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Grapefruit juice, lyophilized grapefruit juice, and powdered whole grapefruit inhibit cytochrome P450 mediated triazolam hydroxylation by beagle dog liver microsomes

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Abstract

Coadministration of grapefruit juice has been proposed to enhance the systemic availability and decrease the required dose of drugs such as cyclosporine that are extensively metabolized in the intestine and liver. Although grapefruit juice inhibits human cytochrome P450 (CYP) 3A, effects on dog CYP have not yet been reported. Consequently, we determined whether grapefruit juice (GFJ) inhibits triazolam hydroxylation by Beagle dog liver microsomes (DLM) using human liver microsomes (HLM) as positive control. Results were compared to effects of lyophilized GFJ and commercially-available powdered grapefruit capsules, which may be more convenient dosage forms. GFJ inhibited α -hydroxytriazolam formation in both DLM and HLM with similar IC₅₀ values of 0.56 and 0.52% (v/v), respectively. Lyophilized GFJ and powdered grapefruit also inhibited DLM α -hydroxytriazolam formation with IC₅₀ values of 0.76 and 1.2 mg / mL, respectively. Consistent with mechanism-based enzyme inhibition, preincubation of DLM with any of the grapefruit products for 20 minutes resulted in significant enhancement of inhibition of triazolam α -hydroxylation by 8 to 20%. The results indicate that 16 grams of lyophilized GFJ or 23 grams of powdered grapefruit would be equivalent to dosing 100 mL of GFJ. *In vivo* pharmacokinetic interaction studies are needed to confirm these *in vitro* findings.

Keywords

grapefruit juice; cytochrome P450; interaction; triazolam; dog

Introduction

Cyclosporine is an immunosuppressive agent used in both human and veterinary medicine. Although more widely known for its use in organ transplant recipients, there is growing interest in the use of cyclosporine in canine dermatology, including treatment of perianal fistulas and atopic dermatitis (Mathews & Sukhiani, 1997; Mathews et al., 1997; Griffiths et

al., 1999; Fontaine & Olivry, 2001; Olivry et al., 2002a; Olivry et al., 2002b; Mouatt, 2002; Patricelli et al., 2002; Steffan et al., 2003; Doust et al., 2003; Hardie et al., 2005). Unfortunately, the currently approved canine cyclosporine product (Atopica®, Novartis Animal Health, Greensboro, NC) while effective, is also relatively expensive. When given at a 5 mg/kg daily dose, the approximate monthly cost of medication can range from \$60 to \$400 depending on the size of the animal (1800petmeds.com; accessed 7–18–2008).

In dogs and humans, cyclosporine has limited systemic availability in part resulting from extensive presystemic metabolism by cytochrome P450 (CYP) in the intestine and liver (Whalen et al., 1999; Wu et al., 1995). Accordingly, co-administration of CYP inhibitors with cyclosporine would be expected to increase plasma concentrations of cyclosporine. Therapeutic drug-drug interactions of this type have been used to decrease the dose of cyclosporine necessary to achieve effective blood concentrations and, in turn, reduce the overall cost of therapy. For example, in a recent study involving treatment of dogs with perianal fistulas, ketoconazole – a potent CYP3A inhibitor – was successfully shown to “boost” cyclosporine levels, thereby decreasing the cost of treatment by up to 70% (O’Neill et al., 2004). This finding is in agreement with the results of an earlier study where ketoconazole coadministration reduced the cyclosporine dose by 80 to 90% and the cost of therapy by as much as 83% (Mouatt, 2002). Patricelli and colleagues also found this combination to be a cost-saving and effective method for the treatment of perianal fistulas in dogs (Patricelli et al., 2002). In addition to ketoconazole, the calcium channel-blocker and CYP3A inhibitor, diltiazem, has been used to reduce the required dose of cyclosporine in two dogs with pruritic dermatopathies (personal communication by A. Bell cited in Robson, 2003). However, coadministration of cyclosporine with either ketoconazole or diltiazem exposes the animal to the inherent risks associated with the boosting agent. In fact, a recent report found a 15% frequency for adverse effects during ketoconazole therapy, with vomiting, anorexia, lethargy, and diarrhea being the most common (Mayer et al., 2008).

Coadministration of grapefruit juice represents a potentially safer means of “boosting” cyclosporine levels in dogs. Various *in vitro* and *in vivo* investigations have implicated the furanocoumarins as the grapefruit juice constituents responsible for CYP3A inhibition in humans (Schmiedlin-Ren et al., 1997; He et al., 1998; Guo et al., 2000; Tassaneeyakul et al., 2000; Paine et al., 2006). While grapefruit juice drug interactions have been extensively studied in humans, far fewer studies have been conducted in dogs. Orally administered liquid or freeze-dried grapefruit juice increased the maximum observed plasma concentration (C_{max}) and area under the plasma concentration-time curve (AUC) of praziquantel in beagle dogs by approximately 200% and 150%, respectively (Giorgi et al., 2003). In another study in beagle dogs, bergamottin, an inhibitory furanocoumarin found in grapefruit juice, increased diazepam AUC and C_{max} by 89 and 51-fold, respectively (Sahi et al., 2002). The results of these two studies suggest that grapefruit juice is capable of inhibiting canine CYP metabolism. Unfortunately, the clinical use of grapefruit juice (up to 100 mL dose is required for an inhibitory effect) is limited by its poor palatability in dogs, while bergamottin is relatively expensive and not currently available in a form for routine clinical use.

A product containing powdered whole grapefruit in capsule form is currently being marketed as a dietary supplement for weight loss (Citrasens™ Grapefruit Solution™ capsules; <http://www.vitareason.com/>; accessed 3–3–2009). This product might represent a convenient method for boosting cyclosporine levels in dogs, assuming that the capsule contents are capable of inhibiting canine CYP metabolism. Consequently, the main objective of the present study was to use a validated bioassay to determine the potential for inhibition of CYP metabolism in dog liver by powdered grapefruit capsules. Triazolam hydroxylation was used as a surrogate for cyclosporine oxidation since the triazolam hydroxylation assay was readily available, technically less challenging, and both triazolam and cyclosporine are specifically metabolized by the same CYP isoforms (CYP3A subfamily) in humans (Kronbach et al., 1988; Kronbach et al., 1989; Ducharme et al., 1995; von Moltke et al., 1996; Greenblatt et al., 2000; Perloff et al., 2000). Effects of white grapefruit juice and lyophilized ruby red grapefruit juice on triazolam hydroxylation were also evaluated for comparison. The results of this study provide a starting point for clinical investigations examining cost-effective methods for boosting cyclosporine levels in dogs. However, pharmacokinetic data from *in vivo* studies will be necessary before GFJ-boosted cyclosporine regimens can be adopted.

Materials and Methods

Reagents

Triazolam, β -nicotinamide adenine dinucleotide phosphate, isocitric acid, isocitric dehydrogenase, ketoconazole, and 6',7'-dihydroxybergamottin (dihydroxybergamottin) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solvents for HPLC analysis were of HPLC grade and obtained from Fisher Scientific (Pittsburgh, PA, USA). Powdered whole grapefruit capsules were purchased from a commercial source (<http://www.vitareason.com/>). White grapefruit juice produced by Ocean Spray (Lakeville-Middleboro, MA, USA) was purchased from a local grocery store and stored at 4°C until use. Ruby red grapefruits were purchased from a local grocery store, the juice extracted by squeezing, and then dried down in a lyophilizer. Human liver microsomes (HLM) and dog liver microsomes (DLM) were obtained from frozen liver banks maintained in the Department of Pharmacology and Experimental Therapeutics, Tufts University, Boston, MA, USA. The preparation of microsomes has been described previously (Court & Greenblatt, 1997). HLM and DLM were suspended in 0.1 M phosphate buffer containing 20% glycerol and were stored at –80°C until use. Pooled DLM were prepared by combining individual preparations from four male beagle dogs. The collection and use of these livers for this purpose was approved by the Tufts University Institutional Review Board, and Institutional Animal Care and Use Committee, respectively.

Microsomal incubations

Microsomal incubations were carried out in a final volume of 250 μ l as described in detail previously (von Moltke et al., 1996; Perloff et al., 2000). Incubations contained 50 mM phosphate buffer (pH 7.5), 5 mM magnesium, 0.5 mM nicotinamide adenine dinucleotide phosphate, and an isocitrate dehydrogenase regenerating system. For studies using white grapefruit juice, a series of incubation tubes containing 0, 0.05, 0.1, 0.25, 0.5, 1, and 2.5%

(volume/volume) juice were preincubated with HLM or DLM (0.25 mg of microsomal protein per mL of incubation volume) for 20 minutes at 37°C. Tubes containing 0.1 μ M ketoconazole or 2.5 μ M of the inhibitory furanocoumarin, dihydroxybergamottin, were also included as positive control inhibitors. At the conclusion of the preincubation period, the triazolam hydroxylation reaction was initiated by transferring the mixture to tubes containing triazolam (250 μ M final concentration). The incubation mixtures were immediately returned to the 37°C water bath for an additional 20 minutes. Reactions were then stopped by the addition of 100 μ l of ice-cold acetonitrile containing 500 ng of the internal standard, diazepam. After centrifuging, 300 μ l of the supernatant was transferred to an HPLC vial for quantitation of hydroxytriazolam content. Incubations were performed in duplicate and results averaged.

For studies using powdered grapefruit capsules, the contents of each of 8 randomly selected capsules were weighed; 10 mg from each capsule was combined, and then added to 8 mL of Millipore-filtered water to yield a 10 mg/mL stock solution. Since the powder did not completely dissolve after vigorous mixing, the stock solution was centrifuged and the supernatant stored at -20°C until use. Incubations using the supernatant were then performed as described for the aforementioned grapefruit juice assays. The final concentrations of powdered grapefruit tested were 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.25, 2.5, and 5 mg / mL.

For studies using lyophilized ruby red grapefruit juice, a 10 mg/mL stock solution was prepared in Millipore-filtered water, centrifuged after vigorous mixing, and the supernatant was stored at -20°C until use. The final concentrations of lyophilized ruby red grapefruit juice tested were 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.25, 2.5, and 5 mg / mL.

Effect of inhibitor preincubation on the extent of inhibition

The effect of 20 minutes preincubation at 37°C of DLM with inhibitor and cofactor mix (but without triazolam) on the extent of inhibition of triazolam hydroxylation was tested by comparing rates of hydroxylation with and without a 20 minute preincubation period. Incubations were performed as described above, except that for incubations without preincubation, both triazolam and inhibitor were added at the same time. Reactions (performed in triplicate) were initiated using DLM at a final concentration of 0.25 mg of microsomal protein per mL of incubation volume. Inhibitor concentrations tested were 0.1 μ M for ketoconazole, 2.5 μ M for dihydroxybergamottin, 0.5% (v/v) for grapefruit juice, 0.7 mg / mL for powdered grapefruit, and 0.375 mg / mL for lyophilized ruby red grapefruit juice.

Capsule-to-capsule variability in CYP inhibition by powdered grapefruit

A 10 mg / mL powdered grapefruit solution was prepared from each of eight capsules as described above. The effects of each of these 8 solutions at a final concentration of 1.25 mg / mL on triazolam hydroxylation by DLM were determined in triplicate.

Quantitation of α -hydroxytriazolam and 4-hydroxytriazolam formation by HPLC

Incubation mixtures were analyzed using a Thermo-Finnigan Surveyor HPLC system operated by the ChromQuest software package, version 4.0 (Thermo Fisher Scientific, Waltham, MA, USA) as previously described with minor modifications (von Moltke et al., 1996). The column was a 4 micron 250×4.60 mm Phenomenex Synergi Hydro-RP (Phenomenex, Torrance, CA, USA). The HPLC mobile phase consisted of 50% (v/v) 20 mM phosphate buffer (pH = 2.2), 31% methanol, and 19% acetonitrile delivered at a flow rate of 0.850 mL/min for 40 minutes. The injection volume was 20 μ L. Column effluent was monitored by measuring ultraviolet absorbance at a wavelength of 220 nm. Analyte peaks were identified by injecting pure standards. Typical retention times were 17.2 minutes for α -hydroxytriazolam, 18.1 minutes for 4-hydroxytriazolam, 26.8 minutes for triazolam, and 35 minutes for diazepam.

Data Analysis

Peak height ratios of α -hydroxytriazolam and 4-hydroxytriazolam relative to the internal standard, diazepam, were calculated for each incubation mixture. Relative reaction velocities were calculated using the average peak height ratio for each inhibitor concentration expressed as a percentage of the analogous ratio resulting from control incubations that contained no inhibitor. The concentration producing a 50% decrease in reaction velocity (IC_{50}) was determined by nonlinear regression analysis using SigmaPlot 11.0 (Systat Software, San Jose, CA, USA) as previously described (von Moltke et al., 2002). In the preincubation study, differences in the α -hydroxytriazolam formation rate with and without preincubation were compared with a two-tailed Student's t-test. Differences with a P value < 0.05 were considered statistically significant.

Results

In agreement with their known ability to inhibit human CYP3A metabolism (Guo et al., 2000; Tassaneeyakul et al., 2000; Venkatakrishnan et al., 2000), the positive control inhibitors, ketoconazole (0.1 μ M) and dihydroxybergamottin (2.5 μ M), reduced the rate of α -hydroxytriazolam formation by HLM to $63 \pm 2\%$ and $41 \pm 2\%$ of control (respectively). 4-hydroxytriazolam formation was reduced to $65 \pm 0.5\%$ (ketoconazole) and $47 \pm 1\%$ of control (dihydroxybergamottin). In DLM, ketoconazole reduced α -hydroxytriazolam and 4-hydroxytriazolam formation to $78 \pm 3\%$ and $76 \pm 3\%$ of control, respectively. The analogous values for dihydroxybergamottin were $53 \pm 2\%$ and $75 \pm 4\%$ of control.

As shown in Figure 1, white grapefruit juice inhibited α -hydroxytriazolam formation in both HLM and DLM in a concentration-dependent manner with inhibition potency (IC_{50}) values (Table 1) that were essentially identical (about 0.5% v/v) for both species. On the other hand, the potency of inhibition by white grapefruit juice of 4-hydroxytriazolam formation in DLM was almost half that observed for inhibition of 4-hydroxytriazolam formation in HLM and also half that for inhibition of α -hydroxytriazolam formation in DLM (Table 1). Lyophilized ruby red grapefruit juice and powdered grapefruit also inhibited both α -hydroxytriazolam and 4-hydroxytriazolam formation by DLM in a concentration-dependent manner (Figure 2). However, the potency of inhibition was about 40% less for powdered

grapefruit as compared with lyophilized ruby red grapefruit juice (Table 1). Again, 4-hydroxytriazolam formation was less potently inhibited than was α -hydroxytriazolam formation in DLM for both grapefruit products.

DLM assays conducted to examine the impact of preincubation on the inhibition of α -hydroxytriazolam formation revealed a greater degree of inhibition when dihydroxybergamottin (by 27%), white grapefruit juice (by 10%), lyophilized ruby red grapefruit juice (by 8%), and powdered grapefruit (by 20%) were preincubated with microsomal protein prior to the addition of triazolam (Figure 3). No difference in the inhibitory effect of ketoconazole was noted when incubations were conducted with or without preincubation. Similar results were observed for 4-hydroxytriazolam formation (data not shown).

Since natural products and dietary supplements are not as stringently regulated as pharmaceuticals, an important concern regarding their potential use in clinical patients is variability between preparations. Consequently, we weighed the contents of 8 randomly selected grapefruit powder capsules as well as determined the ability of the contents of each of these 8 capsules to inhibit triazolam hydroxylation by DLM. As shown in Table 2, capsule content weights ranged from 533 to 591 mg, with a coefficient of variation of only 3%. Inclusion of 1.25 mg / mL (final concentration) powdered grapefruit resulted in decreased α -hydroxytriazolam formation ranging from 55 to 63% of control rates, while 4-hydroxytriazolam formation decreased to 51 to 62% of control rates with coefficients of variation of 4 and 6%, respectively.

Discussion

The results of the present study indicate that white grapefruit juice, lyophilized ruby red grapefruit juice, and powdered whole grapefruit are all capable of inhibiting triazolam hydroxylation by CYP in beagle DLM, albeit with differing inhibition potencies. Substantiating our assay system, IC_{50} values obtained for inhibition by grapefruit juice of triazolam hydroxylation in HLM are in good agreement with previously published values (Farkas et al., 2007). Grapefruit juice also inhibited triazolam hydroxylation in DLM with generally similar IC_{50} values to HLM, suggesting that triazolam is metabolized by enzymes with similar catalytic properties in both species. The only difference was somewhat less potent inhibition of 4-hydroxytriazolam formation in DLM compared with HLM, which suggests that this metabolic pathway could be different in dogs compared with humans. Future studies could resolve this by evaluation of recombinant canine CYP isoforms.

An important objective of this study was to approximate the dose of powdered grapefruit capsules that would be needed to achieve the same level of drug metabolism inhibition as a dose of grapefruit juice. In the study by (Giorgio et al 2003), a grapefruit juice dose of 100 mL given to adult beagle dogs was sufficient to cause a 3-fold increase in the AUC of praziquantel. Using the IC_{50} values we obtained for inhibition of α -hydroxytriazolam formation in DLM, we estimate the concentration of powdered grapefruit that is equivalent to grapefruit juice to be 231 mg / mL – (IC_{50} powdered grapefruit (1.2 mg/mL)) / (IC_{50} grapefruit juice (0.52 ml / 100 ml). Consequently, the amount of powdered grapefruit

equivalent to 100 mL of grapefruit juice would be 23.1 grams or 41 capsules, assuming an average of 565 mg contents per capsule as we had measured. A similar calculation for lyophilized ruby red grapefruit juice yields a dose equivalent mass of 15.7 grams (about 31 capsules at approximately 500 mg each). Both of these estimates appear to be similar to, although somewhat higher (i.e. less potent) than, the dose of 10 grams of lyophilized grapefruit juice reported by Giorgio et al (2003) to result in an increase in praziquantel AUC (similar increase to that of 100 mL of juice). Reasons for these differences may be related to the type of grapefruit used, and the grapefruit parts used to prepare the juice and/or powdered extract.

The powdered grapefruit product uses the entire fruit including the albedo (white rind) which is generally low in inhibitory furanocoumarins (Decastro et al., 2006). This may explain the lower inhibition potency of powdered grapefruit. The furanocoumarin content of grapefruit can also vary depending on variety and also from lot to lot (Ho et al., 2000; Decastro et al., 2006). Ruby red grapefruit have been reported to contain higher amounts of furanocoumarins than pink or white grapefruit (Decastro et al., 2006). Although our study was not designed to make this comparison directly, assuming the white grapefruit juice we used contained 10 grams of dry matter per 100 mL of liquid and there is no potency loss from lyophilization (as reported by Giorgio et al, 2003), then the dry weight IC₅₀ value for inhibition of triazolam α -hydroxylation by DLM would be 0.52 mg / mL. This is slightly lower than the value we obtained for lyophilized ruby red grapefruit juice (0.76 mg / mL) and suggests that there may be no advantage in using ruby red over white grapefruit for inhibition. We also evaluated the variability in capsule content weight and inhibition capacity, and showed remarkably low variability between the capsules obtained for the present study. However, these capsules were from a single lot and it is possible that more variability could be encountered between lots differing by year of production. Consequently, if such products are to be used as pharmaceutical boosting agents it may be necessary to standardize the dose by either measurement of furanocoumarin content or inhibition capacity by bioassay (as in this study).

Another important result is that we observed time-dependent enhancement of canine CYP inhibition by all grapefruit products tested, as well as by dihydroxybergamottin, but not by ketoconazole. Such findings are consistent with mechanism-based inhibition (metabolic activation), which is typical of inhibition by furanocoumarins of CYP3A in humans and other species (Letteron et al., 1986; Schmiedlin-Ren et al., 1997; He et al., 1998; Guo et al., 2000; Tassaneeyakul et al., 2000; Greenblatt et al., 2003; Kakar et al., 2004). Ketoconazole, while a relatively potent CYP inhibitor, is not a mechanism-based inhibitor and preincubation results in unchanged (as in this case) or even decreased inhibition associated with conversion of ketoconazole to inactive metabolites. Time-dependent furanocoumarin inhibition tends to be irreversible, requiring *de novo* synthesis of CYP3A enzyme for recovery of CYP3A function in affected tissues. In humans, the half-life of recovery of CYP3A activity following grapefruit juice inhibition is approximately 23 hours (Greenblatt et al., 2003).

In conclusion, the results of this study demonstrate that a variety of grapefruit products are capable of mechanism-based inhibition of CYP mediated triazolam hydroxylation in dog

liver. Since triazolam and cyclosporine are substrates for the same enzyme (CYP3A) in humans and other species, concomitant administration of these grapefruit products with cyclosporine to dogs might be expected to enhance cyclosporine systemic availability through decreased enteric metabolism. In turn, this would decrease the required dose of cyclosporine and treatment cost. Powdered grapefruit capsules represent a more convenient dosing form, as compared with grapefruit juice or lyophilization. Although the number of grapefruit capsules that we predict are needed to have an effect equivalent to 100 mL of grapefruit juice is relatively high (41 capsules), it is possible that lower doses might also be effective. A clinical study investigating the impact of coadministration of these grapefruit capsules on the pharmacokinetics of cyclosporine in dogs is currently in progress.

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List of Abbreviations

CYP	cytochrome P450
C_{max}	maximum plasma concentration
AUC	area under the plasma concentration-time curve
dihydroxybergamottin	6',7'-dihydroxybergamottin
HLM	human liver microsomes
DLM	dog liver microsomes
IC₅₀	inhibitor concentration producing a 50% decrease in reaction velocity

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Figure 1:

Inhibition by white grapefruit juice of α -hydroxytriazolam formation in human liver microsomes and pooled dog liver microsomes. Each data point represents the mean of two preincubation experiments in which each inhibitor concentration was assayed in duplicate. Nonlinear regression analysis was used to derive the curve and IC_{50} estimate.

Figure 2: Inhibition by lyophilized ruby red grapefruit juice and powdered whole grapefruit of α -hydroxytriazolam formation in pooled dog liver microsomes. Each data point represents the mean of duplicate incubations. Non-linear regression analysis was used to derive the curve and IC_{50} estimate.

Figure 3:

Effect of 20 minute preincubation on inhibition of α -hydroxytriazolam formation measured in pooled dog liver microsomes. For preincubation assays, the inhibitor was preincubated with microsomal protein for 20 minutes prior to the addition of triazolam. The inhibitor concentrations tested were 0.1 μ M for ketoconazole, 2.5 μ M for 6',7'-dihydroxybergamottin (DHB), 0.5% of incubation volume for white grapefruit juice (GFJ), 0.7 mg/mL for powdered grapefruit (PGF) and 0.375 mg/mL for lyophilized ruby red grapefruit juice (LGFJ). Data are presented as mean $\pm$ SEM for incubations performed in triplicate. * denotes a significant difference ($P < 0.05$) between the two conditions for a given inhibitor tested by Student's unpaired t-test.

Table 1:

IC₅₀ values for inhibition by different grapefruit preparations of triazolam hydroxylation in human and dog liver microsomes.

Preparation	Triazolam α -hydroxylation (IC ₅₀ $\pm$ SE)	Triazolam 4-hydroxylation (IC ₅₀ $\pm$ SE)
Human liver microsomes		
White grapefruit juice	0.56 $\pm$ 0.05 % (v/v)	0.58 $\pm$ 0.08 % (v/v)
Dog liver microsomes		
White grapefruit juice	0.52 $\pm$ 0.18 % (v/v)	1.1 $\pm$ 1.1 % (v/v)
Lyophilized ruby red grapefruit juice	0.76 $\pm$ 0.14 mg/mL	1.1 $\pm$ 0.31 mg/mL
Powdered whole grapefruit	1.2 $\pm$ 0.38 mg/mL	1.7 $\pm$ 1.2 mg/mL

IC₅₀ values were determined by nonlinear regression and represent the inhibitor concentration producing a 50% reduction in triazolam hydroxylation rate as compared to the rate measured without added inhibitor. SE denotes the standard error of the IC₅₀ estimate.

Table 2:

Variability in powdered grapefruit capsule content weight and inhibition of triazolam hydroxylation by dog liver microsomes for 8 randomly selected capsules.

Capsule	Content weight (mg)	Triazolam α -hydroxylation (% of control without inhibitor)	Triazolam 4-hydroxylation (% of control without inhibitor)
#1	557	63	62
#2	533	57	54
#3	558	55	51
#4	557	57	56
#5	585	58	55
#6	578	59	58
#7	591	58	58
#8	558	61	59
Mean:	565	59	57
Standard deviation:	19	3	3
Coefficient of variation (%):	3	4	6

A 10 mg of capsule contents per mL of water mixture was prepared for each capsule. The resulting supernatant, at a final concentration of 1.25 mg/mL, was then used in preincubation assays with pooled dog liver microsomes. Values are expressed as the percentage ratio of the reaction velocity for incubations containing inhibitor to the control velocity obtained when no inhibitor was present and are the mean of triplicate incubations per capsule.