29].

A value of $P<0.05$ was required to reject the null hypothesis.

Results were expressed as mean value \pm s.d..

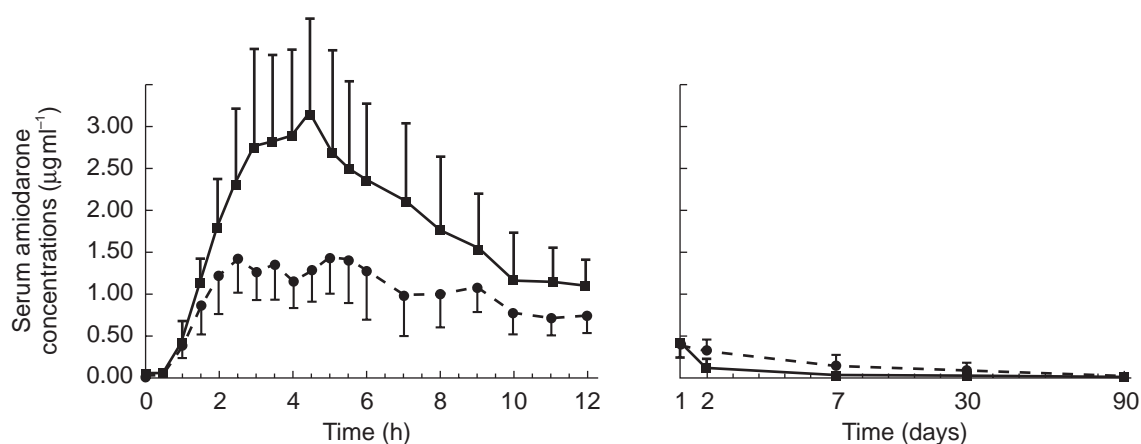


Figure 2 Mean serum concentration *vs* time graph for amiodarone in 11 subjects receiving amiodarone with grapefruit juice (solid line and full squares) or with water (broken line and full circles). Each point is mean \pm s.e.mean.

Results

Grapefruit juice totally inhibited the production of N-DEA in all subjects, with no significant trace of N-DEA being found in any of the samples (Figure 1). As compared with the control administration, grapefruit juice also significantly increased the AUC of amiodarone (Figure 2) by 50% (35.9 ± 14.3 *vs* 23.9 ± 11.2 $\mu\text{g ml}^{-1}$ h, mean \pm s.d.; $P < 0.005$; 95% CI on mean difference 6.14–17.84). C_{max} increased by 84% (3.45 ± 1.7 *vs* 1.87 ± 0.6 $\mu\text{g ml}^{-1}$, mean \pm s.d.; $P < 0.02$; 95% CI on mean difference 0.67 *vs* 2.42) (Table 1).

During the water period, the ratio N-DEA/amiodarone, which was about 0.17 6 h after amiodarone intake, reached a value of 1.00 on the 7th day.

Mean amiodarone t_{max} and $t_{1/2}$ were not significantly altered (3.63 ± 0.96 *vs* 4.08 ± 1.66 and 9.53 ± 5.50 *vs* 11.56 ± 8.00 h, respectively).

No difference was observed between the two baseline periods before amiodarone intake (with water or grapefruit juice) for ECG parameters and arterial pressure data.

The greatest ECG changes were seen at 6 h postdose: during the water period, amiodarone intake caused ECG variations in all subjects with an mean increase of PR and QTc intervals by $+17.9\% \pm 6.6$ ($P < 0.001$) and $+11.3\% \pm 8.0$ ($P < 0.001$), respectively (Table 2).

During the grapefruit juice period, however, these variations were only $+10.2\% \pm 8.8$ ($P < 0.01$) and $+3.3\% \pm 4.5$ (NS), respectively.

For both periods, RR intervals and QRS durations were not statistically altered and systolic arterial pressure decreased slightly (-6% , NS). It should be noted nevertheless that for some of the subjects the decrease in systolic arterial pressure went as far as 25% without any clear relation to the type of amiodarone administration.

Discussion

These results show for the first time that grapefruit juice given simultaneously with amiodarone dramatically alters N-DEA production from the parent drug, with complete inhibition of the formation of this major metabolite. Because it is not possible to control every CYP3A4 modulation from xenobiotic factors, we cannot quantify the influence of such factors on intrasubject variability. Nevertheless, the use of calibrated lunches and of a 14 h fasting period preceding the evaluations should have reduced such variability.

As grapefruit juice is already known to inhibit CYP3A4 activity, these results are in agreement with previous experimental data which have suggested that N-DEA is produced mainly by the CYP3A4 in humans [9, 10]. The

Table 1 Pharmacokinetic data of amiodarone (mean \pm s.d.).

	Period 1 (water)	Period 2 (grapefruit juice)	95% CI on mean difference	P
C_{max} ($\mu\text{g ml}^{-1}$)	1.87 ± 0.6	3.45 ± 1.7	0.67, 2.42	< 0.02
t_{max} (h)	4.08 ± 1.66	3.63 ± 0.96	$-1.79, 0.89$	NS
AUC ($\mu\text{g ml}^{-1}$ h)	23.9 ± 11.2	35.9 ± 14.3	6.14, 17.84	< 0.005
$t_{1/2}$ (h)	11.56 ± 8.0	9.53 ± 5.5	$-5.21, -1.18$	NS

Table 2 ECG alterations during period 1 (water) and period 2 (grapefruit juice) at 6 h postdose (mean \pm s.d.).

	Period 1			Period 2		
	Baseline	Amiodarone and water	% of increase	Baseline	Amiodarone and grapefruit	% of increase
PR (ms)	166.2 \pm 14.2	196.3 \pm 23.7	17.9 $P < 0.001$	168.2 \pm 14.8	186.1 \pm 28.8	10.2 $P < 0.01$
RR (s)	0.89 \pm 0.17	0.895 \pm 0.12	2.86 NS	0.876 \pm 0.15	0.930 \pm 0.10	8.70 NS
QTc	0.38 \pm 0.03	0.42 \pm 0.03	11.28 $P < 0.001$	0.39 \pm 0.03	0.40 \pm 0.02	3.33 NS

exact mechanism of inhibition of this isoenzyme activity remains unclear: it has been suggested that flavonoids present in grapefruit juice could be involved in this alteration [11–15, 30], but other inhibitors like furanocoumarins have also been cited [31–35]. Metabolism alteration by inhibitors, such as those found in grapefruit or citrus juices and probably in many fruit and vegetable juices including red wine [36], could well explain unexpected variations of amiodarone bioavailability. The pronounced interindividual variability of naringin and naringenin production by intestinal bacteria after grapefruit juice intake could also provide a possible explanation for the wide range of amiodarone metabolism [37].

Since the gut wall contains CYP3A4, it is highly probable that these inhibitors act, at least in part, on the prehepatic amiodarone biotransformation [2–7]. These induced alterations of amiodarone metabolism could have clinical consequences: like amiodarone, N-DEA has class III antiarrhythmic properties, but it also has some electrophysiological properties which differ from the parent drug, notably by a higher fast sodium channel blockade and lower class IV effects [38–43]. Experimental data have shown a correlation between N-DEA plasma concentration and antiarrhythmic properties of amiodarone [18, 19]. During chronic treatment, it has also been shown that the ratio of metabolite to parent compound is ≈ 1 at the plasma level, but go up to 4.8 at the myocardial level [20, 22]. These ratios could be significantly dependent on flavonoids or other CYP3 A inhibitor intake.

In spite of the lack of statistically significant arterial pressure alterations and although the extent of the ECG changes observed in these healthy volunteers was not of concern, these changes could be important in patients suffering from cardiomyopathy and/or from conduction or rhythmic disorders. As it has been shown that during the grapefruit juice period amiodarone concentrations reached values exceeding therapeutic concentrations [44, 45], this could result in significant electrophysiological and clinical consequences linked to the parent drug. Furthermore, inhibition of N-DEA production could decrease the antiarrhythmic action of amiodarone as evoked by

experimental data showing the effect of N-DEA on ventricular defibrillation threshold [18] but this could conversely decrease the arrhythmogenic effects linked to QT prolongation and thus lead to beneficial effects. These consequences are worth further investigation.

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