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Grapefruit Juice and Orange Juice Effects on the Bioavailability of Nifedipine in the Rat

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ABSTRACT: Previous studies with rats indicate that nifedipine undergoes both hepatic and extrahepatic presystemic metabolism after peroral (po) administration, and that its bioavailability is increased and absorption delayed by concomitant administration of grapefruit juice concentrate (GJC). Hence, the effects of GJC could be to delay stomach emptying and inhibit nifedipine metabolism in the small-intestinal wall and liver or, alternatively, to impede nifedipine absorption until reaching the large intestine where gut wall presystemic metabolism is not a factor. The mechanism(s) of action of GJC might be partially resolved by comparison with orange juice concentrate (OJC), which has a similar consistency but lacks inhibitory effects on nifedipine presystemic metabolism, and also by giving regular-strength solutions of the two juices, both on which should not significantly affect stomach emptying. This study compared the po bioavailability of nifedipine (6 mg kg^{-1}) in male Sprague–Dawley rats coadministered GJC, OJC, grapefruit juice regular strength (GJRS), orange juice regular strength (OJRS), or (tap) water. Nifedipine plasma concentration–time profiles in the GJRS, OJRS, and (tap) water groups displayed a single peak. Both GJC and OJC groups have double-peak profiles (indicating delayed gastric emptying); however, the majority of the nifedipine dose in both cases was absorbed during the interval of the second peak, which occurred several hours postdosing. GJC significantly increased nifedipine bioavailability (relative bioavailability 2.02, compared with (tap) water), indicating that GJC may affect both extrahepatic and hepatic first-pass metabolism, although a reduction in systemic nifedipine clearance cannot be ruled out. Surprisingly, GJRS had no significant effect on nifedipine bioavailability. OJC did not increase nifedipine bioavailability, further suggesting that the delay in nifedipine absorption by GJC or OJC results from delayed gastric emptying. © 1998 John Wiley & Sons, Ltd.

Key words: bioavailability; bioflavonoids; extrahepatic metabolism; first-pass metabolism; gastric emptying; pharmacokinetics

Introduction

Grapefruit and its juice are common constituents of a healthy balanced human diet and are typically consumed at breakfast, leading to potential grapefruit–drug interactions since drugs are also often taken in the morning. Grapefruit juice has been shown to augment the bioavailability or plasma concentrations of many different classes of compounds in human beings and rats [1–13], including the 1,4-dihydropyridine class of calcium channel antagonists (e.g. nifedipine, felodipine, nimodipine, nisoldipine and nitrendipine) [1,14–24]. However, to date, the inhibitory substance(s) or mechanism(s) of action of grapefruit juice has not been conclusively identified and the effects are not duplicated by orange juice [1].

Several *in vitro* study reports [9,25–28] speculate that bioflavonoids (e.g., hesperidin, naringin, quercetin, kaempferol, etc) may inhibit *in vivo* cytochrome P450 (CYP) isozyme mediated drug oxidation (including CYP3A4, CYP1A2, and CYP2A6) via the intestinal wall or the liver [1,5]. This is potentially clinically important because bioflavonoids are found in many fruits and vegetables including the grapefruit and orange [29]. Citrus fruits and their juice, however, contain specific bioflavonoids that are relatively uncommon in the plant kingdom [30]. For example, naringin, a bitter tasting flavanone neohesperidoside, is the major bioflavanoid found in grapefruit juice [31]. Interestingly, naringin is not reported to be a component of orange juice [1,30,31], although some conflicting reports exist [29,32]. Several *in vivo* studies [1,16,17,27] have shown no or little evidence of an interaction between individual flavonoids (including, naringin, quercetin, and

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kaempferol) and different 1,4-dihydropyridine compounds; nonetheless, naringenin, the aglycone and human metabolite of naringin, could be the inhibitory substance responsible for the 'effect' of grapefruit juice [1,33]. In fact *in vitro* inhibition of nifedipine metabolism by naringenin in liver microsomes has been demonstrated [28].

Nifedipine is well absorbed ($\geq 90\%$) from the gut lumen of human beings and rats [34–40]. After absorption, nifedipine is metabolized by oxidative mechanisms—involving CYP 3A isozymes—to a pharmacologically inactive nitropyridine analog, which is subsequently metabolized to more polar compounds [41–43]. The absolute bioavailability of nifedipine is similar in both humans and rats ranging from about 0.45 to 0.68 [37,38,44–47]. A previous study [47] from our laboratory using male Sprague–Dawley rats indicated that nifedipine undergoes substantial extrahepatic first-class elimination, in addition to hepatic extraction, presumably within the wall of the small intestine. Similarly, in man, enzymes located both in the gut wall and liver are thought to be involved in the interaction of grapefruit juice with felodipine. [48] Miniscalco *et al*. [28] showed that naringenin, quercetin, and kaempferol separately inhibited 1,4-dihydropyridine metabolism (nifedipine and (R)- and (S)-felodipine) in an *in vitro* study using rat liver microsomes. Hence, these observations suggest that the rat may be a good model species with which to further investigate the *in vivo* interaction of grapefruit juice with nifedipine. In fact, a preliminary *in vivo* study from our laboratory [14] indicates that grapefruit juice concentrate (GJC) delays nifedipine absorption and may increase nifedipine bioavailability in rats.

In man, grapefruit juice does not affect systemic nifedipine clearance [24]; assuming the same for rats, the mechanism(s) by which GJC affects nifedipine absorption and bioavailability in rat could involve (i) delaying gastric emptying and subsequently inhibiting nifedipine first-pass metabolism in the small-intestinal wall or liver (or both) or (ii) delaying absorption until the drug reaches the large intestine—where drug bioavailability may be higher due to the absence of gut wall presystemic metabolism. [47] The mechanism(s) of action of GJC might be partially resolved by comparing GJC with orange juice concentrate (OJC) which has a similar consistency and sugar content but lacks inhibitory effects on nifedipine presystemic metabolism [1], and also by administration of regular-strength solutions of the juices, both of which should not influence gastric emptying to a significant extent.

Based on these considerations, a bioavailability study was conducted using adult male Sprague–Dawley rats. Standard pharmacokinetic measures of nifedipine given by the peroral (po) route were compared following coadministration of either GJC, OJC, grapefruit juice regular strength (GJRS), orange juice regular strength (OJRS), or (tap) water.

Materials and Methods

Chemicals

Nifedipine powder was purchased from Sigma (St. Louis, MO, USA). Nisoldipine powder (internal standard, IS) was obtained from Miles Canada (Etobicoke, Ont., Canada). Unsweetened grapefruit juice and orange juice pure frozen concentrates (sugars 0.47 and 0.49 g mL^{-1}, respectively) were purchased from a local supermarket (Bel Air®, Lucerne Foods, Vancouver, BC, Canada) and contained no artificial colours, flavours or preservatives. Heparin (Hepalean®, 1000 USP units mL^{-1}) was supplied by Organon Teknika (Toronto, Ont., Canada); sodium pentobarbital (Somnotol®, 65 mg mL^{-1}) was purchased from MTC Pharmaceuticals (Cambridge, Ont., Canada); and polyethylene glycol-300 and -400 (Carbowax® Sentry®, FCC grade) was obtained from Union Carbide Chemicals (Danbury, CT, USA). All other chemicals and solvents used in this study were either reagent or HPLC grade and obtained from commercial suppliers.

Animals and Surgical Cannulation Procedure

Adult male Sprague–Dawley rats (initial weight 260–310 g) were obtained from the Biosciences Animal Service (University of Alberta, Edmonton, Alta., Canada) and housed for at least 2 days in a clean room and given food and water *ad libitum*. The day prior to dosing experiments, rats were moved to the laboratory and subjected to surgical cannulation. During anesthesia induced by sodium pentobarbital (65 mg kg^{-1}), a small longitudinal incision was made in the skin of each rat over the right jugular vein, which was then made accessible by clearing the surrounding tissues. The vein was catheterized with Silastic® laboratory tubing (i.d. 0.635 mm, o.d. 1.194 mm, Dow Corning, Midland, MI, USA) containing heparinized (100 IU mL^{-1}) normal saline and fixed in place with two nonabsorbable surgical sutures (Surgical Suture USP, Cyanamid Canada, Montreal, Que., Canada). Each cannula was terminated with a suitable length of polyethylene tubing (PE-50, i.d. 0.58 mm, o.d. 0.965 mm, Clay Adams, Parsippany, NJ, USA) and the free end exteriorized to the dorsal side of the neck. The exposed areas were then closed using nonabsorbable surgical suture. Each rat was allowed to recover for the next

16–20 h in a metabolic cage and provided with only drinking water for the remainder of the experiment. The implanted cannulas permitted frequent blood sampling from each rat.

Pharmacokinetic Study: Experimental Design

All drug preparation, dosing, and collection of blood samples was done under sodium lamps to prevent nifedipine photodegradation [49]. A nifedipine dosing solution (5 mg mL^{-1} in polyethylene glycol-400) was prepared the day before each experiment to allow sufficient time for drug solubilization, wrapped in aluminium foil to protect the solution from light, and stored at room temperature. On experimental days, cannulated rats received 6 mL GJC, GJRS, OJC, OJRS, or (tap) water by an oral feeding tube over approximately 15 s. Exactly 15 min later, each rat received a single po dose of nifedipine (6 mg kg^{-1}) also given by an oral feeding tube. About 120 min after the nifedipine dose, the rats received a further 3 mL GJC, GJRS, OJC, OJRS, or (tap) water, as described above. No adverse events or symptoms in the rats were observed, before or after nifedipine dosing or as a result of administration of any of the five treatments. Blood draws (0.25 mL samples) were made at predetermined times (see below) using heparinized 1 mL syringes connected to the jugular cannula. The blood volume drawn was immediately replaced with an equal volume of normal saline. Blood samples were centrifuged (Beckman Microfuge E, Beckman Instruments, Palo Alto, CA, USA) at 15 000 rpm for 4 min and the plasma samples obtained were placed in 1.5 mL poly(propylene) microfuge tubes, stored in light resistant bags and kept at −20°C until needed for analysis.

Grapefruit Juice Concentrate Administration. Six rats (295.5–325.5 g) received both GJC (undiluted) and nifedipine perorally as previously described. Blood samples were drawn at −5, 15, 30, 60, 90, 120, 240, 360, 480, 600, 720, 840, and 1440 min—the time of nifedipine dosing was designated 0 min.

Grapefruit Juice Regular-Strength Administration. Six rats (295.0–330.5 g) received both GJRS (GJC diluted 1:3 with (tap) water) and nifedipine perorally as previously described. Blood samples were drawn at −5, 2, 5, 10, 15, 30, 60, 90, 120, 240, 300, 360, 480, and 600 min—the time of nifedipine dosing was designated 0 min.

Orange Juice Concentrate Administration. Six rats (303.5–354.5 g) received both OJC (undiluted) and nifedipine perorally as previously described. Blood samples were drawn at −5, 15, 30,60, 90, 120, 240, 360, 480, 600, 720, 840, and 1440 min—the time of nifedipine dosing was designated 0 min.

Orange Juice Regular-Strength Administration. Six rats (302.5–339.0 g) received both OJRS (OJC diluted 1:3 with (tap) water) and nifedipine perorally as previously described. Blood samples were drawn at −5, 2, 5, 10, 15, 30, 60, 90, 120, 240, 300, 360, 480, and 600 min—the time of nifedipine dosing was designated 0 min.

(Tap) Water Administration. Six rats (315.5–334.5 g) received both (tap) water and nifedipine perorally as previously described. Blood samples were drawn at −5, 2, 5, 10, 15, 30, 60, 90, 120, 240, 300, 360, 480, and 600 min—the time of nifedipine dosing was designated 0 min.

Chromatography

Nifedipine concentrations in rat plasma samples were determined by means of an established HPLC method (lower limit of quantitation 5 ng mL^{-1}) [50] using 0.1–0.2 mL samples diluted to 1.0 mL with HPLC-grade water. All analyses were conducted under sodium lamps to prevent photodegradation of nifedipine. [49] Calibration curve standards were prepared by adding a known amount of the drug to 0.1 mL of blank rat plasma and diluting to 1.0 mL as described above. The calibration curve (5–2000 ng of nifedipine) was determined from the best-fit regression line calculated using a $1/\chi^2$ weighting factor (where χ corresponds to the amount of nifedipine added; $r^2 > 0.99$; interday variability $< 10\%$). HPLC instrumentation included a model 600E solvent delivery system, a model 717 autosampler, a model 486 tunable UV–VIS absorbance detector (set at 350 nm), and an NEC 486–33 MHz computer running Millennium 2010 Chromatography Manager software Version 1.1 (Waters, Mississauga, Ont., Canada).

Pharmacokinetic Analysis

Standard pharmacokinetic measures from each of the individual rat plasma concentration–time profiles were calculated by non-compartmental methods using the computer progam WinNonlin Standard Edition Version 1.0 (Scientific Consulting, Apex, NC, USA)—the area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule from time zero (t_0) to the time of the last quantifiable concentration (t_{last}), followed by extrapolation to infinity. Relative bioavailability (F_A/F_B) after po nifedipine administration at the same dosing level was determined according to the expression

$$\frac{F_A}{F_B} = \frac{AUC_A}{AUC_B} \quad (1)$$

where AUC_A and AUC_B are the areas under the nifedipine plasma concentration–time curve for treatments A and B, respectively.

Statistical Analysis

All the calculated pharmacokinetic parameters—except t_{max}—were assumed to follow log-normal distribution, and therefore log-transformed before statistical analysis. The pharmacokinetic parameter estimates obtained were expressed as either geometric or arithmetic means with their corresponding 95% confidence intervals, as appropriate. Pharmacokinetic data were analyzed using independent measures one-way analysis of variance (ANOVA) and a *post hoc* test (Duncan's multiple-range test) was used to determine where, if any, significant differences occurred ($p \leq 0.05$). The computer program SPSS for Windows Version 6.1 (SPSS, Chicago, IL, USA) was used for all the statistical tests. For the purposes of generating plasma concentration–time profiles, plasma nifedipine concentrations were expressed as arithmetic means $\pm$ standard error ($s_{\bar{x}}$).

Results and Discussion

Administration of relatively large volumes of the treatment solutions (i.e. GJC, GJRS, OJC, OJRS, and (tap) water), and the timing of the treatments in this study, were done to maximize potential inhibitory effects on nifedipine presystemic metabolism and to ensure their presence in the gut throughout the period of nifedipine absorption. The solution volumes administered in the study did not cause harm to the animals as the typical water consumption of rats is about 80–110 mL kg^{-1} day^{-1} [51], and a safe intragastric injection volume in rats (250 g) is reported to be about 5 mL (depending upon the degree of stomach filling) [52]. Additionally, it is reported that a 300 g rat can be tube fed 13 mL of water at a time and trained to take up to 26 mL [51]. With regard to the timing of treatment administration we used, Lundahl *et al*. [48] studied the relationship between the time of intake of grapefruit juice and the pharmacokinetic effect on felodipine in human subjects. The effect of grapefruit juice was immediate with regard to the increase in AUC and C_{max} of felodipine, with similar effects seen at 0 and 1 h, and reduced but significant effects seen 10 h later compared to controls (note that C_{max} was significantly increased even at 24 h post-dosing). Thus, the administration schedule we used should have allowed for near optimal effects.

The mean ($\pm s_{\bar{x}}$) plasma nifedipine concentration–time profiles obtained from each of the five treatment groups are shown in Figure 1. Individual and mean profiles for the GJRS, OJRS and (tap) water groups displayed single peaks, whereas the GJC and OJC groups displayed double peaks. The first peak in the GJC group exhibited the lowest peak nifedipine concentration (all six rats), but the opposite relationship was found in most rats of the OJC group (four of six rats). Nonetheless, the areas under the curves of the first peaks were about 2.6% and 16.7% of the total areas under the mean curves for the GJC and OJC groups, respectively. This indicates that the majority of nifedipine absorption was significantly delayed in both GJC and OJC treatments, and the position of the second peak (Figure 1) shows that the delay was of about the same extent. Mean nifedipine plasma concentration–time profiles also showed more variability in the GJC and OJC groups, which was expected given the anticipated variability in gastric emptying or colonic arrival times.

Standard pharmacokinetic measures of nifedipine bioavailability calculated from the data obtained from all five treatments are shown in Table 1 and significant differences, where found, are noted. Nifedipine oral clearance in the (tap) water group (see Table 1) was similar to that found in a group of rats administered po nifedipine ((tap) water given *ad libitum*) from a previous study (17.0 mL min^{-1} kg^{-1}, $n = 6$) [47]. Oral nifedipine clearance was significantly lower for the GJC group than the (tap) water group but none of the other treatments had this effect. In the GJRS group, oral clearance was not significantly decreased but the geometric mean value was lower than that of (tap) water, OJC, and OJRS groups.

Bioavailability of nifedipine in the GJC group was about double that of the (tap) water group. It is worth noting that the determination of relative bioavailabilities in this report assumes that nifedipine systemic clearance was unchanged by each treatment. Support for this assumption comes from a human study [24] in which grapefruit juice did not alter the kinetics of an iv nifedipine dose but increased the AUC and bioavailability of nifedipine given perorally, and by the fact that the elimination half-life of the drug was unchanged. Similarly, in other reports [7,8] grapefruit juice did not effect the systemic clearance of cyclosporine or midazolam but did improve oral bioavailability of both agents, suggesting that its mechanism of action is to increase absorption or reduce gut wall metabolism. The apparent increased nifedipine bioavailability found in the GJC group suggests that both hepatic and extrahepatic presystemic metabolism were inhibited by GJC, or that a large portion of the dose bypassed presystemic metabolism (via absorption in the lower gastrointestinal (GI) tract). Surprisingly, GJRS did not significantly increase nifedipine bioavailability in rat (although a small increase was found) as has been shown with regular- or double-strength grapefruit juice in human beings [1,19,22,24], indicating that a relatively large dose of

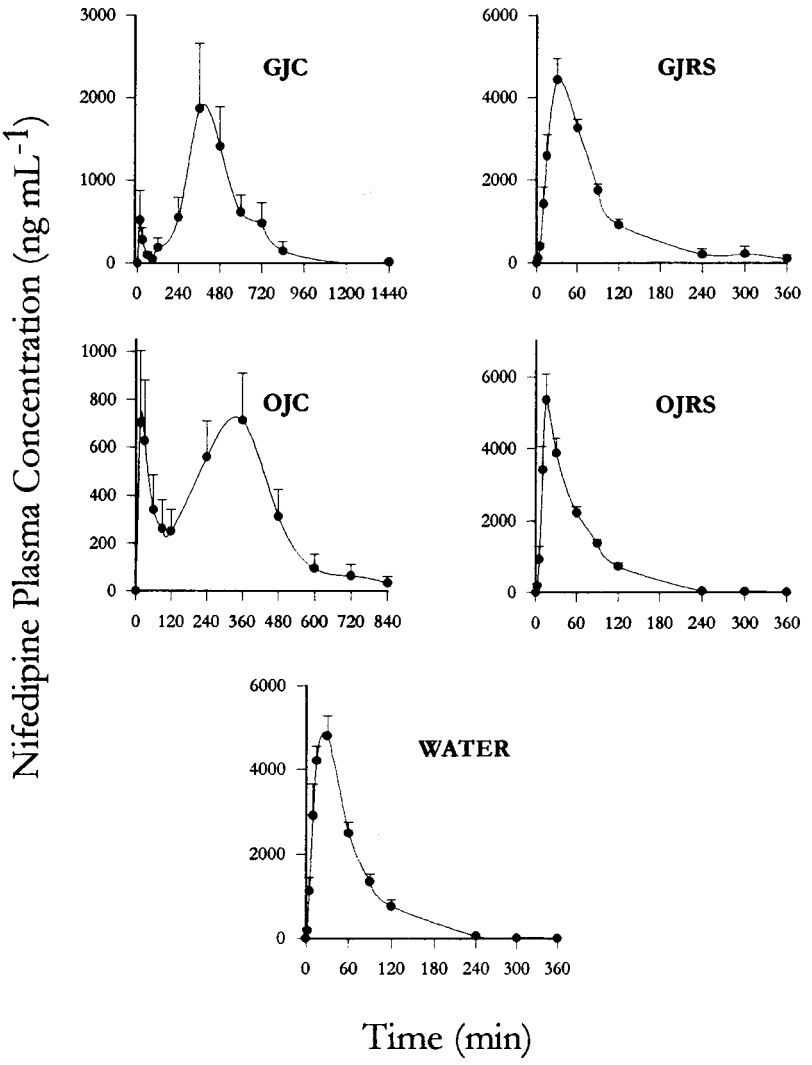

Figure 1. Plasma concentration–time profiles in male Sprague–Dawley rats after a single po dose of nifedipine (6 mg kg^{-1}) was given to rats in the following treatment groups: grapefruit juice concentrate (GJC), grapefruit juice regular strength (GJRS), orange juice concentrate (OJC), orange juice regular strength (OJRS), and (tap) water. For illustration purposes, the axes of each plot were scaled for each treatment group. Data are mean values $\pm s_x$ ($n = 6$ animals group)

the inhibitory substance(s) in grapefruit juice is required to achieve an effect in rat (i.e. GJC). This is in contrast to *in vitro* findings with naringenin which show that rat liver microsomal metabolism of nifedipine is inhibited to a greater extent than when using human microsomal preparations. [28]

In addition to its effect on nifedipine bioavailability, GJC significantly increased the apparent t_{max} of the drug compared to all other groups (Table 1), corroborating the findings of a previous study [14]. The apparent increase in nifedipine t_{max} observed for OJC was not significantly different than (tap) water or regular-strength juice groups. However, this was a result of the double-peaking phenomenon previously described. That is, if the first peak in the OJC rat profiles had been ignored when determining t_{max} then a significant difference would have been observed. The regular-strength solutions of both juices did not significantly alter t_{max} from that of the (tap) water group. These values were not statistically different from that reported in a previous study [47] for po dosing of rats fed water *ad libitum* (t_{max} (95% CI) = 16.7 (9.5–23.8) min). Interestingly, GJRS has been shown to delay the absorption of perorally administered cyclosporine, midazolam, quinidine, and triazolam in human subjects [7,8,13,53]. Variable effects on t_{max} of 1,4-dihydropyridines (nifedipine, felodipine, and nisoldipine) by regular- or double-strength grapefruit juice have been reported in humans including reduced [17], none [15,16,24,48], or increased [18,19,22] effects.

The double-peak phenomenon noted in both the GJC and OJC nifedipine plasma concentration–time profiles was an interesting finding. A more prominent double-peak effect was produced by OJC, for reasons not clear at present, despite the fact that both juices were of similar consistency and composition (except for differences previously noted). The double-peak phenomenon has been observed for

Table 1. Pharmacokinetic parameters obtained following po administration of nifedipine (6 mg kg^{-1}) to rats coadministered grapefruit juice, orange juice, or (tap) water treatment regimens. Where appropriate, data are expressed as geometric means with corresponding 95% confidence intervals (CI, $n = 6$ animals group). T_{max} is presented as arithmetic mean with 95% CI ($n = 6$ animals per group)

Parameters	GJC	GJRS	OJC	OJRS	(Tap) water
AUC (μg mL^{-1} min)	700 (450–1088)[a]	393 (335–460)[b]	274 (213–354)[c]	327 (280–383)[b,c]	345 (283–419[b,c]
C_{max} (ng mL^{-1})	2097 (1106–3978)[d]	4657 (3976–5453)[e]	1223 (899–1664)[f]	5563 (4260–7264)[e]	5230 (4548–6013)[e]
t_{max} (min)	443 (132–753)[g]	35 (22–48)[h]	126 (0–276)[h]	16 (8–24)[h]	23 (12–34)[h]
$t_{1/2\lambda}$ (min)	70 (39–127)[i]	37 (27–51)[j,k]	56 (30–106)[i,j]	31 (26–37)[k]	31 (25–38)[k]
CL/F (ml min^{-1} kg^{-1})	8.6 (5.5–13.3)[l]	15.3 (13.0–17.9)[m]	21.9 (17.0–28.2)[n]	18.3 (15.7–21.4)[m,n]	17.4 (14.3–21.2)[m,n]
Relative bioavailability[o]	2.02	1.14	0.79	0.95	1.0

[a–n] Identical superscript characters identify non-significant differences ($p > 0.05$).
[o] Relative to the (tap) water group.
AUC, area under the plasma drug concentration–time curve from zero to infinity; CL/F, oral plasma clearance of nifedipine; C_{max}, maximum (peak) plasma drug concentration; F, systemically available fraction of the dose of a drug; GJC, grapefruit juice concentrate; GJRS, grapefruit juice regular strength; iv, intravenous; OJC, orange juice concentrate; OJRS, orange juice regular strength; t_{max}, time to reach the maximum (peak) drug concentration following extravascular drug administration; $t_{1/2\lambda}$, elimination half-life of the drug associated with the terminal slope of a semilogarithmic plasma drug concentration–time curve.

several orally administered drugs such as cimetidine, L-dopa, morphine, pafenolol, and others. [54] Several potential explanations for this phenomenon include enterohepatic circulation, pH-dependent solubility, different permeabilities or extents of presystemic metabolism in various regions of the GI tract, or interruption of gastric emptying. The absence of double peaks in the (tap) water or both regular-strength juice group plasma profiles would tend to rule out the possibility of enterohepatic circulation. As well, nifedipine is rapidly, extensively, and presumably irreversibly metabolized via oxidation in the rat, with the principal metabolite further metabolized to several other biotransformation products which themselves can undergo enterohepatic circulation [34,35]. Given that the pK_a-value of nifedipine is -0.9 [55], the drug exists primarily in its unprotonated form at all pH conditions in the GI tract and thus pH-dependent solubility and absorption can be ruled out. The existence of specific sites of absorption ('absorption windows') for nifedipine can also be ruled out since the drug is well absorbed ($> 90\%$) throughout the upper and lower intestine in both rats and man [36,47,56], although differences in the extent of gut wall metabolism in different regions of the gut could contribute, in part, to the observed double-peak effect [47]. Therefore, the most likely explanation for the double-peak phenomenon is a delay in gastric emptying produced by GJC or OJC.

Commonly used laboratory animals display a cyclic gastric motility pattern (i.e. the unfed state has several phases which repeat every 2 h and empty the stomach contents), but in the fed state gastric retention can be several hours [57]. In the rat, gastric emptying of liquids is relatively rapid. This is illustrated in a study showing the gastric emptying of ^{131}I-polyvinylpyrrolidone to be 75% complete within 15 min [57,58]. Therefore, following coadministration of a nifedipine solution with (tap) water or regular-strength juice, rapid absorption of drug from the small intestine can be expected, and was indeed found (see Table 1). When a nifedipine solution is coadministered with grapefruit and orange juice concentrates (both relatively viscous solutions with high osmolarity), the effect on gastric emptying rate is difficult to predict since the exact mechanism of inhibition by liquid and solid meals is not yet known in rats or humans [59]. Human studies have shown gastric emptying to be delayed by hot meals, fatty foods, high-protein or carbohydrate meals, and viscous solutions [59]. As well, citric acid is used to inhibit gastric motility in ^{13}C-urea breath tests for detection of *Helicobactor pylori* infection [60,61]. On the other hand, large fluid volumes can increase gastric emptying rate [59].

If GJC and OJC were assumed to have no effect upon gastric emptying then the observed delay in nifedipine absorption would have to involve a physical effect such as nifedipine incorporation in the concentrates. This could delay nifedipine absorption until the juice concentrates were broken down, presumably in the small intestine or colon. The transit time of material through the rat small intestine is reported to be only about 88 min [62]. Similarly, another report [63] showed that 50% of ^{125}I-PVP given intraduodenally passed through the small intestine in only 20 min. Hence, given the above assumption and the kinetic profiles obtained in the GJC and OJC groups, one would expect the majority of the nifedipine absorption to occur in the colon. Data from intracolonic and rectal

nifedipine dosing in rats shows that bioavailability increases relative to po dosing [44,47]. However, nifedipine bioavailability in the OJC group was less than that in the (tap) water group (Table 1), indicating that physical incorporation of nifedipine by the concentrates is unlikely (assuming nifedipine absorption was complete in all cases). This observation, taken with the finding of double peaking, strongly indicates that both GJC and OJC affected the rate of gastric emptying in this study.

Interestingly, du Souich *et al*. [64] noted a lack of intestinal, hepatic, or pulmonary presystemic metabolism of nifedipine in the rabbit, leading to speculation by the authors that nifedipine may be decomposed in the GI lumen of rats and humans to account for its poor oral bioavailability. The results of the present study seem to contradict this theory, as nifedipine appears to be completely absorbed from the GI tract of rats when coadministered with GJC. This is despite the fact that GJC greatly delays the absorption of nifedipine which might be expected to lead to increased decomposition of the drug in the GI tract.

In conclusion, the results of this study showed that GJC acts to inhibit presystemic metabolism of nifedipine (both hepatic and extrahepatic), and that both GJC and OJC slow the rate of gastric emptying in rats. As expected, OJC and OJRS did not significantly increase nifedipine bioavailability compared to (tap) water. Surprisingly, GJRS had no significant effect upon nifedipine bioavailability. Nonetheless, it was demonstrated that, under appropriate experimental conditions, the rat may be a suitable model for *in vivo* investigation of the interaction of grapefruit juice with nifedipine.

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References

1. D.G. Bailey, J.M. Arnold, and J.D. Spence, Grapefruit juice and drugs. How significant is the interaction? [Review]. *Clin. Pharmacokinet*., **26,** 91–98 (1994).
2. M.P. Ducharme, R. Provenzano, M. Dehoorne-Smith and D.J. Edwards, Trough concentrations of cyclosporine following administration with grapefruit juice. *Br. J. Clin. Pharmacol*., **36,** 457–459 (1993).
3. U. Fuhr, K. Klittich and A.H. Staib, Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *Br. J. Clin. Pharmacol*., **35,** 431–436 (1993).
4. S.I. Sharif and B.H. Ali, Effect of grapefruit juice on drug metabolism in rats. *Food Chem. Toxicol*., **32,** 1169–1171 (1994).
5. U. Merkel, H. Sigusch and A. Hoffman, Grapefruit juice inhibits 7-hydroxylation of coumarin in healthy volunteers. *Eur. J. Clin. Pharmacol*., **46,** 175–177 (1994).
6. S.E. Rau, D.G. Bailey, L.T. Tran, J.D. Spence and J.R. Bend, Inhibition of terfenadine metabolism by co-administration of grapefruit juice in humans. In *Abstract Book of the 10th International Symposium on Microsomes and Drug Oxidation*, G.D. Bellward (Ed), Toronto, 1994, p. 310.
7. H.H. Kupferschmidt, H.R. Ha, W.H. Ziegler, P.J. Meier and S. Krahenbuhl, Interaction between grapefruit juice and midazolam in humans. *Clin. Pharmacol. Ther*., **58,** 20–28 (1995).
8. M.P. Ducharme, L.H. Warbasse and D.J. Edwards, Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. *Clin. Pharmacol. Ther*., **57,** 485–491 (1995).
9. A.A. Hollander, J. van Rooij, G.W. Lentjes, F. Arbouw, J.B. van Bree, R.C. Schoemaker, L.A. van Es, F.J. van der Woude and A.F. Cohen, The effect of grapefruit juice on cyclosporine and prednisone metabolism in transplant patients. *Clin. Pharmacol. Ther*., **57,** 318–324 (1995).
10. W. Schubert, G. Cullberg, B. Edgar and T. Hedner, Inhibition of 17 beta-estradiol metabolism by grapefruit in ovariectomized women. *Maturitas*, **20,** 155–163 (1994).
11. R.E. Benton, P.K. Honig, K. Zamani, L.R. Cantilena and R.L. Woosley, Grapefruit juice alters terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. *Clin. Pharmacol. Ther*., **59,** 383–388 (1996).
12. A. Balogh, A. Weber and G. Klinger. Can grapefruit juice influence the bioavailability of ethinylestradiol? *Eur. J. Clin. Pharmacol*., **47,** A93 (1994) (Abstract).
13. S.K. Hukkinen, A. Varhe, K.T. Olkkola and P.J. Neuvonen, Plasma concentrations of triazolam are increased by concomitant ingestion of grapefruit juice. *Clin. Pharmacol. Ther*., **58,** 127–131 (1995).
14. J.S. Grundy and R.T . Foster, The effect of grapefruit juice concentrate (GJC) on the pharmacokinetics (PK) of oral nifedipine (NIF) administered to the rat. *Pharm. Res*., **12,** S-425 (1995) (Abstract).
15. D.G. Bailey, M.O. Arnold, J.R. Bend, L.T. Tran and J.D. Spence, Grapefruit juice–felodipine interaction: reproducibility and characterization with the extended release drug formulation. *Br. J. Clin. Pharmacol*., **40,** 135–140 (1995).
16. D.G. Bailey, J.M. Arnold, .C Munoz and J.D. Spence, Grapefruit juice–felodipine interaction: mechanism, predictability, and effect of naringin. *Clin. Pharmacol. Ther*., **53,** 637–642 (1993).
17. D.G. Bailey, J.M. Arnold, H.A. Strong, C. Munoz and J.D. Spence, Effect of grapefruit juice and naringin on nisoldipine pharmacokinetics. *Clin. Pharmacol. Ther*., **54,** 589–594 (1993).
18. B. Edgar, D. Bailey, R. Bergstrand, G. Johnsson and C.G. Regardh, Acute effects of drinking grapefruit juice on the pharmacokinetics and dynamics of felodipine—and its potential clinical relevance. *Eur. J. Clin. Pharmacol*., **42,** 313–317 (1992).
19. D.G. Bailey, J.D. Spence, C. Munoz and J.M. Arnold, Interaction of citrus juices with felodipine and nifedipine. *Lancet*, **337,** 268–269 (1991).
20. B. Edgar, D.G. Bailey, R. Bergstrand, G. Johnsson and L. Lurje, Formulation dependent interaction between felodipine and grapefruit juice. *Clin. Pharmacol. Ther*., **47,** 181 (1990) (Abstract).
21. P.A. Soons, B.A. Vogels, M.C. Roosemalen, H.C. Schoemaker, E. Uchida, B. Edgar, J. Lundahl, A.F. Cohen and D.D. Breimer, Grapefruit juice and cimetidine inhibit stereoselective metabolism of nitrendipine in humans. *Clin. Pharmacol. Ther*., **50,** 394–403 (1991).
22. H. Sigusch, M. Hippius, L. Henschel, K. Kaufmann and A. Hoffmann, Influence of grapefruit juice on the pharmacokinetics of a slow release nifedipine formulation. *Pharmazie*, **49,** 522–524 (1994).

23. U. Fuhr, A. Maier, H. Blume, W. Mück, S. Unger and A.H. Staib, Grapefruit juice increases oral nimodipine bioavailability. *Eur. J. Clin. Pharmacol.*, **47,** A100 (1994) (Abstract).
24. T.J. Rashid, U. Martin, H. Clarke, D.G. Waller, A.G. Renwick and C.F. George, Factors affecting the absolute bioavailability of nifedipine. *Br. J. Clin. Pharmacol.*, **40,** 51–58 (1995).
25. M.T. Obermeier, R.E. White and C.S. Yang. Effects of bioflavonoids on hepatic P450 activities. *Xenobiotica*, **25,** 575–584 (1995).
26. M.K. Buening, R.L. Chang, M.-T. Huang, J.G. Fortner, A.W. Wood and A.H. Cooney, Activation and inhibition of benzo(a)pyrene and aflatoxin B_1 metabolism in human liver microsomes by naturally occurring flavonoids. *Cancer Res.*, **41,** 67–72 (1981).
27. J. Rashid, C. McKinstry, A.G. Renwick, M. Dirnhuber, D.G. Waller and C.F. George, Quercetin, an *in vitro* inhibitor of CYP3A, does not contribute to the interaction between nifedipine and grapefruit juice. *Br. J. Clin. Pharmacol.*, **36,** 460–463 (1993).
28. A. Miniscalco, J. Lundahl, C.G. Regardh, B. Edgar and U.G. Eriksson, Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. *J. Pharmacol. Exp. Ther.*, **261,** 1195–1199 (1992).
29. J. Kühnau. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet.*, **24,** 117–191 (1976).
30. R.L. Rouseff. Liquid chromatographic determination of naringin and neohesperidin as a detector of grapefruit juice in orange juice. *J. Assoc. Off. Anal. Chem.*, **71,** 798–802 (1988).
31. R.L. Rousef, S.F. Martin and C.O. Youtesey, Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. *J. Agric. Food Chem.*, **35,** 1027–1030 (1987).
32. F. Drawert, G. Leupold and H. Pivernetz, Quantitative determination of rutin, hesperidin, and naringin in orange juice by gas chromatography. *Chem. Mikrobiol. Technol. Lebensm.*, **6,** 189–191 (1980).
33. U. Fuhr and A.L. Kummert, The fate of naringin in humans: a key to grapefruit juice–drug interactions? *Clin. Pharmacol. Ther.*, **58,** 365–373 (1995).
34. B. Duhm, W. Maul, H. Medenwald, K. Patzschke and L.A. Wegner, [Animal experiments on pharmacokinetic and biotransformation of radioactively labelled 4-(2′-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester]. *Arzneim. Forsch.*, **22,** 42–53 (1972) (in German).
35. D. Scherling, W. Karl, M. Radtke, H.J. Ahr and H.-M. Siefert, Biotransformation of nifedipine in rat and dog. *Arzneim. Forsch.*, **42,** 1292–1300 (1992).
36. C.H. Kleinbloesem, J. van Harten, L.G.J. de Leede, P. van Brummelen and D.D. Breimer, Nifedipine kinetics and dynamics during rectal infusion to steady state with an osmotic system. *Clin. Pharmacol. Ther.*, **36,** 396–401 (1984).
37. T.S. Foster, S.R . Hamann, V.R. Richards, P.J. Bryant, D.A. Graves and R.G. McAllister, Nifedipine kinetics and bioavailability after single intravenous and oral doses in normal subjects. *J. Clin. Pharmacol.*, **23,** 161–170 (1983).
38. J.G. Kelly and K. O'Malley, Clinical pharmacokinetics of calcium antagonists. An update. [Review]. *Clin. Pharmacokinet.*, **22,** 416–433 (1992).
39. E.M. Sorkin, S.P. Clissold, R.N. Brogden, Nifedipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in ischaemic heart disease, hypertension and related caridiovascular disorders. [Review]. *Drugs*, **30,** 182–274 (1985).
40. C.H. Kleinbloesem, P. van Brummelen and D.D. Breimer, Nifedipine. Relationship between pharmacokinetics and pharmacodynamics. [Review]. *Clin. Pharmacokinet.*, **12,** 12–29 (1987).
41. J. Dokladalova, J.A. Tykal, S.J. Coco, P.E. Durkee, G.T. Quercia, J.J. Korst, Occurrence and measurement of nifedipine and its nitropyridine derivatives in human blood plasma. *J. Chromatogr.*, **231,** 451–458 (1982).
42. J.H. Schellens, I.M. Van Haelst, J.B. Houston and D.D. Breimer, Nonlinear first-pass metabolism of nifedipine in healthy subjects. *Xenobiotica*, **21,** 547–555 (1991).
43. F.A. Tucker, P.S. Minty. G.A. MacGregor, Study of nifedipine photodecomposition in plasma and whole blood using capillary gas–liquid chromatography. *J. Chromatogr.*, **342,** 193–198 (1985).
44. S. Kondo and I. Sugimoto, Moment analysis of intravenous, intraduodenal, buccal, rectal and percutaneous nifedipine in rats [published erratum appears in J. Pharmacobio-Dyn., **10,** 767 (1987)]. *J. Pharmacobio-Dyn.*, **10,** 462–469 (1987).
45. C.H. Kleinbloesem, P. van Brummelen, J.A. van de Linde, P.J. Voogd and D.D. Breimer, Nifedipine: kinetics and dynamics in healthy subjects. *Clin. Pharmacol. Ther.*, **35,** 742–749 (1984).
46. J.S. Grundy, L.A. Eliot and R.T. Foster, The pharmacokinetics (PK) of nifedipine (NIF) in the rat following intravenous (IV), intraperitoneal (IP), and oral (PO) dosing. *Pharm. Res.*, **12,** S-427 (1995) (Abstract).
47. J.S. Grundy, L.A. Eliot and R.T. Foster. Extrahepatic first-pass metabolism in nifedipine in the rat. *Biopharm. Drug Disposit.*, **18,** 509–522 (1997).
48. J. Lundahl, C.G. Regardh, B. Edgar and G. Johnson, Relationship between time of intake of grapefruit juice and its effect on pharmacokinetics and pharmacodynamics of felodipine in healthy subjects. *Eur. J. Clin. Pharmacol.*, **49,** 61–67 (1995).
49. J.S. Grundy, R. Kherani and R.T. Foster, Photostability determination of commercially available nifedipine oral dosage formulations. *J. Pharm. Biomed. Anal.*, **12,** 1529–1535 (1994).
50. J.S. Grundy, R. Kherani, R.T. Foster, Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection. *J. Chromatogr.* B, **654,** 146–151 (1994).
51. W.S. Bivin, M.P. Crawford and N.R. Brewer, Morphophysiology. In *The Laboratory Rat*, H.J. Baker, J.R. Lindsey, S.H. Weisbroth (Eds), Academic, Toronto, 1979.
52. R. Remie, J.J. Van Dongen, J.W. Rensema and G.H.J. Van Wunnik, General techniques. In: *Manual of Microsurgery on the Laboratory Rat*, J.P. Huston (Ed), Elsevier, Amsterdam, 1990.
53. D.I. Min, Y.-M. Ku, D.R. Geraets and H.-C. Lee, Effect of grapefruit juice on the pharmacokinetics and pharmacodynamics of quinidine in healthy volunteers. *J. Clin. Pharmacol.*, **36,** 469–476 (1996).
54. H. Lennernäs and G.-G. Regårdh, Regional gastrointestinal absorption of the beta-blocker pafenolol in the rat and intestinal transit rate determined by movement of 14C-polyethylene glycol (PEG) 4000. *Pharm. Res.*, **100,** 130–135 (1993).
55. S.L. Ali, Nifedipine. In *Analytical Profiles of Drug Substances*, K. Florey (Ed), Academic, New York, pp. 221–288, 1989.
56. J.S. Grundy and R.T. Foster, The nifedipine gastrointestinal therapeutic system (GITS): evaluation of pharmaceutical pharmacokinetic and pharmacological properties. *Clin. Pharmacokinet.*, **30,** 28–51 (1996).
57. T.T. Kararli, Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Disposit.*, **16,** 351–380 (1995).
58. F. Nilsson and H. Johansson, A double isotope technique for the evaluation of drug action on gastric evacuation and small bowel propulsion studied in the rat. *Gut*, **14,** 475–477 (1973).
59. P.G. Welling, Effects of food on drug absorption. *Pharmacol. Ther.*, **43,** 425–441 (1989).
60. E. Lotterer, J. Ramaker, F.E. Ludtke, R. Tegeler, J.V. Geletneky and F.E. Bauer, The simplified 13C-urea breath test—one point analysis for detection of *Helicobacter pylori* infection. *Z. Gastroenterol.*, **29,** 590–594 (1991).

61. E. Lotterer, F.E. Ludtke, R. Tegeler, G. Lepsien and F.E. Bauer. The 13C-urea breath test—detection of Helicobacter pylori infection in patients with partial gastrectomy. *Z. Gastroenterol.*, **31**(2), 115–119 (1993).
62. B. Davies and T. Morris, Physiological parameters in laboratory animals and humans. *Pharm. Res.*, **10,** 1093–1095 (1993).
63. G. Pettersson, H. Ahlman and J. Kewenter, A comparison of small intestinal transit time between the rat and the guinea-pig. *Acta Chir. Scand.*, **142,** 537–540 (1976).
64. P. du Souich, L. Héroux, H. Maurice, M. Dépôt and G. Caillé, Lack of presystemic metabolism of nifedipine in the rabbit. *J. Pharmacokinet. Biopharm.*, **23,** 567–580 (1995).