Disposition of Bisantrene in Humans and Rabbits: Evidence for Intravascular Deposition of Drug as a Cause of Phlebitis¹

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ABSTRACT

The investigational antitumor agent bisantrene (9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1H-imidazol-2-yl)hydrazene dihydrochloride] causes frequent local complications of phlebitis and thromboses in patients receiving the drug by peripheral venous infusion. Bisantrene pharmacokinetics was studied in five patients. Plasma elimination was biphasic with t₁/₂ α of 65 min and t₁/₂ β of 1142 min; the mean apparent volumes of distribution of the central compartment and peripheral compartments were 185 and 1662 liters/sq m, suggesting extensive uptake, binding, or deposition of drug. Total body clearance was 735 ml/min/sq m, and 11.3% of drug was excreted in urine. One hr after bisantrene (260 mg/sq m) at 1 mg/ml in 5% dextrose was infused into the marginal ear vein of a rabbit, the vein was congested with blood and contained 2.1 mg precipitated bisantrene. After 24 hr, the vein was clotted and contained 1.18 mg precipitated drug. Precipitation of bisantrene appears to be related to the low solubility of the drug at physiological pH. Maximum solubility of bisantrene in human and rabbit serum was 12.7 μg/ml. Intravascular precipitation of bisantrene may be responsible for phlebitis and thromboses in humans receiving the drug by i.v. infusion.

INTRODUCTION

Bisantrene (9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1H-imidazol-2-yl)hydrazene dihydrochloride] is an anthracene bishydrazone derivative undergoing clinical evaluation for activity against a wide spectrum of human cancers. The drug was initially placed in clinical trials because of activity against many model murine tumor systems (3). Special clinical interest in bisantrene has been generated by reports of responses in patients with advanced cancers including renal carcinoma and melanoma (1, 7, 8).

Myelosuppression was the major toxicity noted in large-animal studies (3) and has been reported as the dose-limiting toxicity in 3 Phase I studies (1, 7, 8). More troublesome than reversible leukopenia have been local complications of phlebitis and thromboses in up to two-thirds of patients given bisantrene by infusion into a peripheral vein. These toxicities occurred on 3 different schedules, 1 day every 4 weeks, weekly for 3 weeks, and daily for 5 days every 4 weeks (1, 7, 8). As little as 80 mg bisantrene per sq m in 500 ml 5% dextrose in water, which is about one-third of the dose recommended for Phase II trials on the single-day schedule, produced immediate local toxicity in some patients (7). Local complications of bisantrene therapy prompted Speigel et al. (7) to speculate that the drug may exert a direct effect on vascular endothelium leading to loss of integrity of the vein and local inflammation.

We became interested in the mechanism by which bisantrene produces local toxicity because of our difficulties in administering the drug without producing pain, venospasm, and redness over the infused vein in some patients. Dilution of bisantrene at 260 mg/sq m in volumes up to 1500 ml 5% dextrose in water and administration over 3 hr in as large a peripheral vein as possible decreased the frequency and severity of signs of acute local irritation but did not prevent the occurrence of thrombosis in several patients 7 to 10 days later (6). Because bisantrene appeared to be exerting a toxic effect locally, we reexamined some physicochemical characteristics of the drug in vitro and in vivo in animal models, in addition to studying the pharmacokinetics of the drugs in humans as part of a recently completed Phase II trial in advanced colorectal carcinoma (6).

MATERIALS AND METHODS

Bisantrene was obtained from Division of Drug Treatment, National Cancer Institute, Bethesda, Md. Human studies on the pharmacokinetics of bisantrene were conducted in patients with colorectal or bladder cancer receiving the drug in Phase II clinical trial. All patients had no direct-reacting serum bilirubin and a serum creatinine less than 1.5 mg/dl. Patients were hospitalized in a clinical studies unit for 36 hr for the pharmacokinetic study. Bisantrene was dissolved in 500 ml 5% dextrose and water (approximately 1 mg/ml), and 260 mg/sq m were administered over 90 min into a peripheral vein. Blood samples were collected in heparinized tubes from the opposite arm used for infusion before and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 12, 18, and 24 hr after starting infusion. Plasma was stored frozen at −70°C. Urine was collected before and for 24 hr after administration of drug. Male New Zealand White rabbits, weighing 1.75 to 3.0 kg, were infused through a 23-gauge needle (Miniset Infusion Set; Travenol, Deerfield, Ill.) with bisantrene at a dose of 260 mg/sq m into a peripheral ear vein over 1, 24, or 72 hr with a solution of bisantrene in 5% dextrose in water at 1 mg/ml, 50 μg/ml, and 15 μg/ml, respectively. The flow rate for each infusion was approximately 0.5 ml/min. After administration of bisantrene, the ear vein was infused with 5 ml 0.9% NaCl solution at the same flow rate, the infusion needle was removed, and the animal was sacrificed 1 or 24 hr later. Both ears were removed, and a 0.5-cm-wide strip of skin overlying the peripheral ear vein was peeled back to expose approximately 6 cm of vein from just above the point of infusion to the base of the ear. The vein was examined under low-power light microscopy and cut into 1-cm lengths. Each 1-cm segment was extracted for 1 hr with 0.1 N HCl with vigorous shaking, and the extracts were assayed for bisantrene. Bisantrene was assayed by a reversed-phase high-pressure liquid chromatographic procedure described previously (5).

Solubility studies were conducted using bisantrene supplied as the dihydrochloride salt and bisantrene base prepared by adjusting a

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solution of bisantrene dihydrochloride to pH 7.0, collecting the precipitated base by filtration, washing extensively with water, and drying under vacuum. Fresh human serum, phosphate-buffered saline (Dulbecco's), and 10% crystalline human serum albumin (Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline were each mixed with a solution of bisantrene dihydrochloride (10 mg/ml water; 25 µl/ml serum or 0.9% NaCl solution) and gently agitated for 30 min at room temperature. The pH was adjusted to 7.3 with 0.1 N NaOH. The sample was centrifuged twice at 15,000 rpm for 15 min, and aliquots were taken for determination of bisantrene. Fresh human serum and phosphate-buffered saline were shaken with an excess of bisantrene base (1 mg/ml) for 2 hr at 37°C in the dark. The pH was adjusted at intervals to 7.3. Serum samples were also filtered through a 0.45-µm filter (Millipore HA Filter Unit; Millipore, Bedford, Mass.). Results were corrected for 16.6% binding of bisantrene to the filter determined in separate experiments with saturated solutions of bisantrene in serum. Ultrafiltrates were prepared from solutions of bisantrene in serum and albumin using Amicon ultrafiltration cones (Amicon, Lexington, Mass.). To determine the extent of binding or uptake of bisantrene by blood cells, washed cells from 1.5 ml heparinized homologous blood were mixed with 1.5 ml bisantrene-saturated serum and incubated with gentle shaking at 37°C for 30 min or at room temperature for 60 min. Cells were removed by centrifugation, and the amount of bisantrene in the serum was determined.

Analysis of pharmacokinetic data for short-term infusions were conducted using the NONLIN least-squares regression analysis program (2) on a CDC Cyber 170–720 computer with interactive graphic analysis. Pharmacokinetic parameters were calculated with allowance for 16.6% binding of bisantrene to blood cells. The biexponential decline in the plasma concentrations of bisantrene was fitted to the equation

\[ C = Ae^{-\alpha t} + Be^{-\beta t} \]

with a weighing factor of 1/y², where C is the plasma concentration of bisantrene at time t after completion of infusion, A and B are intercepts at this time, and \( \alpha \) and \( \beta \) are the fast and slow disposition constants.

RESULTS

Pharmacokinetic Studies in Humans. Plasma concentrations of bisantrene in 5 patients receiving a 90-min infusion of bisantrene at 260 mg/sq m declined in a biphasic manner after cessation of the infusion (Chart 1) with a mean initial half-life of 65 ± 15 (S.E.) min and a mean terminal half-life of 1142 ± 226 min. The peak plasma concentration during infusion was approximately 5 µg/ml (Table 1). The mean apparent volume of distribution of the central compartment (Vc) was 185 ± 46 liters/sq m, and the mean apparent volume of distribution of the peripheral compartment (Vp) was 1662 ± 486 liters/sq m. Mean total body plasma clearance (Cl) was 735 ± 125 ml/min/sq m, and 11.3 ± 1.8% of the total dose was excreted unchanged in the urine in 24 hr.

Solubility of Bisantrene in Human Serum. A study of the solubility of bisantrene in human plasma was prompted by our observation that some bisantrene precipitated and remained as a precipitate in the peritoneal cavity of mice 24 hr after i.p. injection of 260 mg/sq m (0.1 ml of a 23-mg/ml solution in water). We found that a solution of bisantrene dihydrochloride as prepared for administration to patients, 1 mg/ml in 5% dextrose in water, has a pH of 4.2. As the pH of this solution is increased with 0.1 N NaOH, bisantrene precipitates as a bright yellow amorphous material. When a 1-mg/ml solution of bisantrene dihydrochloride in 5% dextrose in water was mixed with heparinized human plasma in ratios of 1:1 or 1:4, plasma cloudiness developed after a few min and amorphous yellow material could be sedimented by centrifugation. We determined the maximum solubility of bisantrene in human serum by adding an excess of bisantrene dihydrochloride in solution (1 mg/ml in 5% dextrose in water) and an excess of bisantrene base as a solid to fresh human serum at 37°C. The maximum concentration of bisantrene in human serum after addition of the drug in solution and after addition of the drug as a solid was 13 µg/ml (Table 2). We assumed that most of the drug in serum was bound to protein because an ultrafiltrate of serum had no detectable drug (<10 mg/ml).

The solubility of bisantrene in phosphate-buffered saline, pH 7.3, was no more than 0.43 ± 0.05 µg/ml (n = 3). This may be an overestimate since most of the drug was removed by a 0.45-µm filter. The solubility of bisantrene in a 10% solution of crystalline human serum albumin in phosphate-buffered saline, pH 7.3, was 77 µg/ml. Little bisantrene (<10 ng/ml) was present in an ultrafiltrate of this solution. Incubation of RBC with an equal volume of a saturated solution of bisantrene in serum reduced the concentration of drug in serum. The ratio of the concentration of bisantrene in RBC to the concentration in serum was 3:1.

Intravascular Deposition of Bisantrene in Rabbit. We studied the effect of bisantrene on the ear veins of rabbits after infusing the drug at the dose used in our clinical studies, 260 mg/sq m (about 40 mg/rabbit). The concentration of bisantrene in rabbit plasma at the end of infusion of 260 mg/sq m over 1 hr was 10.7 ± 2.2 µg/ml (n = 3), and at the end of infusion of 260 mg/sq m over 24 hr it was 0.26 ± 0.02 µg/ml.

The ear vein of a rabbit 1 hr after infusion of bisantrene (260 mg/sq m over 1 hr) contained deposits of bright yellow material along its length. A vein from the opposite ear contained no yellow deposits and was indistinguishable in appearance from an ear vein of an untreated rabbit (Fig. 1). Twenty-four hr after a 1-hr i.v. infusion of bisantrene dihydrochloride, the infused ear vein was distended with liquid and clotted blood and contained deposits of yellow material. To establish that the deposits were precipitated bisantrene, 1-cm segments of infused veins were extracted with 0.1 N HCl and assayed for bisantrene. Infused ear veins contained bisantrene along their entire lengths (Chart 2). One hr after administration of 40 mg of bisantrene by 60-min infusion, 6-cm lengths of infused vein contained a total of 0.09 ± 0.63 mg (n = 3) bisantrene.
Intravascular Deposition of Bisantrene

Table 1
Pharmacokinetic parameters of bisantrene in humans

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Concentration (µg/ml)</th>
<th>t(^1/2)a (min)</th>
<th>t(^1/2)b (min)</th>
<th>V1 (liters/sq m)</th>
<th>V2 (liters/sq m)</th>
<th>Cl(^0) (ml/min/24 hr)</th>
<th>Urinary excretion (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>4.6</td>
<td>76</td>
<td>685</td>
<td>320</td>
<td>1414</td>
<td>1200</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>4.4</td>
<td>91</td>
<td>1870</td>
<td>206</td>
<td>1415</td>
<td>451</td>
<td>10.4</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>2.6</td>
<td>37</td>
<td>1006</td>
<td>146</td>
<td>1533</td>
<td>836</td>
<td>8.5</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>7.7</td>
<td>23</td>
<td>735</td>
<td>35</td>
<td>488</td>
<td>348</td>
<td>13.1</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>5.7</td>
<td>100</td>
<td>1435</td>
<td>218</td>
<td>3460</td>
<td>842</td>
<td>16.8</td>
</tr>
</tbody>
</table>

Mean ± S.E. 5.0 ± 0.7 65 ± 15 1142 ± 226 185 ± 46 1662 ± 486 735 ± 125 11.3 ± 1.8

Table 2
Solubility of bisantrene

<table>
<thead>
<tr>
<th>Preparation</th>
<th>No. of preparations</th>
<th>Maximum solubility (µg/ml)</th>
<th>Ultrafiltrate (µg/ml)</th>
<th>Blood cell:serum concentration ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisantrene dihydrochloride</td>
<td>4</td>
<td>12.6 ± 1.9(^a)</td>
<td>0.6 ± 0.3</td>
<td>3.0 ± 0.6(^b)</td>
</tr>
<tr>
<td>Bisantrene base</td>
<td>3</td>
<td>12.8 ± 1.3(^c)</td>
<td>0.0</td>
<td>2.15 ± 0.8(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± S.E. 15.4 ± 1.5 0.6 ± 0.3 3.0 ± 0.6 12.9 ± 1.3
\(^b\) Homologous blood cells incubated with saturated serum for 1 hr at room temperature.
\(^c\) Filtered through a 0.45-µm filter, corrected for 16.6% binding to filter.
\(^d\) Homologous blood cells incubated with saturated serum for 30 min at 37\(^\circ\)C.

Maximum solubility was determined by mixing an excess of bisantrene dihydrochloride in solution with serum and allowing it to stand at room temperature for 30 min or shaking an excess of bisantrene base with serum for 2 hr at 37\(^\circ\)C. In both cases, pH was adjusted to 7.3. Serum was centrifuged twice at 15,000 rpm for 15 min to remove undissolved drug.

DISCUSSION

Our studies of bisantrene dissolved in various media demonstrate that it is very insoluble at a pH greater than approximately 4.5 and is virtually insoluble at pH 7.4. The maximum solubility of bisantrene dihydrochloride in rabbit and in human serum is approximately 13 µg/ml. Most of the drug in serum appears to be bound to protein because ultrafiltration of plasma saturated with bisantrene removed all detectable drug. The low solubility of bisantrene at physiological pH makes it likely that, when the drug is administered at concentrations in the infusate which produce i.v. concentrations greater than 13 µg/ml, precipitation of the drug occurs intravascularly.

In our Phase II study of bisantrene in patients with advanced colorectal carcinoma, the drug was given at 260 mg/sq m, the total dose being dissolved in a maximum of 1500 ml of 5% dextrose in water and infused over 1.5 to 3 hr (6). Peak plasma concentrations of bisantrene after 260 mg/sq m given over 90 min ranged from 4 to 8 µg/ml. The magnitude of these values is comparable to that reported by Peng et al. (4), 6 µg/ml, at the end of a 30-min infusion of 110 mg/sq m. The secondary phase of plasma elimination of bisantrene ranged from 7 to 30 hr with a mean of 17 hr. The value of the terminal half-life is an estimate based upon blood samples collected for only 24 hr. Although there were considerable differences in the volumes of distribution among the 5 patients studied, all volumes of distribution were extremely large. They reflect, we believe, precipitation of drug in vivo rather than entry of soluble drug into some large compartment such as drug binding to tissue and/or plasma macromolecules. When bisantrene was administered to rabbits via an ear vein at a total dose, concentration, and rate used for administration of the drug to humans (260 mg/sq m as a 1-mg/ml solution over 60 min), the drug precipitated in the infused vessel and adhered to the vessel walls. We believe that the same phenomena occur in humans and are responsible for at least the local toxicity reported in humans.

The fate of the precipitated bisantrene in humans is not known. We believe that it is unlikely that large amounts of drug are released from potential depot sites of precipitated material because of the virtual insolubility of the drug at pH 7.4. This speculation is supported by the fact that in no clinical study in which courses of drug were repeated every 3 to 4 weeks has there been unequivocal evidence of cumulative toxicity of bisantrene or has leukopenia been prolonged (7, 8).

Reducing the concentration of bisantrene in the infusion...
vehicle and increasing the duration of infusion into the ear veins of rabbits decreased the amount of drug recoverable from the infused vessels. The mean amounts of drug extractable from a 6-cm segment of vein immediately proximal to the site of infusion 1 hr after 260 mg/sq m infused over 1 hr (1 mg/ml), 24 hr (0.05 mg/ml), and 72 hr (0.015 mg/ml) were 2091, 134, and 7 μg. These data suggest that it may be advantageous to administer bisantrene as a dilute solution so as not to exceed its solubility in blood. Prolonged infusion may allow cytotoxic effects of bisantrene upon tumor and upon bone marrow to be achieved with little local toxicity by minimizing local precipitation and maximizing bioavailability. We are now studying the drug by 72-hr infusion in a Phase I study.

REFERENCES


Intravascular Deposition of Bisantrene

Fig. 1. Top, peripheral ear vein from a control rabbit. The vein is collapsed and contains little blood. Bottom, peripheral ear vein from a rabbit infused with bisantrene (260 mg/sq m) over 60 min. The animal was killed 1 hr after completion of infusion. The vein is congested with blood and contains bright yellow deposits of drug on the wall.
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