Verapamil disposition clearance has been described as decreased in older as compared with younger subjects and patients (Abernethy et al., 1986, 1993; Schwartz, 1990). This is apparently true after single doses of either i.v. or oral verapamil and during chronic i.v. infusion or oral administration of verapamil as would more commonly occur during therapy. Verapamil biotransformation in humans is predominantly mediated by CYP3A species, with gut wall CYP3A thought to contribute substantially to oral clearance (Kroemer et al., 1992, 1993; Fromm et al., 1996). Previous reports indicate that formation of D-617 (N-dealkylnorverapamil) \(^1\) may be in part mediated by CYP1A2 (Kroemer et al., 1992); however, the involvement of CYP2C8 has also been implicated in the formation of both D-617 and D-620 (N-dealkylverapamil) in human liver microsomal studies (Tracy et al., 1999). In addition, indirect studies have implicated hepatic flavin-containing monoxygenase in the \(\hat{N}\)-oxygenation reaction that leads to formation of both D-617 and D-620 (Cashman, 1989). Therefore, it remains uncertain which drug metabolizing enzyme activities may be decreased in older individuals, and which result in the observed decreases in parent drug verapamil clearance. Although norverapamil has usually been reported as the formed metabolite that attains substantial plasma concentrations in humans after verapamil exposure (Abernethy et al., 1984), D-617 and D-620 have also been noted in patients and healthy volunteers in concentrations of the same magnitude as verapamil and norverapamil (Barbieri et al., 1985; Padrini et al., 1985; Piotrovskii et al., 1986).

Pharmacological activity of the metabolites has been studied in the dog (Neugebauer, 1978) and perfused rabbit heart (Johnson et al., 1991). Norverapamil may have about 20% of the potency of verapamil to block cardiac atrioventricular conduction and mediate coronary vasodilatation in these preparations, whereas D-617 may have limited effects on cardiac contractility and D-620 may have some effect on slowing atrioventricular conduction. Both are much less potent than parent drug verapamil and somewhat less potent than norverapamil for any of the measured pharmacodynamic effects.

This study is a further analysis of our previous report (Abernethy et al., 1993), which noted markedly decreased clearance of both verapamil enantiomers in older as compared with young healthy male volunteers (Table 1). Here we report the analysis of plasma concentrations of racemic verapamil, norverapamil, D-617, D-620, (2-[4-hydroxy-3-methoxyphenyl]-8-[3,4-dimethoxyphenyl]-6-methyl-2-isopropyl-6-azaoctanitrile) (PR-22), and ([3,4-dimethoxyphenyl]-acetic acid) (PR-25) and note that exposure to all detectable species is substantially greater (verapamil, norverapamil) or tending to be greater (D-617, D-620) in older individuals.

Materials and Methods

Samples were analyzed from 11 older (aged 65–75 years) and 8 younger (aged 20–28 years) healthy males who were on no medications and in good health. All were nonsmokers and of similar height (67–73 inches, older; 68–76

\(^1\) Abbreviations used are: D-617, N-dealkylnorverapamil; D-620, N-dealkylverapamil; AUC, area under the plasma concentration time curve; PR-22, 2-[4-hydroxy-3-methoxyphenyl]-8-[3,4-dimethoxyphenyl]-6-methyl-2-isopropyl-6-azaoctanitrile; PR-25, ([3,4-dimethoxyphenyl]-acetic acid).
TABLE 1

Subject characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Older</th>
<th>Younger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>69 (65–75)</td>
<td>23 (20–28)</td>
</tr>
<tr>
<td>Weight (kilograms)</td>
<td>81 (69–100)</td>
<td>77 (61–91)</td>
</tr>
<tr>
<td>Body mass index (kilograms per square meters)</td>
<td>26.2 (22.6–30.5)</td>
<td>23.0 (20.5–25.7)</td>
</tr>
</tbody>
</table>

Verapamil and its metabolites were kindly provided by Dr. James Longstreth (G.D. Searle and Co.). The assay was developed to measure total verapamil and its metabolites, norverapamil, of a 7-day administration of 240 mg of verapamil (Calan SR, G.D. Searle, Skokie, Illinois). The design was as previously described (Abernethy et al., 1993), and subjects included younger and weight (75–100 kg, older; 61–91 kg, younger). Overall study inclusion criteria for verapamil and its metabolites, as well as for the internal standard, gallopamil, were determined and all were stable at 80.0. Stability studies of verapamil and metabolites over time in plasma and 22°C could not be used in this study because the expected plasma concentrations of D-620, PR-22, and PR-25 were too low to be detected by this assay.

In the method developed for this study, verapamil and its metabolites were separated from each other and from matrix interferences on a 5-μm C18 support (ODSII column; Regis Chemical Company, Morton Grove, IL). The mobile phase consisted of 0.3% diethylamine in water modified with acetonitrile (69:31, v/v). The pH of the aqueous portion of the mobile phase was adjusted to 4 with glacial acetic acid before the addition of acetonitrile. The flow rate was 1.0 ml/min, and ambient temperature was used throughout the study. The analytes were detected using a fluorescence detector, excitation wavelength 236 nm and emission wavelength 530 nm. Under these conditions, baseline separation was achieved for verapamil and its metabolites, as well as for the internal standard, gallopamil. The observed relative retentions, expressed as capacity factors (k values) were: k'(PR-25) = 6.0, k'(D-620) = 8.0, k'(D-617) = 10.0, k'(PR-22) = 30.0, k'(verapamil) = 60.0, k'(norverapamil) = 70.0, k'(gallopamil) = 80.0. Stability studies of verapamil and metabolites over time in plasma and standard solvent were determined and all were stable at −20°C for more than 4 years. All analyses were performed within 3 years of sample collection, with samples maintained at −20°C from the time of collection to analysis.

Plasma samples were assayed by transferring a 600-μl aliquot to a 1.5-ml amber polypropylene microcentrifuge tube followed by the addition of 60 μl of the internal standard gallopamil (0.5 μg/ml in water). The resulting solution was centrifuged for 30 min at 13,000 rpm, and 1 ml of the supernatant was transferred to a 1-ml C-18 extraction column (Wenick Scientific, Ottawa, Canada). The solid-phase extraction column had been previously conditioned with 2 ml of water, the analytes were eluted with 2 ml of methanol (containing 0.5% diethylamine), the methanol was evaporated to dryness under a stream of air, the resulting residue was dissolved in 250 μl of 0.01 M HCl for 10 min at 10°C, and a 50-μl aliquot was injected into the chromatographic system.

Standard curves were prepared for verapamil and norverapamil using concentrations of 500, 400, 300, 200, 100, and 50 ng/ml. Standard curves for the remaining metabolites were constructed using concentrations of 120, 100, 80, 60, 40, 20, and 10 ng/ml. Triplicate samples were prepared for each concentration. Calibration curves plotting analyte plasma concentration as a function of the analyte/internal standard peak area ratios were derived for verapamil and each of the metabolites. All of the standard curves were linear with the after regression equations: verapamil y = 0.0065x − 0.0814, R² = 0.9966; norverapamil y = 0.0063x − 0.0912, R² = 0.9965; D-620 y = 0.0047x − 0.0025, R² = 0.9793; D-617 y = 0.004x − 0.0058, R² = 0.9881; PR-22 y = 0.0052x − 0.0048, R² = 0.9862; PR-25 y = 0.0029x − 0.0115, R² = 0.985.

Plasma recoveries were determined by using spiked plasma concentrations for verapamil and norverapamil of 600, 300, and 50 ng/ml whereas the recoveries of the other analytes, D-617, D-620, PR-22, and PR-25, were examined at 120, 60, and 10 ng/ml. The peak area ratios of the three extracted samples of the analytes were compared with two aqueous unextracted samples to derive percent recovery. The recoveries were above 80% for all analytes at all levels except for PR-22 and PR-25, where the recoveries from serum samples containing 10 ng/ml of each analyte were 65 and 66%, respectively. A dilution experiment was also assessed to verify whether dilution of samples off the curve could be reliably assayed. This became an issue during the quantification of subject samples. Occasionally, a subject would have a value for D-617 or D-620 that exceeded the maximum value on the standard curve. The results indicated that concentrations that exceeded the maximum value on the standard curve could be determined with an accuracy of 93% and a c.v. of 6%.

Area under the plasma concentration time curve (AUC) for each species from 0 to 24 h was determined by the linear trapezoidal method. Comparisons between older and younger subjects for the various parameters were made by the Student’s t test assuming unequal variances between groups. A two-sided P value of less than .05 was accepted as significant.

Results

The human metabolic pathway of verapamil is shown in Fig. 1. The plasma concentration data reported here represent exposure to either

Species measured in this study were verapamil, norverapamil, D-617, D-620, PR-22, and PR-25. PR-22 and PR-25 were not detectable in any subject (<10 ng/ml at all time points).

![Fig. 1. Metabolic pathway for verapamil in humans.](Image)
verapamil or the metabolite, and reflect both formation and clearance rate of these various intermediate metabolites. Plasma concentrations of verapamil, norverapamil, D-617, and D-620 at each time point are shown in Fig. 2(A–D). At the same dose, older subjects appear to have greater exposure to verapamil and norverapamil, and the same or somewhat greater exposure to D-617 and D-620 as compared with younger subjects. As previously reported, intersubject variability is large for both groups. To compare exposure to the various species within a subject age group, mean concentrations of each are demonstrated in Fig. 3 (A and B). Within each group, exposure to verapamil, norverapamil, and D-617 is similar, and to D-620 approximately one-third that of the other species. AUC comparisons reflected these observations, with exposure to verapamil and norverapamil greater in older subjects and exposure to D-617 and D-620 tending to be greater in older subjects, although this was not statistically significant (Table 2; Fig. 4). To further explore the relative changes in metabolite formation as reflected by steady-state plasma concentrations in older versus younger subjects, the metabolite AUC/verapamil AUC ratio was determined and compared between groups (Fig. 5). The ratio of norverapamil to verapamil AUC was decreased in older subjects, as was that of D-620 to verapamil AUC, whereas that of D-617 to verapamil AUC tended to be lower in older subjects, although not significantly so. There are many limitations on interpretation of such data; however, they suggest in general that the greater exposure to norverapamil and tendency to greater exposure to D-617 and D-620 in older subjects is due to decreased verapamil clearance and the attendant higher concentrations. Additionally, such findings support the possibility that enzyme activities that mediate clearance of norverapamil, D-620, and perhaps D-617 are decreased in older as compared with younger subjects.

**Discussion**

These data indicate that with chronic dosing of sustained release verapamil at a dose of 240 mg/day healthy older men have greater exposure to verapamil and norverapamil, tend to have increased exposure to D-617 and D-620 as compared with young men. The clinical importance of such an observation is unknown, but unlikely to be associated with the altered verapamil responses observed in older individuals (Abernethy et al., 1986, 1993; Schwartz, 1990) due to the limited pharmacological activity of these metabolites (Neugebauer, 1978; Johnson et al., 1991). In contrast, these findings do add insight regarding which enzymes of drug biotransformation have diminished activity with increasing age. CYP3A4 and CYP3A5 have been implicated in the formation of both
norverapamil and D-617 (Kroemer et al., 1992, 1993; Fromm et al., 1996; Tracy et al., 1999) and they have an important role in verapamil gut wall metabolism (Fromm et al., 1996). These findings are consistent with decreased clearance in older male subjects of other CYP3A substrates such as midazolam and triazolam (Greenblatt et al., 1983, 1984). Previous reports of CYP1A2 and CYP2C8 having a role in verapamil and metabolite biotransformation (Kroemer et al., 1992, 1993; Tracy et al., 1999) are difficult to place into the context of the present data as the in vivo contribution of these CYP enzymes is uncertain. Confounding these and other clinical pharmacokinetic findings that rather consistently note decreased CYP3A activity with age is the report of no age-related change in CYP3A activity as measured by erythromycin N-demethylation in resected human liver preparations (Hunt et al., 1992). The more recently described feature of verapamil disposition is the role of P-glycoprotein in its gut absorption and distribution (Doppenschmitt et al., 1999). There are no data

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**Fig. 3. Pharmacokinetic curves which represent:**

A, mean verapamil and metabolite concentrations for elderly subjects over one dose interval; B, mean verapamil and metabolite concentrations for young subjects over one dose interval.
available that relate to age-related changes in expression or activity of P-glycoprotein; therefore, any potential contribution to the present data cannot be predicted.

In summary, these data indicate that exposure to not only verapamil, but also its metabolite norverapamil is increased during chronic oral verapamil administration to older subjects. In addition, exposure to D-617 tends to be increased with age and exposure to D-620 is similar in younger and older individuals when the same dose regimen of oral verapamil is administered to both groups, whereas PR-22 and

**FIG. 4.** AUC over the 24-h dose interval for verapamil and metabolites in elderly and young subjects. Error bars indicate S.D. of the mean. *P < .05.

**FIG. 5.** Ratio of metabolite AUC to verapamil AUC over the 24-h dose interval for elderly and young subjects. Error bars indicate S.D. of the mean. *P < .05.

<table>
<thead>
<tr>
<th>Measured Species</th>
<th>Older Mean ± S.D. (ng/ml · h⁻¹)</th>
<th>Younger Mean ± S.D. (ng/ml · h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>2815 ± 733*</td>
<td>1639 ± 466</td>
</tr>
<tr>
<td>Norverapamil</td>
<td>2927 ± 655**</td>
<td>2143 ± 471</td>
</tr>
<tr>
<td>D-617</td>
<td>2386 ± 772</td>
<td>1894 ± 418</td>
</tr>
<tr>
<td>D-620</td>
<td>897 ± 366</td>
<td>757 ± 104</td>
</tr>
</tbody>
</table>

*P < .0007; **P < .007.

**TABLE 2**

AUC (0–24 h) for verapamil and metabolites in older and younger male subjects

In summary, these data indicate that exposure to not only verapamil, but also its metabolite norverapamil is increased during chronic oral verapamil administration to older subjects. In addition, exposure to D-617 tends to be increased with age and exposure to D-620 is similar in younger and older individuals when the same dose regimen of oral verapamil is administered to both groups, whereas PR-22 and
PR-25 are not detectable in the plasma of older or younger subjects during steady-state oral verapamil treatment.

Acknowledgments. We thank Nektaria Markoglou for assistance in determination of verapamil and metabolite plasma concentrations.

References


