Eur J Clin Pharmacol (1996) 50 : 501–508

J. Vanakoski · M. J. Mattila · T. Seppälä

Grapefruit juice does not enhance the effects of midazolam and triazolam in man

Received: 4 September 1995 / Accepted in revised form: 5 January 1996

Abstract *Objectives*: Since grapefruit juice (Gra) inhibits hepatic P450 (CYP3A4), we studied its potential to enhance the effects of midazolam (Mid) and triazolam (Trz), which are metabolized by the CYP3A4 isoenzyme.
Methods: In Study I parallel groups of healthy students were given orally Mid 10 mg with water or grapefruit juice (GraMid), two placebo groups receiving water or Gra. The effects of Mid were measured by psychomotor tests and by self-rating on visual analogue scales before and 30 and 90 min after intake. Study II was similar, but the post-treatment tests were at 45 and 90 min, and the active drugs used were 0.250 mg Trz, GraTrz, and Mid 10 mg. In the crossover Study III, 6 subjects took Mid 10 mg alone and with Gra (GraMid) and 750 mg erythromycin (EryMid). Performance tests were made and blood was sampled before and 30, 60 and 90 min after intake. Midazolam and its active metabolite α-OH-midazolam were assayed by gas chromatography (GC) and radioreceptor assay (RRA).
Results: In Study I, both Mid and GraMid impaired digit symbol substitution (DSS), letter cancellation (LC) and flicker fusion (CFF) at 90 min. GraMid had more effect ($P < 0.05$) than Mid on the DSS performance. Mid caused drowsiness at 30 and 90 min. Both Mid and GraMid caused clumsiness and a feeling of impaired performance at 90 min. In Study II, the active drugs impaired objective test performances (DSS, LC, CFF) at 90 min, without having a clear subjective effect. In Study III, Mid, EryMid and GraMid impaired performance in the DSS, LC and CFF tests. EryMid proved stronger than Mid and GraMid on DSS and LC tests at 30 min. Mean values of plasma midazolam (and α-OH-midazolam) at 30, 60, 90 and 120 min after Mid 10 mg were 68 (19), 61 (19), 43 (14) and 42 (12) $\mu g \cdot l^{-1}$. The corresponding values after EryMid were 164 (14), 137 (13), 104 (10) and 89 (10) $\mu g \cdot l^{-1}$, and after GraMid 60 (12), 69 (16), 61 (15) and 57 (14) $\mu g \cdot l^{-1}$.
Conclusions: The grapefruit juice used did have any particular interaction with oral doses of 10 mg midazolam and 0.25 mg triazolam in healthy young subjects.

Key words Grapefruit juice, Midazolam, Triazolam; drug-interaction, drug metabolism, psychomotor performance

J. Vanakoski · M. J. Mattila (✉)
Institute of Biomedicine, Department of Pharmacology and Toxicology, University of Helsinki, Siltavuorenpenger 10, FIN-00014 Helsinki, Finland

T. Seppälä
National Public Health Institute, Helsinki, Finland

Introduction

Grapefruit juice inhibits hepatic P450 isoenzyme (CYP3A subfamily) and interacts with compounds such as cyclosporin and the calcium entry blocking dihydropyridines, both of which being metabolised via CYP3A4. This interaction may take place during the first pass of the drugs concerned. It has been attributed to certain active aglycones (naringenin, quercetin, kaempferol) formed in the gut from the corresponding bioflavonoid glycosides in grapefruit juice [1–5]. The inhibitory effect of naringenin on CYP3A enzymes has been clearly documented [2, 5], but the overall inhibitory effect of grapefruit juice on CYP isoenzymes may also include CYP1A2 [6]. The concentrations of the flavonoids in juice can vary quantitatively and qualitatively (chiral isomers), presumably due to different kinds of grapefruit, the method of juice extraction from the fruits and, perhaps, on the subsequent processing of juice to branded consumer products. There are also considerable interindividual differences in the amount of intestinal and hepatic CYP3A4 [7], which could modify drug interactions with this enzyme.

Since the ultra-short acting hypnotics midazolam and triazolam are also metabolised by CYP3A4

isoenzyme [8], we studied the potential of grapefruit juice to enhance the effects of these hypnotics. This pharmacodynamic approach comprised two studies with parallel groups of subjects, completed with a crossover study in which plasma midazolam concentrations were measured, and erythromycin was used as a positive control for drug interaction. Some of the results have previously been published [9].

Subjects and methods

Subjects and design

120 healthy medical students participated in two double-blind studies (Study I and Study II), with parallel treatment groups roughly matched for weight and gender, during their practical class exercise in pharmacology. The protocol was approved by the Ethics Committee of the Department of Pharmacology and Toxicology, University of Helsinki; the authors did not belong to that committee. All the subjects had to take the tests, but with no obligation to take capsules: the vast majority of students gave their oral informed consent to take the capsules as well. Each subject received one treatment only. Studies were carried out between 13.30 and 15.30 h. Study III, conducted at 10.00 h, was a balanced crossover study in 6 subjects, who gave their written informed consent and were paid for their time.

In Study I, 49 subjects (34 women and 15 men), aged 20–30 y and weighing 43–88 kg, took single oral doses of 10 mg midazolam (Mid) with 300 ml (150 + 150 ml) water (n = 12; 4 men) or grapefruit juice (GraMid; n = 13; 3 men). The two control groups received water (n = 12; 4 men) or Gra (n = 12; 4 men). These treatments were spread over five sessions, each with 10–12 subjects, all treatments being available in each session. A set of psychomotor tests was performed at baseline and 30 and 90 min after intake of the gelatine capsule. The first drink, water or Gra, was given 40 min before the intake of capsule and the second drink.

Study II was similar to Study I except that the hypnotic given was 0.25 mg triazolam (Trz) and that the post-treatment tests were made at 45 and 90 min; midazolam was given as a positive control. A total of 57 subjects (34 women and 23 men) aged 20–36 y and weighing 50–84 kg, were allocated to receive placebo (n = 15; 10 men), Trz 0.25 mg (n = 14; 5 men), GraTrz (n = 13; 6 men) and Mid 10 mg (n = 15; 6 men).

Study III was conducted in 6 subjects, 3 men and 3 women, aged 23–31 y and weighing 55–81 kg. They were given 10 mg Mid with water, with Gra (GraMid) and with 750 mg erythromycin (EryMid), in a crossover desigh and in balanced order, at weekly intervals. Erythromycin was used, in a double blind manner, as a positive control for the interaction [10]. Performance tests were made and venous blood was sampled before the Mid intake and after 30, 60 and 90 min blood was also sampled at 120 min. The drinks were given as in Study I.

The drug capsules of identical appearance were filled with ground tablet material by the University Pharmacy. The grapefruit juice (Gra), sold in plastic-laminated cardboard packages (Valio, Helsinki), originated from a concentrate (US and German standards) of juice extracted from Floridan grapefruit by vacuum filtration, the commercial "Gra" referred to the original fresh juice. Routine assays of similar dilutions made in the Customs Laboratory revealed the concentrations of 2.5 to 3.0 $g.l^{-1}$ of naringin + neohesperidin. Since the naringin: neohesperidine ratio in these mixtures averaged over 20 (range 14 to 84) [11], the naringin dose in 300 ml Gra would have been about 700 mg.

Tests

The set of performance tests included, as previously described [10, 12], digit symbol substitution with pencil and paper for 3 min, letter cancellation (Russian text) for 2 min, flicker fusion with red light (Leeds tester), tapping with a hand tally counter for 30 s, and short-term memory (digit list). Subjective effects were assessed on visual analogue scales (VAS); the pairs of adjectives recorded were drowsy/alert, calm/nervous, mentally slow/quick-witted, clumsy/skillful, discontented/contented, and poor/good overall performance. Background vigilance in the parallel groups was moderate. In Study III, digit symbol substitution (1.5 min), letter cancellation and flicker fusion tests were used.

Assay of plasma midazolam in Study III

Plasma samples were stored at $-20°$ C until assayed for midazolam and α-hydroxy-midazolam by gas chromatography as derivatives of N-methyl-N-(trimethylsilyl)-2,2,2-trifluoroacetamide (MSTFA) (Vanakoski and Seppälä, 1995, unpublished). Briefly, plasma 1 ml buffered with Na_2HPO_4 was extracted with 5 ml toluene containing diazepam as internal standard. The organic layer was separated and evaporated to dryness and MSTFA (20 µl) was added for derivatisation for 30 min, at room temperature. The solution was evaporated to dryness, the residue eluted with 100 µl toluene, and 2 µl eluate was injected into a Hewlett-Packard 5880A gas chromatograph with electron capture detector. Separation was achieved on a 50 m × 0.32 mm I.D. HP-5 capillary column coated with SE-54 (5% phenyl methyl silicone). Injection was made at the oven temperature of 300° C. The limit of detection was 1 ng/ml for both compounds, and CV% values (at the 50 $ng \cdot ml^{-1}$ level) were 3.1% for midazolam and 5.9% for α-OH-midazolam.

Benzodiazepine activity in plasma was measured by a radioreceptor assay (RRA) [13]. Briefly, ^{3}H-flunitrazepam (0.7 nmol), 50 µl plasma and rat cerebral cortex (0.25 mg protein) in TRIS buffer (1 ml) were incubated on ice for 60 min, the samples were vacuum filtered, and the radioactivity of the brain tissue and filter was counted with Wallac liquid scintillator. Four samples of midazolam (25, 50, 250 and 500 $ng \cdot ml^{-1}$) in plasma were run in each assay, and the results were read from a log-probit scale. The concentration/probit graphs in consecutive assays were closely parallel, the mean probits being 5.65, 5.35, 4.40 and 4.10, respectively. The presence of erythromycin tended to modify the level of standard graphs towards underestimation of midazolam in samples by 10–20% . We measured the EryMid samples against a standard containing erythromycin.

Statistics

Mean (SEM) values were computed for absolute performances and their Δ-values (change from baseline). In order to confirm the hypothesis that Gra interacted with midazolam, the ranked Δ-values were analysed by one-way ANOVA and Scheffé's multiple comparison tests in Studies I and II. This analysis, which we have used before [10, 12], was adopted because the baseline performances and the responses to treatment (Δ-values) deviated from a Gaussian distribution. In the crossover Study III, two-way ANOVA and Scheffe's test were computed for unranked Δ-values. All three studies were analysed separately, defining digit symbol substitution, letter cancellation, flicker fusion and subjective drowsiness as primary dynamic variables, and the plasma midazolam as a primary kinetic variable. Analyses with 95% confidence intervals (CI) were also computed (General linear model) for the Δ-values at the level of important drug effects. The standard power estimation, based on the results from previous studies [10, 12], indicated that the groups were sufficiently large for $P < 0.05$. A Spearman matrix was computed to interrelate different variables of plasma midazolam.

Results

In the parallel group Studies I and II, there was non-significant variation between the groups at baseline, and obvious practice effects were recorded in the digit symbol substitution test after placebo and Gra (Tables 1–2). The drug effects in Study I were clearer than in Study II; no significant subjective effects were found in the latter. As reported in our previous studies, digit symbol substitution and letter cancellation proved the most sensitive and reliable objective test of benzodiazepine effects.

Midazolam (Mid) alone and with grapefruit juice (GraMid) in Study I

As seen in Table 1, Mid reduced the number of digits correctly substituted, lowered the flicker fusion threshold, and shifted the assessments towards drowsiness on the drowsy/alert VAS scale. It also caused clumsiness (F_D 6.76), mental slowness (F_D 4.76) and impaired overall performance (F_D 7.05) at 90 min. Grapefruit juice alone did not differ from placebo.

GraMid modified performance in most of the variables measured. The GraMid-induced impairments differed from placebo and/or Gra, and also from the Mid effect in the digit symbol substitution test (Table 1). GraMid shared the effect of Mid on subjective clumsiness and on overall subjective impairment of performance at 90 min.

Some drug effects were also analysed by comparing the 95% confidence intervals (CI) computed for the Δ-values. The mean (and CI) values for Δ-digit substitutions at 90 min were 10.200 (0.331 to 20.000) after placebo, 9.250 (−0.586 to 19.100) after Gra, −11.700 (−21.500 to −1.830) after Mid, and 31.000 (−40.450 to −21.6000) after GraMid. Thus, both Mid and GraMid differed from placebo, and GraMid also

Table 1 Effects of 10 mg midazolam alone (Mid) and with grapefruit juice (GraMid), and corresponding placebos (Plac, Gra) on performances of healthy students in Study I. Ranked Δ-values (changes from baseline) were analysed by one-way ANOVA and Scheffe's test; significant ($P < 0.05$) differences **v** Δ-placebo (#), **v** Gra (§) and **v** Mid (*); a, b and c refer to the changes from baseline at the 5%, 1% and 0.1% levels (paired *t*-test)

Test/time	Mean (SEM) values of performances; [N]				F_D
	Plac [14]	Mid [12]	Gra [12]	GraMid [13]	
Digits substituted in 3 min					
BL	133 (6)	134 (4)	135 (5)	140 (6)	
30 min	144 (6) c	135 (5)	143 (5) b	147 (5) a	NS
90 min	143 (6) a	122 (6) b#	145 (5) a	109 (6) c#§*	45.74
Letters cancelled in 2 min					
BL	20 (1)	19 (1)	20 (1)	18 (1)	
30 min	18 (2)	16 (1) b	16 (1) c	15 (1) a	NS
90 min	16 (1) b	13 (1) c	16 (1) b	10 (1) c#§	7.79
CFF (10 × Hz)					
BL	226 (7)	236 (6)	234 (8)	236 (7)	
30 min	231 (7)	231 (5)	239 (8)	239 (8)	NS
90 min	223 (5)	223 (6) b#§	241 (7)	210 (6) c#§	10.19
Alertness (Mm on VAS)					
BL	47 (5)	59 (6)	55 (4)	54 (5)	
30 min	40 (5)	36 (5) b§	58 (5)	43 (3) b	7.08
90 min	43 (5)	30 (5) #§	56 (4)	32 (5) b	5.96

Table 2 Effect of 0.25 mg triazolam (Trz) alone and with grapefruit juice (GraTrz) and 10 mg midazolam (Mid) on performance of healthy students in Study II. Data were analyzed as in Table 1; [a] refers to significant ($P < 0.05$) difference **v** Δ-placebo

Test/time	Mean (SEM) values of performances; [N]				F_D
	Plac [15]	Trz [14]	GraTrz [13]	Mid [15]	
Digits substituted (3 min)					
BL	124 (5)	131 (4)	127 (5)	128 (6)	
45 min	130 (6)	128 (4)	125 (7)	119 (5)	NS
90 min	135 (5)	126 (5) #	116 (8) #	126 (6)	6.50
Letters cancelled (2 min)					
BL	16 (1)	17 (1)	17 (1)	20 (1)	
45 min	15 (1)	15 (1)	13 (1) c	15 (1) c	NS
90 min	17 (1)	13 (1) b*	12 (1) c*	14 (1) c*	7.97
CFF (10 × Hz)					
BL	226 (10)	235 (7)	225 (6)	236 (8)	
45 min	236 (9)	229 (7)	229 (7)	232 (8)	3.17
90 min	231 (7)	225 (8)	220 (7)	238 (8)	3.00

differed from Mid. Borderline differences of Mid and GraMid from placebo were found in the corresponding letter cancellation test: −3.890 (−6.440 to −0.894) after placebo, −6.66 (−9.440 to −3.890 after Mid, and −8.230 (−10.900 to −5.570) after GraMid. In the flicker fusion test GraMid differed significantly from Gra but not from placebo.

When applying this CI analysis after the repeated measures ANOVA to objective primary tests, GraMid differed from placebo in digit substitution (F_D12.32; $P < 0.001$) and flicker fusion (F_D5.83; $P < 0.01$), but not in letter cancellation (F_D4.20; $P < 0.01$) tests. The corresponding Mid effects showed only borderline differences from placebo. The mean and (CI) values of repeated measures subjective drowsiness (F_D11.46; $P < 0.001$) were 2.00 (−9.750 to 13.700) after Gra, −5.958 (−17.700 to 5.790) after placebo, −16.900 (−28.200 to −5.600) after GraMid, and −26.500 (−38.200 to −14.700 after Mid. Mid and GraMid did not differ from each other, and Mid differed from Gra but not from placebo. As a whole, the CI analysis without ranking the Δ-values proved somewhat less sensitive than the ranked values ANOVA + Scheffé's test (Table 1) in revealing significant drug effects.

After the sessions, 10 subjects felt sufficiently tired to stay in the department for a further hour, during which they were given coffee as needed. After the study, it appeared that 8 out of these 10 subjects had been treated with GraMid. No case of anterograde amnesia was noted.

Triazolam, midazolam and grapefruit juice in Study II

Significant drug effects in Study II were fewer and of lower statistical significance than in Study I. With regard to drug effects at separate test times (Table 2), Trz and GraTrz impaired digit substitution at 90 min. The borderline drug effects on flicker fusion at 90 min and clumsiness at 45 min were not attributable to any particular drug. When combining the 45 and 90 min tests for repeated measures ANOVA, all active drugs impaired digit symbol substitution (F_D6.95; $P < 0.01$), Mid and GraTrz impaired letter cancellation (F_D10.09; $P < 0.01$), and Trz impaired flicker fusion (F_D5.07; $P < 0.01$

In the CI analysis of the Δ-values of the drug effects at 90 min in Table 2, the mean (and CI) Δ-values of letter cancellations were 0.600 (−2.440 to 3.640) after placebo, −3.930 (−7.080 to −0.778) after Trz, 5.540 (−8.810 to −2.270) after TrzGra, and −5.600 (−8.640 to −2.560) after Mid. All active drugs showed a borderline difference from placebo. As to the corresponding values in the digit substitutions, GraTrz (mean −11.300; CI −23.800 to 1.200) showed a borderline difference from placebo (mean 10.500; CI −1.110 to 22.200); the other treatments did not differ from placebo.

Midazolam, erythromycin and grapefruit juice in Study III

As seen in Table 3 and Fig. 1, the plasma midazolam concentrations measured by GC after 10 mg Mid alone were as expected. The corresponding values after erythromycin (EryMid) were much higher (+ 150%), and a smaller increase (+ 45%) was seen at 90 and 120 min after grapefruit juice (GraMid). The concentrations of α-OH-midazolam after Mid were about 30% of the corresponding doses of midazolam and were not much reduced either by erythromycin or grapefruit juice,

Table 3 Concentrations of plasma midazolam and α-OH-midazolam assayed by GC or by RRA after 10 mg midazolam alone (Mid) and with 750 mg erythromycin (EryMid) or grapefruit juice (GraMid) in six healthy subjects. Differences between the treatments were analysed by two-way ANOVA and Scheffe's test; significant ($P < 0.05$) differences **v** Mid (*) and GraMid (**)

Treatment and Time	Midazolam in plasma; $\mu g \cdot l^{-1}$		
	Midazolam	α-OH-midazolam	RRA-Midazolam
Mid			
BL	0	0	
30 min	68 (11)	19 (4)	92 (22)
60 min	61 (5)	19 (1)	68 (7)
90 min	43 (6)	14 (1)	46 (5)
120 min	42 (10)	12 (1)	40 (5)
EryMid			
BL	0	0	
30 min	164 (21)**	14 (2)	91 (14)
60 min	137 (19)**	13 (1)*	79 (12)
90 min	104 (10)**	10 (1)**	64 (9)
120 min	89 (11)**	10 (2)	64 (4)*
GraMid			
BL	0	0	
30 min	60 (11)	12 (3)*	62 (23)
60 min	69 (7)	16 (2)	71 (11)
90 min	61 (9)*	15 (1)	63 (10)
120 min	57 (10)*	14 (1)	54 (5)

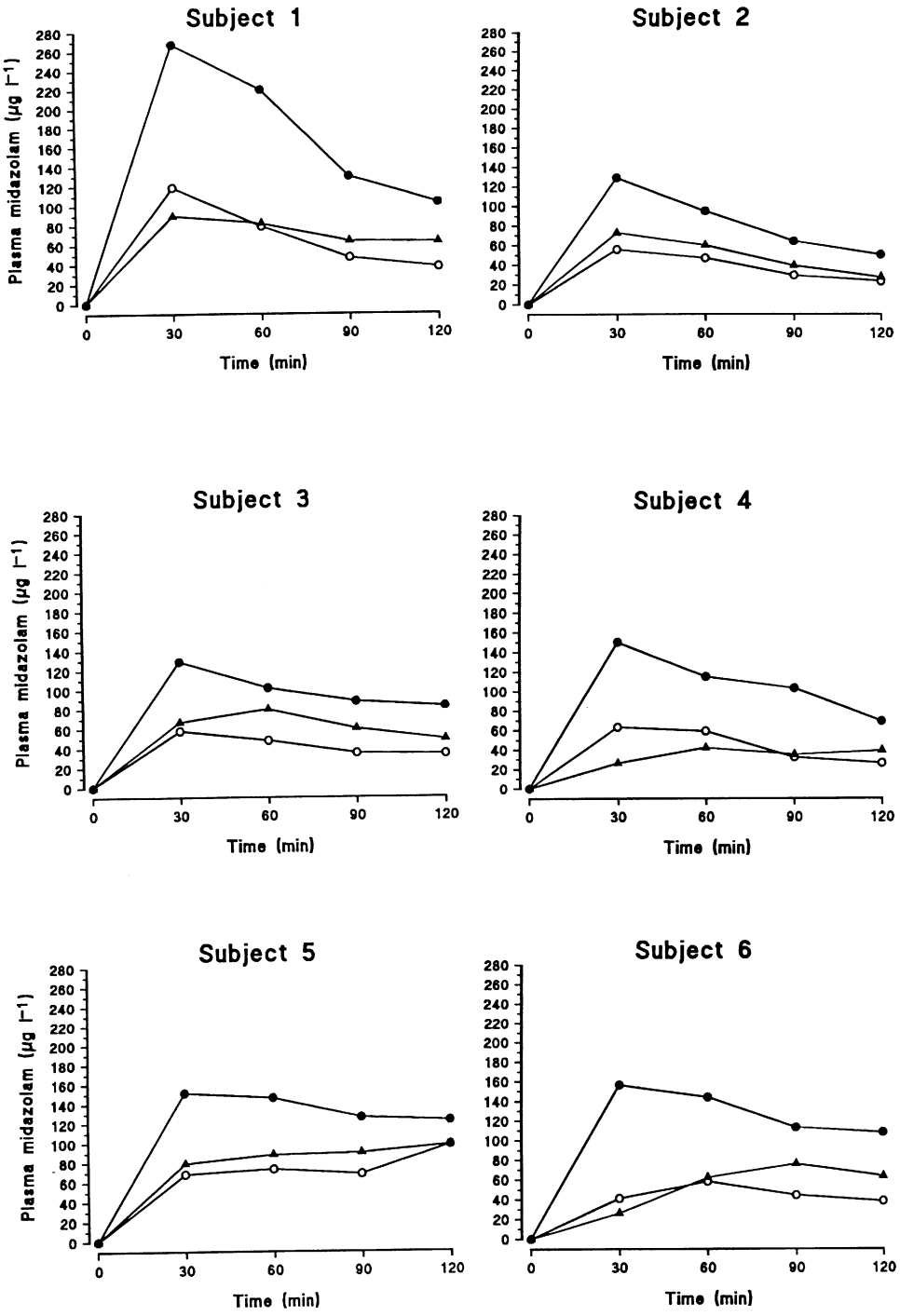

Fig. 1 GC-assay of plasma midazolam in individual subjects after 10 mg midazolam alone (open circles), after erythromycin (solid circles) and after grapefruit juice (solid triangles) in Study II

although the difference occasionally reached statistical significance (Table 3).

In the RRA-assay for plasma midazolam (plus α-OH-midazolam), the concentrations measured after Mid alone were similar to the GC-assay of the sum of these drugs at 30 min. Afterwards, the concentrations assayed by RRA declined to the levels of midazolam alone assayed by GC. The corresponding RRA-concentrations after EryMid matched those found after Mid at 30 min, but were relatively larger at 60, 90 and 120 min (Table 3). However, the RRA assay results after EryMid remained lower than the sums of midazolam and α-OH-midazolam measured by GC at the same times. The RRA assay concentrations after GraMid, compared with those after Mid alone, were less at 30 min and somewhat higher at 90 and 120 min.

Drug-induced impairment (−10% to −50%) of the digit substitution, letter cancellation and flicker fusion performance at after treatment is illustrated in

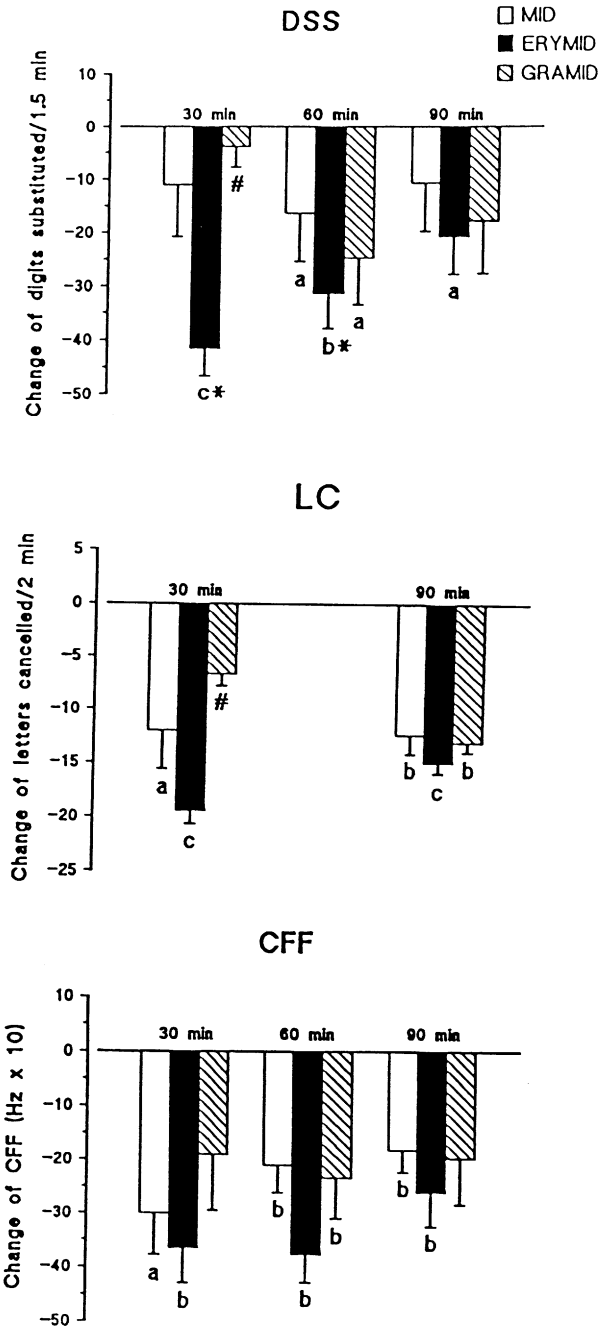

Fig. 2 Effects of oral 10 mg midazolam alone (Mid) and in combination with 0.75 g erythromycin (EryMid) or grapefruit juice (GraMid) on digit substitution (DSS), letter cancellation (LC) and flicker fusion (CFF) in Study II. Significant differences refer to Δ-Mid (*) and Δ-EryMid (#); a ($P < 0.05$), b ($P < 0.01$) and c ($P < 0.001$) refer to changes from baseline

Fig. 2. With the times combined, F_D-value for digit substitution was 14.20, and EryMid differed from both Mid and GraMid. The same applied to the letter cancellation and flicker fusion tests, even though the F_D-values were smaller. Lowered flicker fusion values after different treatments were similar, with no significant between-drug differences.

Subject 1 (see Fig. 1) showed a high peak in plasma midazolam after Mid alone, associated with strong drowsiness. In general, strong sedation and some anterograde amnesia were associated with large plasma midazolam levels in Study II. In these cases the subjects stayed in the laboratory for an extra hour, and were given coffee.

In order to relate the change in plasma midazolam levels to its central effects, the results of the GC assay of midazolam (M), α-OH-midazolam (OH-M), their sum (Σ-M) and the RRA assay of midazolam (RRA), as well as the corresponding drug responses (Δ-values) in digit substitution, letter cancellation and flicker fusion tests were cross-correlated in a simple Spearman matrix. It appeared that the responses in the digit substitution and letter cancellation tests were correlated with chemically assayed M, OH-M and Σ-M ($r = 0.796$ to 0.673; $P < 0.001$ to 0.01) better than with the RRA results ($r = 0.557$; $P < 0.05$) at 30 min but not later. Comparison of different drug concentration variables with each other revealed that chemically assayed M was correlated with Σ-M ($r > 0.98$) better than to OH-M ($r = 0.811$ to 0.604) or RRA ($r = 0.788$ to 0.639). RRA ("midazolam effect in plasma") was moderately ($r = 0.822$ to 0.685) well correlated with chemically assayed M, OH-M and Σ-M at any test time. The r-values between concentration variables refer to *P*-values of < 0.001 to 0.01.

Discussion

Our data suggest that grapefruit juice, in the amount and quality used, slightly interacted with midazolam but not to a practically important extent. The lack of an important interaction with grapefruit juice also applies to triazolam. Both of these ultra-short hypnotics are metabolised by the CYP 3A4 isoenzyme, and so they may interact with other drugs, such as erythromycin and some azole antifungal agents, in both on acute and subacute treatment [8, 12, 14, 15]. Since grapefruit juice increases the bioavailability of several calcium entry blocking dihydropyridines, and of cyclosporine [1–5], the lack of a clearcut interaction in our study with midazolam and triazolam suggests qualitative or quantitative differences in the experimental set-up, and/or in the amount and quality of grapefruit juice compared to the documented interactions quoted above. The clinical relevance of the established kinetic interactions of grapefruit juice has not been fully established, and it may partly depend, for example, on the dihydropyridine concerned [5].

Since our approach was primarily a pharmacodynamic one, the parallel group studies were important. This is because they dealt with large numbers of subjects, who took the hypnotics under ordinary conditions and not, for example, after extensive fasting. Even without blood sampling they were a valuable

complement to the crossover Study III performed in six subjects. Possible α-inflation resulting from numerous statistical tests is a draw-back of such an experimental design, but the low efficacy of grapefruit juice in our study did not result from the use of parallel groups. In fact, a significantly increased midazolam effect after grapefruit juice was documented in the parallel group Study I but, not in the crossover Study III.

Two reports [16, 17] were published during preparation of our manuscript. Kupferschmidt et al. [16], in a crossover balanced study, showed that grapefruit juice increased the peak level and AUC of plasma midazolam by about 50% after oral intake of 15 mg midazolam. The corresponding effects were increased and they were prolonged as well. Hukkinen et al. [17] showed that plasma concentrations after oral 0.25 mg triazolam were increased by concomitant ingestion of grapefruit juice, the AUC values being increased by 50%. However, grapefruit juice only slightly increased the triazolam-induced impairment in objective and subjective tests.

Our pharmacodynamic results with triazolam do not disagree with those of Hukkinen et al. [17], because they did not find a major pharmacodynamic interaction despite the increase in plasma triazolam. The authors attributed the lack of stronger sedation to tolerance resulted from the slightly slower absorption rate in the presence of grapefruit juice. This is possible, but we must bear in mind the basic rule of the concentration/effect relationship: concentrations move along the logarithmic scale and effects along an arithmetic scale. The same rule complicates interpretation of the RRA-effect relationship, as in considering the RRA assay of midazolam ("drug effect in plasma") in Study III (Table 3).

Our results with midazolam do not agree with those reported by Kupferschmidt et al. [16]. The difference probably resulted from use of different experimental conditions and disparate doses of midazolam. The fairly long fast before and after the intake of midazolam, as well as the collection of numerous consecutive blood samples no doubt increased pharmacokinetic accuracy but they do not match the conventional pattern of hypnotic intake. The single oral 15 mg dose of midazolam employed by Kupferschmidt et al. might cause a stronger kinetic and clearer dynamic interaction in comparison with our dose of 10 mg. It is also possible that different formulations of midazolam (tablet versus capsule) led to slightly different rates of absorption. Thus, 10 mg midazolam given as a capsule and 15 mg given as a tablet might differ from each other more than the plain numbers indicate. Further, due to intersubject differences in the amount of intestinal CYP3A4 [7, 18], small groups of subjects volunteering for two different crossover studies may respond differently.

Most of the published reports of the interaction of grapefruit juice refer to brand names but with no idea of the quality of the juice used. It is possible that juice extracted from fruits by simple pressing has the largest content of flavonoids and other unknown substances, and so might exhibit the clearest inhibition of CYP3A4. Investigators have recently been urged to declare the origin, extraction process and chemical characteristics of their grapefruit juice. Kupferschmidt et al. [16] strictly analysed their juice, more completely than we did. The Customs Laboratories have a keen interest in mixed orange and grapefruit juices. Our juice was definitely from grapefruit and was sold in commercial one-liter household packs for everyday use; it might not have been essentially different from the juice used by Kupferschmidt et al [16]. Bailey et al. [18] measured the naringin concentration (450 $mg \cdot l^{-1}$) in the grapefruit juice they used, but this concentration of naringin in water did not produce a significant interaction with felodipine. In any case, no general conclusion on the drug metabolism inhibiting effect of grapefruit juice can be drawn until all its active ingredients have been identified.

The results of Kupferschmidt et al. [16] are against the view that grapefruit juice has little effect on CYP 3A4-mediated metabolism of drugs with rapid absorption and metabolism, although it avidly inhibits this metabolic route for drugs with a slow absorption and metabolism, such as calcium entry blocking agents [19]. The effects of grapefruit juice and erythromycin on the pharmacokinetics of felodipine have been reported to be almost identical [5]. This refers solely to felodipine and we presume that if Kupferschmidt et al. [16] had given 750 mg erythromycin to their subjects as we did, their subjects would have fallen in to deep sedation or sleep.

Are single oral doses of 750 mg erythromycin and 300 ml grapefruit juice commensurable as inhibitors of CYP3A4? The effect of erythromycin in our present study was similar to at previously found for the erythromycin–midazolam interaction [10, 12, 20]. The degree of enhancement by erythromycin was similar to or less than that reported by Olkkola et al. [14] in their crossover study, in which oral 15 mg doses of midazolam were given after 1 week treatment with erythromycin. They also found stronger enhancement of the effects of midazolam, several subjects showing deep sedation. In our Study III, the effect of 750 mg erythromycin on plasma midazolam and midazolam effects was greater than that of grapefruit juice (Table 3, Fig. 2). These findings indirectly indicate that (i) oral 750 mg erythromycin or maintenance with erythromycin inhibits the CYP3A4 isoenzyme more effectively than 300 ml grapefruit juice, and (ii) grapefruit juice interacts with midazolam to a lesser extent than it does with, for example, felodipine, which interacts to the same degree with erythromycin and grapefruit juice [5]. Thus, well-known attributes for the important interactions between erythromycin and midazolam may not apply to grapefruit juice and midazolam.

Bailey et al. [18] have recently found that the felodipine-grapefruit juice interaction varied between individuals whilst being reproducible within individuals; grapefruit juice inhibited the presystemic elimination of felodipine at the primary and secondary metabolic

steps. Ducharme et al. [21] interpreted their cyclosporine–grapefruit juice interaction as taking place predominantly in the intestinal wall. The activity of the intestinal CYP3A4 isoenzyme varies from 6- to 11-fold but does not match the corresponding hepatic enzyme, as estimated by the erythromycin breath test as showing 10-fold variation [15]. The active principles of grapefruit juice inhibit other CYP isoenzymes than just CYP3A4, thus contributing to increased bioavailability of calcium entry blocking dihydropyridines and other drugs. The effect of grapefruit juice on CYP1A2-mediated metabolism is also controversial, since grapefruit juice has reduced the clearance of caffeine whereas no effect on theophylline pharmacokinetics was observed [6, 22].

In conclusion, our results suggest that 300 ml ordinary commercial grapefruit juice may slightly inhibit midazolam metabolism, and it causes a minor increase in the effects of midazolam in healthy young subjects. Although the dose of juice was limited and its quality was not selected, a clinically significant interaction of grapefruit juice with 10 mg midazolam seems unlikely.

Under our experimental conditions 750 mg erythromycin elevated plasma midazolam and enhanced it effects, thus demonstrating inhibition of the CYP3A isoenzyme family in an acute, single-dose study. The concentrations in Table 3 suggest that erythromycin inhibits the 4-hydroxylation of midazolam rather than its α-hydroxylation.

References

1. Bailey DG, Spence JD, Munoz C, Arnold JM (1991) Interaction of citrus juices with felodipine and nifedipine. Lancet 337: 268–269
2. Minisalco A, Lundahl J, Redgårdh CG, Edgar B, Olsson Eriksson UG (1992) Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. J Pharmacol Exp Ther 261:1196–1199
3. Ducharme MP, Provenzano R, Dehoorne-Smith M, Edwards DJ (1993) Through concentrations of cyclosporine in blood following administration with grapefruit juice. Br J Clin Pharmacol 36:457–459
4. Hollander AAMJ, Vanrooij J, Lentjes EGWM, Arbow F, Vanbree JB, Schoemaker RC, Vanes LA, Vanderwoude FJ, Cohen AF (1995) The effect of grapefruit juice on cyclosporine and prednisone metabolism in transplant patients. Clin Pharmacol Ther 57:318–324
5. Bailey DG, Arnold JMO, Spence D (1994) Grapefruit juice and drugs. How significant is the interaction? Clin Pharmacokinet 26: 91–98.
6. Fuhr U, Klittich K, Staib AH (1993) Inhibitory effect of grapefruit juice and the active component, naringenin, on CYP1A2 dependent metabolism of caffeine in man. Br J Clin Pharmacol 35:431–436
7. Lown KS, Kolars JC, Thummel KE, Barnett JL, Kunze KL, Wrighton SA, Watkins PB (1994) Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel. Lack of prediction by the erythromycin breath test. Drug Metabol Dispos 22:947–955
8. Kronbach T, Mathys M, Umeno M, Gonzales FJ, Meyer UA (1989) Oxidation of midazolam and triazolam by human liver cytochrome P450IIIA4. Mol Pharmacol 36:89–96
9. Vanakoski J, Mattila MJ, Seppälä T (1995) Grapefruit juice does not interact with midazolam in man. Br J Clin Pharmacol 40:520P
10. Mattila MJ, Vanakoski J, Idänpään-Heikkilä J-J (1994) Azithromycin does not alter the effects of oral midazolam on human performance. Eur J Clin Pharmacol 47: 49–52
11. Rouseff RL (1988) Liquid chromatographic determination of naringin and neohesperidin as a detector of grapefruit juice in orange juice. J Assoc Anal Chem 71:798–802
12. Mattila MJ, Idänpään-Heikkilä JJ, Törnwall M, Vanakoski J (1993) Oral single doses of erythromycin and roxithromycin increase the effects of midazolam on human performance. Pharmacol Toxicol 73: 180–185
13. Aranko K, Seppälä T, Pellinen J, Mattila MJ (1985) Interaction of diazepam or lorazepam with alcohol. Psychomotor effects and bioassayed serum levels after single and repeated doses. Eur J Clin Pharmacol 28:559–565
14. Olkkola KT, Aranko K, Luurila H, Hiller A, Saarnivaara L, Himberg J-J, Neuvonen P (1993) A potentially hazardour interaction between erythromycin and midazolam. Clin Pharmacol Ther 53:298–305
15. Olkkola KT, Backman JT, Neuvonen PJ (1994) Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole and itraconazole. Clin Pharmacol Ther 55:481–485
16. Kupferschmidt HHT, Riem Ha H, Ziegler WH, Meier PJ, Krähenbühl S (1995) Interaction between grapefruit juice and midazolam in humans. Clin Pharmacol Ther 58:20–28
17. Hukkinen SK, Varhe A, Olkkola KT, Neuvonen PJ (1995) Plasma concentrations of triazolam are increased by concomitant ingestion of grapefruit juice. Clin Pharmacol Ther 58:127–131
18. Bailey DG, Arnold JM, Munoz C, Spence JD (1993) Grapefruit juice–felodipine interaction: mechanism, predictability, and effect of naringin. Clin Pharmacol Ther 53:637–642
19. Bailey DG, Arnold JMO, Bend JR, Tran LT, Spence JD (1995) Grapefruit juice-felodipine interaction: reproducibility and characterization with the extended release of drug formulation. Br J Clin Pharmacol 40:135–140
20. Vanakoski J, Mattila MJ, Vainio P, Idänpään-Heikkilä JJ, Törnwall M (1995) 150 mg fluconazole does not substantially increase the effects of 10 mg midazolam or the plasma midazolam concentrations in healthy subjects. Int J Clin Pharmacol Ther 33:518–523
21. Ducharme MP, Warbasse LH, Edwards DJ (1995) Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. Clin Pharmacol Ther, 57:485–491
22. Fuhr U, Maier A, Keller A, Steinijans VW, Sauter R, Staib AH (1995) Lacking effect of grapefruit juice on theophylline pharmacokinetics. Int J Clin Pharmacol Ther 33:311–314