Antitumor Activity and Preliminary Drug Disposition Studies on Chartreusin (NSC 5159)

J. Patrick McGovren, Gary L. Nell, Sheri L. Crampton, Maxine I. Robinson, and John D. Douros

Cancer Research Unit, The Upjohn Company, Kalamazoo, Michigan 49001 (J. P. M., G. L. N., S. L. C., M. I. R.) and National Cancer Institute, NIH, Silver Spring, Maryland 20910 (J. D. D.)

SUMMARY

Chartreusin has exhibited significant therapeutic activity against three experimental mouse tumors (ascitic P388, L1210 leukemia, and B16 melanoma) when tumor cells were inoculated i.p. and drug was administered i.p. In further testing against P388 leukemia, no activity was observed when drug was administered p.o., s.c., or i.v.

Chartreusin was very slowly absorbed from the small intestine, thus explaining the lack of activity when administered p.o. When given i.p., the drug precipitated in the peritoneal cavity and was slowly absorbed over several hr. The strong activity observed by this route was related to the prolonged and intimate contact of drug with tumor cells in the peritoneal cavity. Upon s.c. administration, extensive precipitation occurred. Subsequent dissolution and absorption from the injection site were very slow, and measured plasma and tissue levels were quite low. Bilary excretion of chartreusin, the predominant route of elimination, was very rapid, with 80 to 100% of the dose appearing as unchanged drug in the bile within 6 hr after i.v. administration. Rapid biliary excretion after i.v. administration was reflected in a rapid decline in plasma and tissue concentrations to levels (shown by in vitro cell kill experiments) less than those necessary to kill P388 cells. When the bile ducts of i.v.-dosed leukemic mice were ligated, therapeutic activity was observed, confirming that the physiological disposition of chartreusin exerts a major influence on its therapeutic utility.

INTRODUCTION

Chartreusin is a Streptomyces-produced antibiotic which was originally reported in 1953 (7). Upon its discovery, the biological activity of chartreusin was investigated in a variety of screening systems at The Upjohn Company. It was also submitted to the National Cancer Institute and the Sloan-Kettering Institute for antitumor testing in the systems then in use. Except for a limited spectrum of antimicrobial activity (2), little or no activity was observed in any screen, and antitumor testing was suspended in 1957. The chemical structure of chartreusin was elucidated and published in 1964 (3, 12) (Chart 1). Recently, a reevaluation of older natural products in new in vivo screening systems has revealed that chartreusin has significant chemotherapeutic activity in mice against the P388 and L1210 leukemias and the relatively refractory B16 melanoma. Biochemical studies have revealed that chartreusin binds to DNA and inhibits RNA and DNA synthesis (8). This report documents the antitumor activity of chartreusin in experimental mouse tumor systems and presents drug disposition and kinetic cell kill data that explain the dependence of activity on the route of drug administration.

MATERIALS AND METHODS

Chartreusin. Chartreusin was isolated and purified from fermentations carried out at The Upjohn Company, Kalamazoo, Mich. Chemical identity and purity were determined by IR, UV, nuclear magnetic resonance, and mass spectral methods and elemental analysis.

Formulation for Administration to Animals. Chartreusin solutions for i.p., p.o., or s.c. administration to rats or mice were prepared by dissolving crystals in 0.01 N NaOH at 3 to 4 mg/ml, adjusting the pH to 9.2 to 9.5, and bringing to desired volume with 0.2 M Na2HPO4 (v/v). At this pH, a maximum solubility of 2.0 mg/ml could be achieved. Solutions for i.v. administration were prepared at 5 mg/ml in 20% N,N-dimethylacetamide:0.2 M Na2HPO4 (v/v).

Chartreusin suspensions for i.p. or s.c. administration were prepared with the use of a Potter-Elvehjem type tissue homogenizer (Tri-R Instruments, Rockville Center, N. Y.) in either 0.9% NaCl solution:0.1% Tween 80 or 0.3% hydroxypropylcellulose:0.9% NaCl solution:0.1% Tween 80.

Chemotherapy Studies. The animals used in chemotherapy experiments were C57BL/6 × DBA/2 F1 (hereafter called B6D2F1) mice, 6 to 8 weeks of age (18 to 25 g). Female mice were used in the B16 melanoma studies; male mice were used in all others. Groups of 6 to 10 mice were housed in plastic cages and given pelleted feed and water ad libitum. L1210 and P388 leukemias were maintained by continuous i.p. passage in syngeneic DBA/2 female mice. B16 melanoma and Lewis lung carcinoma were carried s.c. in syngeneic C57BL/6 male mice. Standardized protocols of the Drug Research and Development Program, National Cancer Institute, were followed for continuous passage of the tumor and for implantations of tumors into B6D2F1 mice for chemotherapy experiments (4). L1210 and P388 leuke-
Absorption, Distribution, Metabolism, and Excretion Studies. Male Sprague-Dawley rats (160 to 300 g) and male B6D2F1 mice (18 to 25 g) were used in all disposition studies.

Oral absorption was studied by administering p.o. doses of 50 mg/kg by gavage to bile duct-cannulated rats and measuring the cumulative amount of chartreusin appearing in the bile, feces, and urine at intervals to 48 hr. After dosing, rats were placed in special restrainer cages (Stoelting Co., Chicago, Ill.) which were modified with devices to allow separate collection of urine and feces.

Biliary excretion studies were conducted in bile duct-cannulated rats. Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and bile ducts were cannulated with PE10 tubing (Intramedic polyethylene tubing; Clay Adams, Parsippany, N. J.). Abdominal incisions were closed with animal wound clips (Autoclips; Clay Adams). Body temperature was maintained by directing heat lamps onto the rats. Bile was collected into tared plastic tubes which were reweighed at the end of a collection interval and frozen until assay.

Absorption of chartreusin after i.p. administration was studied by administering doses of 10 and 50 mg/kg to bile duct-cannulated rats. The rats received the same volume of chartreusin solution at each dose level (as 0.4 or 2.0 mg/ml) to duplicate the conditions of the therapy studies in mice in which a constant volume was administered to each animal at each dose. The rats were kept unconscious and lying on their backs for the 1st 6 hr of the experiment so that drug would not leak out through the abdominal incision. After 6 hr, the rats were placed in restrainer cages and allowed to regain consciousness. Bile was collected at intervals to 48 hr after administration.

Metabolism cages (having open mesh flooring with devices to separate urine from feces) were used to study urinary and fecal excretion in rats and mice.

The time course of tissue distribution of chartreusin was measured in mice after s.c. administration and in rats after i.v. administration. At selected intervals after administration, groups of animals were anesthetized with ether or methoxyflurane, blood was withdrawn from the inferior vena cava into heparinized syringes, and the following tissues were dissected and stored frozen until assay: kidneys, spleen, liver, small intestine, heart, lung, thymus, muscle from leg, and brain. Blood was centrifuged to obtain plasma, which was frozen until assay.

In vitro liver metabolism was investigated by incubating chartreusin (100 and 200 µg/ml) with the supernatant from a mouse liver homogenate centrifuged at 9000 × g and a NADPH-generating system (9).

Assay for Chartreusin. Bile and urine samples were assayed undiluted or diluted with 0.2 M Na2HPO4 by applying 80-µl aliquots to wells formed in Sarcoma lutea-seeded agar trays. After incubation (37°, 14 to 20 hr), the diameters of the zones of growth inhibition were measured. These zones were compared with those obtained with chartreusin standards prepared in drug-free bile and urine at the same dilution and applied to each agar tray.

Tissue and fecal samples were diluted in pH 7, 0.1 M sodium phosphate buffer and homogenized with a Tekmar Tissumizer (Tekmar Co., Cincinnati, Ohio).

Plasma, tissue, and fecal samples were assayed by extracting chartreusin into chloroform, evaporating the solvent, reconstituting the extracted material in 0.01 N NaOH, and applying to Sarcoma lutea-seeded agar trays as described above. Standards were prepared by the addition of known quantities of chartreusin to drug-free tissue homogenates for each tissue analyzed and extracted in the same manner as described above. A separate tissue standard curve was applied to each agar tray.

The specificity of the microbiological assay for unchanged chartreusin was evaluated by TLC and bioautography. Aliquots of whole bile and urine collected for 6 hr from rats dosed i.v. with chartreusin were extracted with chloroform. The concentrated extract was applied to flexible plastic-backed TLC plates (Bakerflex silica gel IB-F; J. T. Baker Chemical Co., Phillipsburg, N. J.) which were developed in chloroform: methanol:water (85:14:1). Separated bands were visualized by their fluorescence under 360 nm UV. The silica gel surfaces of the plates were then placed in contact with S. lutea-seeded agar trays for 30 min and removed. The trays were incubated, and zones of inhibition corresponding to the separated bands were observed. Aliquots of bile and urine were then applied to silica gel-coated glass TLC plates (Uniplate; Analtech, Inc., Newark, Del.) which were developed as before. Bands were visualized and scraped off the glass plate. The separated bioactive materials were eluted from the silica gel and assayed microbiologically. Aliquots of whole bile and urine were also assayed to determine total bioactivity. Bile and urine from untreated animals were processed in the same fashion.

Cell Kill Studies. In studies of P388 cell kill kinetics, cells were maintained in culture in Roswell Park Memorial Institute Medium 1634 supplemented with fetal calf serum (5%), NaHCO3 (0.75 mg/ml), penicillin (0.1 mg/ml), and streptomycin (0.05 mg/ml), as described previously (1). In this medium, the cells grew exponentially with a doubling time of about 12 hr. Chartreusin was dissolved and then diluted...
with medium prior to the addition of cells. At the end of drug exposure, cells were centrifuged and washed to remove drug and then suspended in medium to determine cloning efficiency. Cell survival was determined by cloning in soft agar (5) and has been described previously (1). In the calculation of percentage survivals, the control samples (no drug treatment) were normalized to 100% survival. The cloning efficiency of control cells was 60 to 90%. The relative standard deviation in determining cell survival was about 15%.

Chemotherapeutic Activity. When administered i.p. to mice inoculated i.p. with P388 or L1210 leukemia or B16 melanoma, chartreusin displayed significant activity over a wide dose range (Tables 1 and 2). A maximum increase in life-span of 131% was observed in P388 leukemic mice at a dose of 50 mg/kg. Significant therapeutic activity (over 25% ILS) was observed against P388 leukemia over a range of 0.8 to 100 mg/kg. In the P388 testing, similar results were obtained whether solutions or suspensions were administered.

Significant activity was also exhibited in L1210 leukemic mice over a dose range of 10 to 80 mg/kg (46% maximum ILS at 10 mg/kg).

In B16 melanoma testing, 83 to 99% ILS was obtained at doses of 25, 50, and 100 mg/kg, and 3 cures were observed.

No activity was observed against s.c.-implanted Lewis lung tumor over a dose range of 12.5 to 200 mg/kg administered i.p. (data not presented).

In further testing against P388 leukemia, no activity was observed when tumor cells were inoculated i.p. and drug was given p.o., s.c., or i.v. Also, no activity was seen when P388 was inoculated i.v. and drug was injected i.p. or i.v. (data not presented).

Cell-Kill Studies. The in vitro P388 cell-kill studies revealed that relatively high chartreusin concentrations must be maintained for long times in order to kill tumor cells. Chart 2 shows that 5 μg chartreusin per ml for 24 hr killed 99% of the cells. However, 60% of the cells survived a 6-hr exposure at that same concentration. Similar results were obtained in L1210 cell-kill kinetic studies and will be the subject of a future report (B. K. Bhuyan and M. Robinson, unpublished results).

Assay for Chartreusin. The sensitivity of the microbiological assay (10 μg/ml) was adequate to measure drug concentrations in bile and urine. However, plasma and tissue levels were below this value, and it was necessary to extract relatively large volumes of pooled plasma or tissue homogenates to amplify the sensitivity. Because of extensive biliary excretion, feces contained high levels of chartreusin. However, extractions were necessary to eliminate interfering materials.

Experiments were conducted to determine the specificity of the microbiological assay for chartreusin. When relatively large volumes of bile or urine from chartreusin-treated rats or mice were extracted and applied to TLC plates, one band in addition to chartreusin, which was not present in bile or urine from untreated animals, could be observed under 360 nm light after development. The band traveled more slowly than chartreusin under these conditions, indicating that it was more polar. Bioautography indicated that the compound also inhibited the growth of S. lutea, but when chartreusin and the polar metabolite were eluted from the TLC sorbent and assayed, unchanged chartreusin accounted for virtually all of the total bioactivity present in the original fluids. The quantity of the eluted metabolite was below assay sensitivity limits, suggesting minimal metabolism of chartreusin.

The microbiological method was very reproducible. Standard curves of log chartreusin concentration versus diameter of the zone of growth inhibition gave linear regression correlation coefficients of 0.95 or greater for all tissues and fluids.

In Vitro Liver Metabolism. No decline in total bioactivity was observed when chartreusin was incubated with the 9000 × g supernatant from a mouse liver homogenate preparation.

Biliary Excretion. The most striking feature of the studies of chartreusin disposition was the rapid and extensive biliary elimination of unchanged drug. Chart 3 shows that, with doses of 5 and 10 mg/kg i.v., 80 to 100% was eliminated in the bile over 6 hr. The previously mentioned studies of assay specificity indicated that chartreusin was not being significantly metabolized before biliary excretion. When these data are plotted as biliary excretion rate versus time (Chart 4), a peak is noted shortly after dosing, followed by a biphasic decline with a terminal 1st-order half-life of about 1 hr. The limited tissue distribution data discussed below suggest that the decline in biliary excretion rate paralleled the rapid decline in drug concentrations in tissues.

Tissue Distribution. Following i.v. dosing, tissue concentrations rapidly declined to levels less than those necessary to kill P388 cells. Chart 5 shows liver and small intestine concentrations in bile duct-cannulated (bile-diverted) rats after a 10 mg/kg i.v. dose. Levels in all tissues except liver and small intestine were less than 1 μg/g by 1 hr after administration. Liver concentrations were initially high but declined to 1 μg/g by 4 hr. Because the rats used in this experiment were bile diverted, the measured chartreusin represented the actual tissue concentration rather than drug excreted in bile. The levels in small intestine did not decline in parallel with the other tissues. No chartreusin was detected in brain (assay sensitivity was approximately 0.5 μg/g). The rate of decline of chartreusin in tissues after i.v. dosing roughly paralleled the decline in biliary excretion.
Table 1

Effect of i.p.-administered chartreusin on the life-span of mice inoculated i.p. with P388 leukemia

<table>
<thead>
<tr>
<th>Dose (mg/kg/i.p. injection)</th>
<th>Schedule (Days)</th>
<th>Vehicle</th>
<th>Wt change* (g)</th>
<th>Survival time (days)</th>
<th>Median</th>
<th>Range</th>
<th>ILS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control*</td>
<td>200</td>
<td>Suspension</td>
<td>2.1</td>
<td>9.4</td>
<td>8-13</td>
<td></td>
<td></td>
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<tr>
<td>100</td>
<td>1-9</td>
<td>Suspension</td>
<td>-2.7</td>
<td>16.0</td>
<td>10-21</td>
<td>70</td>
<td></td>
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<tr>
<td>50</td>
<td></td>
<td>Suspension</td>
<td>-2.1</td>
<td>20.8</td>
<td>16-22</td>
<td>121</td>
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<tr>
<td>25</td>
<td></td>
<td>Suspension</td>
<td>0.3</td>
<td>16.3</td>
<td>15-19</td>
<td>73</td>
<td></td>
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<td>Suspension</td>
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<td>19.7</td>
<td>16-22</td>
<td>109</td>
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<tr>
<td>6.3</td>
<td></td>
<td>Suspension</td>
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<td>15.0</td>
<td>13-16</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
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<td>Suspension</td>
<td>0.9</td>
<td>14.0</td>
<td>13-17</td>
<td>48</td>
<td></td>
</tr>
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</table>

Untreated control

| 50                          | 1-9             | Solution   | -1.5         | 21.8                 | 12-25  | 131   |
| 25                          |                 | Solution   | -0.3         | 20.3                 | 14-25  | 115   |
| 12.5                        |                 | Solution   | -0.1         | 19.3                 | 18-25  | 105   |
| 6.3                         |                 | Solution   | 0.1          | 18.6                 | 16-20  | 100   |
| 3.1                         |                 | Solution   | 0.3          | 15.0                 | 12-16  | 59    |
| 1.6                         |                 | Solution   | -0.3         | 13.7                 | 13-14  | 45    |
| 0.8                         |                 | Solution   | 0.8          | 12.3                 | 9-14   | 30    |
| 0.4                         |                 | Solution   | 0.7          | 11.4                 | 11-12  | 21    |

Untreated control

| 128                        | 1, 5, and 9     | Suspension | 0.9          | 17.0                 | 11-23  | 82    |
| 64                          |                 | Suspension | -0.4         | 16.3                 | 13-23  | 75    |
| 32                          |                 | Suspension | -1.1         | 16.0                 | 11-17  | 72    |
| 16                          |                 | Suspension | 0.1          | 16.1                 | 13-17  | 73    |
| 8                           |                 | Suspension | 0.5          | 14.0                 | 11-16  | 50    |

* Average weight change per mouse between Days 1 and 5.
* Untreated controls received injections of the respective vehicles.
* Chartreusin was suspended in 0.9% NaCl solution: 0.3% hydroxypropylcellulose: 0.1% Tween 80 or 0.9% NaCl solution: 0.1% Tween 80.
* Chartreusin was dissolved in 0.01 N NaOH at 4 mg/mI, the pH was adjusted to 9.2 to 9.5, and the solution was brought to the desired volume with 0.2 M Na2HPO4.

Table 2

Effect of i.p.-administered chartreusin on the life-span of mice inoculated i.p. with L1210 leukemia and B16 melanoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Dose* (mg/kg/i.p. injection)</th>
<th>Wt change* (g)</th>
<th>Survival time (days)</th>
<th>Median</th>
<th>Range</th>
<th>ILS (%)</th>
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<tr>
<td>L1210</td>
<td>Untreated control*</td>
<td>0.7</td>
<td>9.5</td>
<td>4.5</td>
<td>12-17</td>
<td>33</td>
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<td>80</td>
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<td>-2.6</td>
<td>12.7</td>
<td>10-17</td>
<td>33</td>
<td></td>
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<tr>
<td>40</td>
<td></td>
<td>-0.7</td>
<td>13.0</td>
<td>11-16</td>
<td>37</td>
<td></td>
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<tr>
<td>20</td>
<td></td>
<td>0.1</td>
<td>12.8</td>
<td>12-15</td>
<td>35*</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>0.2</td>
<td>13.8</td>
<td>10-18</td>
<td>46</td>
<td></td>
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<tr>
<td>5</td>
<td></td>
<td>-0.5</td>
<td>11.0</td>
<td>10-12</td>
<td>46</td>
<td></td>
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<tr>
<td>2.5</td>
<td></td>
<td>0.3</td>
<td>11.7</td>
<td>10-16</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td></td>
<td>0.6</td>
<td>9.3</td>
<td>9-10</td>
<td></td>
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<tr>
<td>B16</td>
<td>Untreated control*</td>
<td>-0.8</td>
<td>20.5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>100</td>
<td></td>
<td>-2.9</td>
<td>38.0</td>
<td>7-54</td>
<td>85</td>
<td></td>
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<tr>
<td>50</td>
<td></td>
<td>-1.7</td>
<td>40.8</td>
<td>8-55</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>-0.3</td>
<td>37.7</td>
<td>17-45</td>
<td>83*</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td></td>
<td>-0.1</td>
<td>30.0</td>
<td>21-36</td>
<td>46</td>
<td></td>
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</tbody>
</table>

* Doses were administered, Days 1 to 9, as suspensions in 0.9% NaCl solution: 0.1% Tween 80.
* Average weight change per mouse between Days 1 and 5.
* Untreated controls received injections of the vehicle.
* One cure (survival to Day 60) was observed.
* Two curves (survival to Day 60) at 100 mg/kg and 1 cure at 25 mg/kg were observed.

rate with a 1st-order half-life of about 1 hr.

Absorption Studies. Knowledge of the extent of biliary excretion after i.v. dosing was used to determine the extent of p.o. absorption. Two bile duct-cannulated rats received 50 mg/kg p.o., and the amount of drug in the bile was measured at intervals. Each rat excreted a total of 6% of the dose in the bile in 72 hr. An additional 0.1% appeared in the urine in each rat, indicating that the total extent of p.o. absorption was only 7 to 8%. The feces collected over 72 hr after drug administration contained 53 and 19% of the dose.
indicating that much of the drug was still in the gastrointestinal tract. The volume of feces excreted by the animals was low, indicating poor gastrointestinal function, probably as a result of surgical trauma.

The extensive biliary excretion of chartreusin suggested that enterohepatic circulation of drug would be possible. However, the very slow p.o. absorption suggested that the amount of drug involved in enterohepatic recycling would be insignificant. This was verified by administering i.v. doses to rats and mice and measuring cumulative urinary and fecal excretion. Urine contained 5% of the dose, and 75% was recovered in the feces in 48 hr.

The s.c. administration of 225 mg/kg to mice resulted in extensive precipitation and very slow dissolution of drug at the injection site. On the basis of observation of the size of the deposit of chartreusin remaining at the injection site, very little drug was absorbed up to 96 hr after administration. Tissue and plasma levels were measured at intervals to 12 hr after dosing. The levels were below assay sensitivity (approximately 0.5 µg/g) in most tissues. In the tissues in which drug could be detected, the levels were similar at each time point. Plasma levels were 1 to 2 µg/ml, liver contained 20 to 40 µg/g, and kidney levels were 2 to 3 µg/g. Assays of segments of small intestine showed significant quantities of drug present, reflecting chartreusin excreted in bile as well as drug actually in the tissue.

After i.p. administration to rats and mice, chartreusin precipitated extensively from solution and was slowly absorbed. In an attempt to quantitate the rate of absorption of drug from the peritoneal cavity, the appearance of chartreusin in the bile after i.p. administration (10 or 50 mg/kg) was measured in bile duct-cannulated rats for 48 hr after dosing. The rats were dissected after 48 hr, and the peritoneal cavity was examined for unabsorbed chartreusin. No drug was observed in the animals that received 10 mg/kg, but deposits of chartreusin were clearly visible adhering to the abdominal viscera in rats that received 50 mg/kg. The results are plotted (Chart 6) as the percentage of the administered dose appearing in the bile as a function of the time after administration. The chart also shows the percentage of a 10-mg/kg i.v. dose appearing in the bile as a reference for the situation in which absorption is considered to be instantaneous. Because biliary elimination of chartreusin was so rapid and extensive after i.v. administration, the rate of drug excretion in bile after i.p. administration reflected the overall rate at which chartreusin dissolved and was absorbed from the peritoneal cavity into the general circulation.
Both groups excreted chartreusin at a much slower rate than rats dosed i.v., indicating that redissolution and absorption were rate controlling in the overall disposition. After 14 hr, 85% of the 10 mg/kg dose had been eliminated, and bile concentrations were too low to be measured. The absorption process was considerably slower in the 50 mg/kg group, as evidenced by the lower extent excreted at each time. A similar phenomenon occurring in i.p.-dosed mice in chemotherapy studies could explain the excellent dose-response relationship observed with i.p.-inoculated P388 leukemia. The drug-cell contact time in the peritoneal cavity would be disproportionately increased with increasing dose.

**Chemotherapeutic Activity in Bile Duct-ligated Mice.** When chartreusin was administered i.v. to i.v.-inoculated P388 leukemic mice that had ligated bile ducts, therapeutic activity was observed (Chart 7). The data are presented as survival curves for 4 treatment groups: (a) leukemic, vehicle-treated; (b) leukemic, chartreusin-treated; (c) ligated, leukemic, vehicle-treated; and (d) ligated, leukemic, chartreusin-treated. Almost one-half of the ligated, leukemic, chartreusin-treated mice died by Day 5, an indication of toxicity resulting from chartreusin, since the mice dosed only with the vehicle survived past Day 4. After Day 5, the survival curve leveled off, and by Day 13, although all of the ligated control mice were dead, 36% of the treated animals remained alive. The remaining mice died between 13 and 19 days, with the exception of 2 mice which survived to Day 30. Normal (nonligated) P388 leukemic mice died between Days 8 and 10, and i.v. chartreusin treatment did not increase their life-span.

**DISCUSSION**

Based on the impressive activity displayed by chartreusin in 3 experimental mouse tumor systems, extensive experimental therapeutic evaluation, biochemical characterization, and drug disposition studies were undertaken. Biochemical studies showed that chartreusin is an effective cytotoxic agent and suggested its mechanism of action (8). However, the therapeutic studies revealed an extreme dependence of activity on the route of drug administration. Chartreusin increased the life-span of P388 leukemic mice only when tumor cells were inoculated i.p. and drug was administered i.p. Studies of chartreusin disposition and cell-kill kinetics have explained this behavior.

Killing of P388 cells in vitro by chartreusin requires a long duration of exposure and/or a high exposure concentration. In therapy studies, chartreusin was quite effective when both tumor cells and drug were given i.p. because of extensive precipitation and slow dissolution of drug in the peritoneal cavity. The tumor cells remaining in the cavity were exposed for relatively prolonged periods to the high local concentration of chartreusin. An observation that tends to support this interpretation is that moribund mice that had been inoculated i.p. with P388 cells and dosed i.p. on Days 1 to 9 with chartreusin had very little withdrawable ascites (<0.2 ml) compared to controls (5 to 7 ml). When tumor cells were inoculated i.v. and drug was injected i.p., no activity resulted because of the combination of slow absorption and rapid elimination in the bile.

Chartreusin was ineffective when administered p.o. or s.c. because of very slow absorption from the gastrointestinal tract or the s.c. injection site.

After i.v. administration, chartreusin was very rapidly eliminated in the bile, therapeutic blood and tissue concentrations were not maintained, and no therapeutic activity was observed in i.p.- or i.v.-inoculated P388 leukemic mice. However, if the bile ducts of P388 leukemic mice were ligated, a therapeutic effect was obtained with i.v. administration, confirming that the physiological disposition of chartreusin exerts a major influence on its therapeutic utility.

When it is desirable or necessary to maintain body levels of a drug above a certain minimum and that drug is too rapidly eliminated to be given by a practical repetitive dosing schedule, a constant-rate i.v. infusion is indicated. Arabinosylcytosine is an example of such a drug (6). Once a steady state is achieved (i.e., the rate of drug elimination equals the rate of drug infusion), a constant amount of drug is maintained in the body, the magnitude of the level being proportional to the infusion rate (mass per time). It should be possible to treat tumorous mice with chartreusin by giving a constant-rate infusion over some length of time.
We are currently attempting to conduct such studies in tumorous mice (11). If this effort is successful, it would provide impetus for further development of chartreusin.

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REFERENCES

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