Distribution and Excretion of $^{3}$H[Vincristine in the Rat and the Dog

Manford C. Castle, David A. Margileth, and Vincent T. Oliverio

INTRODUCTION

VCR, an alkaloid derived from the plant Cantharanthus roseus, has been widely used in cancer chemotherapy for over 1 decade, mainly in the therapy of leukemia and lymphoma. During this time, knowledge concerning the pharmacological disposition of VCR has remained limited (3). With a bioassay method using KB cells, it was found that the distribution and excretion of tritiated vincristine were studied in the rat and the dog. Biphasic curves for the disappearance of the drug from blood were found in both species, with an initial half-life of approximately 15 min and a secondary half-life of approximately 75 min. Tissue levels were high at 1 hr in the rat and declined rapidly, except in the brain where very low levels of drug were found at all times. The bile was found to be the major route of excretion. The peak rate of excretion in bile was found to occur earlier in the rat (10 min) than in the dog (60 min). Rats given a higher dose of vincristine (1.0 mg/kg) excreted a larger percentage of the dose in the bile than rats given a lower dose (0.1 mg/kg). In rats given a low dose of vincristine (0.1 mg/kg), more than 85% of the drug was excreted in the feces and the urine over 72 hr. Less than 10% of the total radioactive activity in the bile and urine was metabolites whereas, in the plasma, metabolites accounted for 40% of the total radioactivity.

SUMMARY

The distribution and excretion of tritiated vincristine were studied in the rat and the dog. Biphasic curves for the disappearance of the drug from blood were found in both species, with an initial half-life of approximately 15 min and a secondary half-life of approximately 75 min. Tissue levels were high at 1 hr in the rat and declined rapidly, except in the brain where very low levels of drug were found at all times. The bile was found to be the major route of excretion. The peak rate of excretion in bile was found to occur earlier in the rat (10 min) than in the dog (60 min). Rats given a higher dose of vincristine (1.0 mg/kg) excreted a larger percentage of the dose in the bile than rats given a lower dose (0.1 mg/kg). In rats given a low dose of vincristine (0.1 mg/kg), more than 85% of the drug was excreted in the feces and the urine over 72 hr. Less than 10% of the total radioactive activity in the bile and urine was metabolites whereas, in the plasma, metabolites accounted for 40% of the total radioactivity.

MATERIALS AND METHODS

$^{3}$HVCR. The $^{3}$HVCR used in these studies was prepared by Dr. James P. Kutney of the University of British Columbia (Vancouver, British Columbia) by means of catalytic exchange (5, 9). This method involves the addition of unlabeled VCR to a vacuum apparatus containing tritiated trifluoroacetic acid. After standing at room temperature for 48 hr, labeled VCR was extracted with methylene chloride. Pure labeled VCR was then obtained through recrystallization in methanol. By this method the tritium appears in the aromatic rings of the VCR molecule and is therefore not available for exchange. A similar synthetic route for preparation of $^{3}$HVCR has been recently reported by Owellen and Donigian (10). The initial specific activity of this aromatically labeled VCR was 53.2 mCi/mmol. The radiochemical purity was greater than 95% by TLC as described elsewhere (10). Injection solutions were prepared immediately prior to use by adding nonradioactive VCR sulfate to $^{3}$HVCR sulfate to give a final specific activity of 44 μCi/mg.

Animals and Tissues. Male and female Sprague-Dawley rats (200 to 250 g) and female beagle dogs (8 to 10 kg) were obtained from NIH stocks. In all cases, $^{3}$HVCR was given i.v. as the sulfate in distilled water. Following the administration of VCR, rats were placed in metabolism cages and urine and feces were collected separately at 24, 48, and 72 hr. Separate groups of rats were sacrificed at 1, 6, 16, and 24 hr after injection of $^{3}$HVCR and the various tissues were removed and assayed for tritium. Tissue levels in dogs were determined at 4 hr after administration of VCR. Blood samples were obtained from rats (50 μl from cut tail vein) and dogs (1.0 ml from femoral artery) under pentobarbital anesthesia. CSF was obtained by cisternal puncture from a separate group of anesthetized dogs. Bile cannulations were performed on dogs and rats anesthetized with pentobarbital. Gallbladder ligations were performed on the dogs. The animals remained anesthetized throughout the collection period.

The CSF, bile, and urine were assayed for tritium by counting an aliquot of each sample in Aquasol (New England Nuclear, Boston, Mass). Tritium levels in the blood, tissues, and feces were determined by first oxidizing the samples to tritiated water in a Packard Model 306 sample oxidizer (Packard Instrument Co., Downers Grove, Ill.). Rat
tissues and feces were oxidized whole. Dog tissues were homogenized, and an aliquot was taken for assay.

For determination of metabolites in the blood, rats were lightly anesthetized with ether and given a dose of 0.5 mg of \[\text{[3H]}\text{VCR}\] per kg by tail vein injection. Five min later the rats were again anesthetized with ether, and 10 ml of blood were drawn by cardiac puncture into a heparinized syringe. The heparinized blood was immediately placed into a centrifuge tube containing 0.5 mg of unlabeled VCR. A control, 5 \(\mu\)g of \[\text{[3H]}\text{VCR}\] were added to 10 ml of heparinized blood drawn from an untreated rat and incubated for 5 min at 37°. This control, as well as those described below, was devised to differentiate metabolism from decomposition. The plasma was removed after centrifugation and extracted as described below.

A separate group of rats was given the same dose of \[\text{[3H]}\text{VCR}\] and urine was collected for 18 hr in a metabolism cage. As a control, urine was collected from untreated rats over the same time period. Both urine collections were made into vessels containing 0.5 mg of unlabeled VCR carrier. In addition, the collection vessels of the control group contained 50 \(\mu\)g of \[\text{[3H]}\text{VCR}\]. The urine of both groups was then extracted as described below.

A separate group of rats was anesthetized with pentobarbital and the bile ducts were cannulated. Prior to the injection of \[\text{[3H]}\text{VCR}\], bile was collected for 15 min. To this bile were added 50 \(\mu\)g of \[\text{[3H]}\text{VCR}\] and 0.5 mg of unlabeled VCR. This control bile was then carried through the same procedures as the bile from animals given \[\text{[3H]}\text{VCR}\]. After the control collection period, the rats were given injections of 0.5 mg of \[\text{[3H]}\text{VCR}\] per kg via tail vein. Bile was then collected for 2 consecutive 30-min periods into collection vessels containing 0.5 mg of unlabeled VCR carrier.

Samples of blood, urine, and bile were then made basic to pH 10 with NH\(_4\)OH and extracted with methylene chloride. The methylene chloride fraction was then evaporated under a stream of nitrogen. The residue was redissolved in a small volume of methylene chloride and applied to silica gel TLC plates. After development in acetone, the plastic plates were cut up and counted for radioactivity.

All samples were allowed to sit in the dark for 48 hr prior to counting to minimize chemiluminescence. Samples were counted in an ambient-temperature Beckman Model LS250 scintillation spectrophotometer. Quench corrections were performed using the external standardization method.

Disintegration rates (dpm) were converted to ng equivalents or \(\mu\)g equivalents of VCR. A paired t test was used to make comparisons of data where appropriate.

RESULTS

Blood. Labeled VCR was given at a dose of 1.0 mg/kg i.v. to 5 rats, and the curve for the disappearance of radioactivity is shown in Chart 1. Data are expressed as \(\mu\)g equivalents of VCR. A biphasic curve for the disappearance of tritium was found. By estimation from a semilogarithmic plot, the \(t_{1/2}\) for the initial steep portion of the curve was approximately 15 min and a \(t_{1/2}\) of approximately 75 min was found for the slower descending 2nd portion of the curve. A consistent rise in the level of radioactivity was noted between 15 and 25 min. Low levels of tritium were still detectable at 4 hr.

Plasma levels of VCR and its metabolites in rats were estimated at 5 min after an i.v. injection of \[\text{[3H]}\text{VCR}\]. As shown in Table 1, 78% of the radioactivity in the plasma of the control group was chromatographically identical to that of VCR. In the experimental group, only 38% of the radioactivity was present as VCR.

A dose of labeled VCR of 0.5 mg/kg was given to 4 dogs, and Chart 2 shows the curve for disappearance of radioactivity. A biphasic curve was found with an estimated \(t_{1/2}\) of 13 min for the initial period of rapid disappearance and an estimated \(t_{1/2}\) of 75 min for the 2nd portion of the curve. A

Table 1

<table>
<thead>
<tr>
<th>TLC of VCR and its metabolites in plasma, bile, and urine of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>% radioactivity</td>
</tr>
<tr>
<td>Area 1: point of application</td>
</tr>
<tr>
<td>Plasma</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Urine</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Bile</td>
</tr>
<tr>
<td>30-60 min</td>
</tr>
<tr>
<td>0-30 min</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
small but consistent rise in the level of radioactivity was seen in the blood of the dog at 30 min.

**Tissue Levels.** Tissue levels of VCR and/or its metabolites in the rat at 1, 6, 16, and 24 hr are shown in Table 2. Data are expressed as ng equivalents of VCR per g wet weight of tissue. At 1 hr the levels of VCR were similar in the major tissues, excluding the brain, with a range of approximately 100 to 200 ng equivalents per g wet weight. The brain showed very little activity at 1 hr compared with the other tissues. The intestinal contents at 1 hr showed a very high level in the small intestine and a low level in the large intestine.

Maximum levels of radioactivity in the major tissues were found to occur at 1 hour followed by a rapid decline to low but detectable levels at 24 hr. Tissues not following this trend included the brain which showed a slight rise at 16 and 24 hr and the large intestinal contents which reached a maximum at 6 hr.

In the dog, tissue levels of VCR and/or its metabolites at 4 hr are shown in Table 3. VCR levels in the brain and CSF were undetectable. In the other tissues, levels ranged from a low of 0.08 μg equivalent per g in the liver to 2.28 μg equivalents per g in the spleen.

**Biliary Excretion.** VCR and/or its metabolites were very rapidly excreted into the bile of both the dog and the rat. Over a 4-hr period, the portion of the injected dose excreted in the bile was slightly smaller in the dog than in the rat. As seen in Charts 3, 4, and 5, the peak excretion period of radioactivity into the bile occurred much earlier in the rat (10 min) than in the dog (60 min). In the rat the excretion pattern was similar at the 2 dose levels (0.1 and 1.0 mg/kg). However, a much larger percentage of the injected dose was excreted at the higher dose level (Charts 6 and 7). Male rats excreted significantly larger amounts of radioactivity in the bile than did females at the 0.1-mg/kg dose, but there were no sex differences at the 1.0-mg/kg dose. In female dogs given a dose of 0.5 mg/kg, the percentage of the injected dose excreted in the bile over a 4-hr period was approximately the same as for female rats given a dose of 0.1 mg/kg (27 and 28%, respectively).

As shown in Table 1, approximately 90% of the radioactiv...
ity in both the 30-min bile collection and the control was unchanged VCR. In the bile collection from the 60-min period, 83% of the radioactivity occurred as VCR. This difference in radioactivity in the VCR spot (compared with the control) appeared in the region between the point of application and the VCR spot.

**Urinary and Fecal Excretion.** As shown in Table 4, the major route of excretion of VCR and/or its metabolites in the rat is in the feces for both sexes. The urinary and fecal excretion in male rats was quantitatively and qualitatively similar to that in females at both 0.1 and 1.0 mg/kg. In both sexes a much larger percentage of the injected dose was excreted over 72 hr at the lower dose level than at the higher dose level.

### Table 4

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>0.1 mg/kg i.v.</th>
<th>1.0 mg/kg i.v.</th>
<th>0.1 mg/kg i.v.</th>
<th>1.0 mg/kg i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24</td>
<td>13.6 ± 2.1*</td>
<td>53.7 ± 5.1</td>
<td>100 ± 14.1</td>
<td>250 ± 22.3</td>
</tr>
<tr>
<td>24-48</td>
<td>2.3 ± 0.5</td>
<td>14.8 ± 2.4*</td>
<td>7.4 ± 1.5</td>
<td>168 ± 27.6</td>
</tr>
<tr>
<td>48-72</td>
<td>1.2 ± 0.1</td>
<td>1.6 ± 0.4</td>
<td>2.2 ± 0.2*</td>
<td>9.6 ± 1.0</td>
</tr>
</tbody>
</table>

**Cumulative at 72**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>0.1 mg/kg i.v.</th>
<th>1.0 mg/kg i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>87.3 ± 7.1</td>
<td>523.4 ± 52.1</td>
</tr>
<tr>
<td>Feces at 72</td>
<td>85.2 ± 6.0</td>
<td>532.7 ± 43.4</td>
</tr>
</tbody>
</table>

* Mean ± S.E. (n = 7).

The value for the males at 45 min is significantly higher than that for the females (p < 0.05). There are no other significant differences.
dose level. Total fecal output over a 72-hr period was markedly less at the 1.0-mg/kg dose (5.31 ± 0.73 g) than at the 0.1-mg/kg dose (13.3 ± 0.84 g). There were no sex differences in fecal output at either dose level.

As shown in Table 1, 80% of the radioactivity in the urine in the treated rats appeared as VCR at 18 hr compared with 88% in the control. All of this difference in radioactivity between the 2 groups appears to be at the point of application.

DISCUSSION

In both the rat and the dog, blood levels of VCR undergo a rapid initial clearance phase followed by a secondary phase of slower clearance. Estimated half-lives for each phase in the 2 animals are very similar. The \( t_{1/2} \) of 75 min for the slower phase agrees closely with Owellen and Donigian (10), who found a \( t_{1/2} \) of 70 min for this phase in the rat. These half-lives also are similar to those reported for vinblastine, a drug of similar structure, in the dog (4) and in man (11). The antimitotic agents, including VCR and vinblastine, have been shown to be bound rapidly by tissues rich in tubulin and to be bound to platelets and WBC (2, 4, 7, 12, 13). These factors undoubtedly contribute to the rapid disappearance of VCR from the blood. Also contributing to the rapid decrease of blood levels is the rapidity of biliary excretion as discussed below.

The rise in the level of radioactivity seen in the blood of the rat and dog at 20 to 30 min may indicate either enterohepatic recirculation of VCR or appearance of metabolites. No studies have been performed with P.O. administration of VCR but a P.O. form of vinblastine has been reported to be absorbed (8). Thus, it is possible that VCR or its metabolites could be absorbed in the intestinal tract.

Tissue levels of radioactivity in the rat follow a similar pattern to blood levels. Maximum levels are found at 1 hr in most tissues, followed by a rapid decline to low levels at 24 hr. This pattern of rapid fall to low levels differs from that previously reported in that there was much less difference between tissue levels at 2 and 24 hr (10). The finding of very low levels of radioactivity in the brain of the rat and the brain and CSF of the dog is similar to previously reported data for the rat brain (10). The tissue levels of VCR in the dog are comparable to levels previously reported (14), except for a lower level in the liver of the dogs in our study. The levels of radioactivity in the intestinal contents of the rat follow a pattern that would be expected with a drug that is rapidly excreted in the bile. The most radioactivity is found in the small intestinal contents at 1 hr, followed by a rapid decline while the peak in the large intestinal contents is delayed until 6 hr.

The cumulative biliary excretion of radioactivity was similar in female dogs and rats. However, the amount that appeared in the bile of the rat during the 1st 30 min was over 5 times greater than that for the dog over the same time period. Since the rat lacks a gallbladder and the gallbladder in the dog had been tied off, this appears to be a species difference in the ability of the animals to excrete the drug into the bile. The fact that rats given a higher dose of VCR (1.0 mg/kg) excreted a larger percentage of the injected dose in the bile than did rats given a lower dose (0.1 mg/kg) is difficult to explain. It is conceivable that binding sites are saturated at the higher dose level leaving more of the drug available to be excreted into the bile. There was also a sex difference in the biliary excretion at the lower dose level, but not at the higher dose level. This enhanced excretion by male rats at the 0.1-mg/kg dose may be due to the well-known sex difference in drug metabolism. This effect may be hidden in the higher dose level due to an overall increased rate of excretion of the drug.

In the rat, VCR and/or its metabolites are excreted very rapidly in the feces and to a much lesser degree in the urine. However, the extent of excretion appears to be dose dependent. During a 72-hr period, more than 85% of the injected dose of VCR was excreted in the urine and feces at 0.1 mg/kg, but only about 50% was excreted at 1.0 mg/kg. This pattern is just the opposite of what occurs in the bile where a larger portion of the administered dose is excreted at the higher dose level. The decreased excretion of VCR at the higher, toxic dose level is probably the result of paralytic ileus, a well-known toxic effect of VCR. Further evidence that this toxic effect was present in our rats was the observation that the amount of feces collected from rats at the higher dose level was less than 40% of that collected from rats at the lower dose. Thus, a significant portion of the injected dose of VCR may have been trapped in the intestines of the rats given the more toxic dose of VCR. The sex differences observed in biliary excretion (at 0.1 mg/kg) were not seen in fecal excretion at either dose level. Since the biliary excretion was studied only for the 1st 4 hr after injection of the drug, this excretion may not accurately reflect the rate of fecal excretion over a 24- to 72-hr period.

The TLC data from our investigations suggest that the bile and urine of rats given \(^{3}H\)VCR contain only small amounts of metabolites. In the urine, these metabolites occur at the point of application of the TLC plates. A large portion of an injected dose of \(^{3}H\)VCR is excreted in the bile during the 1st 30 min. During this time period, TLC data indicate that no significant metabolism has occurred. During the next 30 min, a much smaller amount of radioactivity appears in the bile and approximately 10% of this is present as metabolic products. In contrast to urinary metabolites, the metabolites in the bile appear on the TLC plate between the point of application and the VCR spot. These data may indicate the formation of several metabolites of VCR, the more polar compounds appearing in the urine and the less polar metabolites appearing in the bile.

Contrary to the results with urine and bile, the majority of the radioactivity in the plasma was found to be metabolites, with only 38% occurring as VCR. Due to the rapid clearance of radioactivity from the blood, metabolites in the plasma were studied at 5 min rather than at a later time period. In addition to the VCR spot on the TLC plates, there were 3 other areas of radioactivity. One of these was at the point of application, corresponding to metabolites found in the urine. Another area of radioactivity occurred between the point of application and the VCR spot, similar to metabolites in the bile. Approximately 7% of the radioactivity in the plasma migrated ahead of the VCR. Since the amount of
radioactivity in this area in the urine and bile was no different from their respective controls, this suggests that the plasma contains a metabolite not found in either the urine or the bile. Our data would seem to indicate that the blood contains large amounts of metabolites at an early time period but that very small amounts of these metabolites are excreted in the bile and urine. However, this discrepancy in the amounts of metabolic products in the plasma and that which is excreted in the bile and urine may not be as great as it appears, since at 5 min only 0.5% of the injected dose of radioactivity remains in the blood.

In conclusion, our data suggest that VCR is not metabolized to any great extent in the rat, most of an injected dose being excreted in the bile during the 1st hr. Similar studies with vinblastine in other species suggest that this Vinca alkaloid is metabolized to a greater extent than is VCR (4, 11).

ACKNOWLEDGMENTS

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REFERENCES

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