Cancer Chemother Pharmacol (1985) 15: 192–195

Cancer Chemotherapy and Pharmacology



# Regional targeting of Bisantrene by directed intravascular precipitation

**John S. Kovach[1], Martin Buck[1], Taiji Tsukamoto[2], Arne Odegaard[2], and Michael M. Lieber[2]**

Departments of Oncology[1] and Urology[2], Mayo Clinic and Mayo Foundation, Rochester, MN 55905, USA

**Summary.** Targeting of anticancer drugs to specific organs by directed intravascular precipitation was studied in calves using 9-10 anthracenedicarboxyaldehyde bis [(4,5-dihydro-1H-imidazol-2-yl) hydrazone] dihydrochloride (Bisantrene), a clinically active anticancer drug with limited solubility at physiological pH. Rapid injection of Bisantrene in solution at pH 4.5 into the internal iliac artery resulted in concentrations of drug in the urinary bladder wall supplied by the artery that were more than 1000 times those in the same tissue following injection of the same dose of drug IV, the route of administration used clinically. Localization of the orange fluorescent drug to the ipsilateral bladder wall was easily seen. Fluorescence microscopy revealed deposits of drug along the walls of the arteriolar and capillary bed supplied by the artery into which it had been injected. Concentrations of drug in the systemic circulation and in tissues not supplied by the internal iliac artery used for drug injection were lower after intraarterial (IA) drug administration than after IV administration. Pathological studies of the tissues of calves sacrificed at intervals up to four weeks following rapid injection into the internal iliac artery of the same doses of Bisantrene used IV in cancer patients did not reveal evidence of extensive cytotoxicity to the infused organs.

## Introduction

Poor solubility of pharmacologically active drugs generally hampers their development and use as therapeutic agents. We wished to take advantage of the poor aqueous solubility of certain anticancer drugs by selectively precipitating them in the vasculature supplying malignant tumors arising in specific regions of the body. We expected that rapid intraarterial (IA) injection of a drug poorly soluble at physiologic pH would produce locally maximal concentrations of soluble drug and establish a depot of precipitated drug within the microvasculature of the perfused organs, concurrently minimizing exposure to drug of organs outside the injected target area [10]. We chose injection of the internal iliac artery because this vessel provides the major blood supply to organs of the true pelvis (urinary bladder, prostate, uterus, ovary, and rectum). Local recurrence of malignant tumors arising in these organs after primary surgery or radiotherapy are common clinical problems, for which better methods of management are urgently needed. We studied Bisantrene dihydrochloride because this investigational drug is active against several human cancers, is poorly soluble above pH 4.5, is not metabolized, and is easily measured in blood and tissue [1, 10–12, 14–17]. We previously demonstrated in rabbits that Bisantrene precipitates in veins infused with the drug at concentrations and at rates used in many clinical trials and suggested that intravascular precipitation of drug was responsible for local toxic effects of the drug, such as thrombophlebitis [13].

## Materials and methods

*Animals.* We compared the plasma and tissue concentrations of Bisantrene after IV, and after IA administration at different rates of infusion in male Holstein calves weighing between 45 and 60 kg. The size and configuration of blood vessels in the pelvis of calves are similar to those of humans, with the first large medial branch of the internal iliac artery, the umbilical artery, supplying the dome and lateral wall of the urinary bladder in a manner similar to the distribution of the superior vesical artery in humans. Calves were anesthetized with IV pentobarbital and maintained under general anesthesia with halothane via an endotracheal tube. Each animal received the usual clinical dose of Bisantrene dihydrochloride, 260 mg/m$^2$ body surface area. The total dose was dissolved in 60 ml 5% dextrose in water, pH 4.5. The IA injections were given into the proximal right internal iliac artery, and the IV injections, into the inferior vena cava. Samples of blood for measurement of systemic plasma concentrations of drug were obtained from the abdominal aorta prior to drug administration and every 5 min for 2 h starting from the time of injection of the drug regardless of the duration of infusion, which ranged from 5 to 60 min. Tissue samples for drug measurement and histological examination were obtained from anesthetized animals killed with an overdose of pentobarbital. For studies of longer term toxicity, animals from which no biopsies were taken for examination were allowed to awaken and remain in a closed holding area for at least 24 h before being released to a research farm facility.

*Measurement of Bisantrene.* Bisantrene was measured in hydrochloric acid-ethanol extracts of plasma and tissues by measurement of fluorescence in an Aminco-Bowman

*Offprint requests to:* J. S. Kovach

spectrofluorometer with excitation at 410 nm and emission at 517 nm [4]. This assay is a modification of the procedure developed by Bachur et al. [3] for measurement of anthracyclines. As we recently reported [4], the efficiency of extraction of Bisantrene added to tissues ranged from 89% at 50 ng/ml to 97% at 0.5 ng/ml. The assay is linear from 0.5 ng/mol to 5000 ng/ml.

## Results

Except for higher initial concentrations of drug, systemic plasma concentrations of Bisantrene after rapid IA injection (5 min and 10 min) were lower than after slow IA injection (60 min) (Table 1). The concentrations of Bisantrene in tissues perfused by the arterial system into which the drug was injected were higher than the concentrations of drug in the same tissues after IV injection of the same dose at the same rate of administration. The most striking difference in the concentrations of drug in tissues examined was between that side of the urinary bladder receiving its arterial supply predominantly from the injected artery and the contralateral bladder wall, which receives its arterial supply from the opposite internal iliac artery. Slow IA injection of Bisantrene over 60 min resulted in a three-fold higher concentration of drug in the ipsilateral bladder wall and a concentration of drug that was no higher in the contralateral bladder wall compared with concentrations of drug in the bladder following IV infusion of drug over 60 min. When Bisantrene was administered IA by 10-min infusion or by 5-min infusion the concentration of drug in the ipsilateral bladder was 1000 and 4000 times greater, respectively, than after IV administration. In contrast to the bladder, there was a smaller increase in concentration of drug in the sigmoid colon and the rectus muscle after IA administration of Bisantrene than after IV injection of the drug. These tissues receive only part of their arterial supply from the internal iliac system. As expected, the concentration of Bisantrene in kidney, an or-

**Table 1.** Concentration of Bisantrene in plasma from the abdominal aorta of four calves following injection of 260 mg/m² in 60 ml 5% dextrose into the inferior vena cava over 60 min (IV-60) or into the right internal iliac artery over 60 min (IA-60), 10 min (IA-10), or 5 min (IA-5)

| | Concentration of Bisantrene (μg/ml) Route and rate of drug administration | | | |
|---|---|---|---|---|
| Time (min) | IV-60 | IA-60 | IA-10 | IA-5 |
| 5 | 0.74 | 1.01 | 4.76 | 6.82 |
| 10 | 0.93 | 1.23 | 4.40 | – |
| 15 | 1.09 | 1.40 | 2.18 | 2.30 |
| 30 | 1.25 | 1.83 | 0.90 | 0.72 |
| 45 | 1.52 | 1.53 | 0.83 | 0.67 |
| 60 | 2.80 | 2.54 | 0.74 | 0.61 |
| 90 | 0.64 | 1.11 | 0.66 | 0.55 |
| 110 | 0.65 | 0.77 | 0.44 | – |

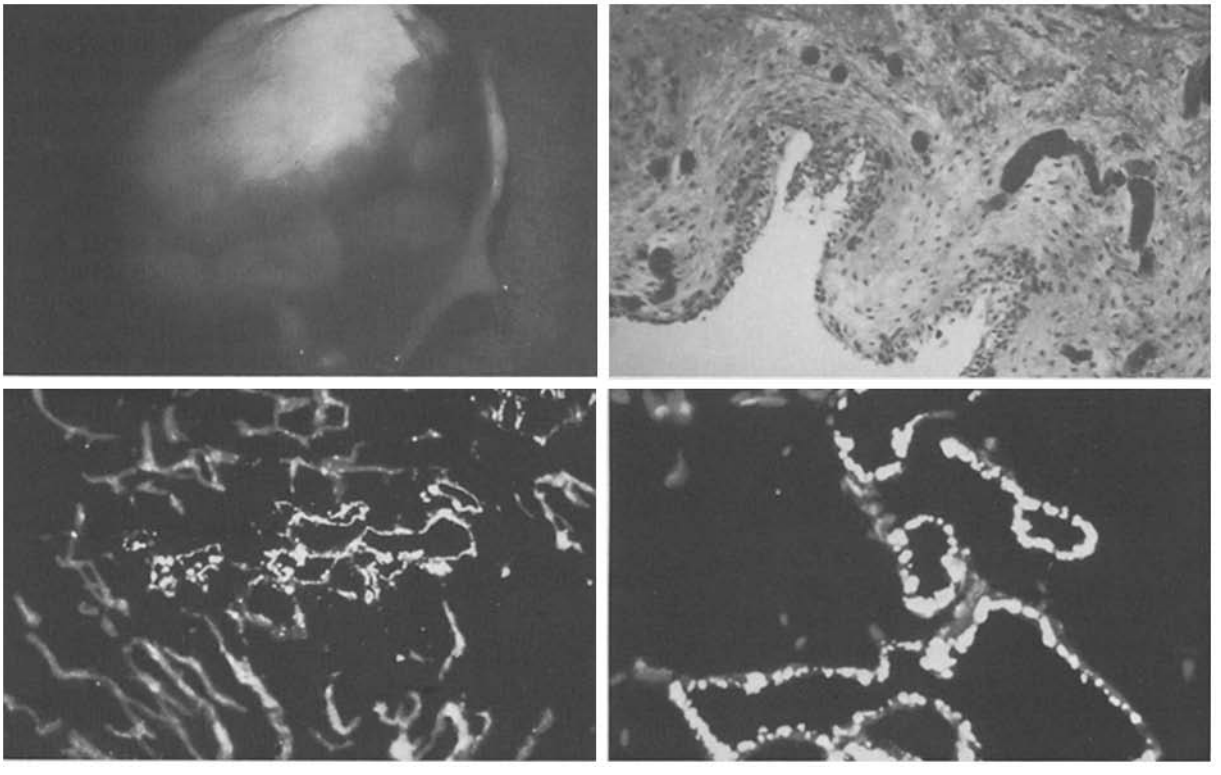

**Fig. 1.** Distribution of Bisantrene in the bladder of a calf after rapid injection of 410 mg (260 mg/m²) in 60 ml 5% dextrose and water into the right internal iliac artery. *Upper left:* The distribution of drug *(light regions)* in unfixed bladder as visualized under ultraviolet light. *Upper right:* Photomicrograph (100×) of sections of the bladder, fixed with formalin and stained with hematoxylin and eosin, reveals normal submucosal vasculature. Photomicrographs *(lower left,* 100×; *Lower right,* 400×) taken under ultraviolet light of unfixed sections of tissue taken from the fluorescent portion of the bladder reveal extensive deposition of drug in most small vessels.

**Table 2.** Concentrations of Bisantrene in tissues of four calves 2 h after the initiation of injection of 260 mg/m² in 60 ml 5% dextrose into the inferior vena cava over 60 min (IV-60) or into the right internal iliac artery over 60 min (IA-60), 10 min (IA-10), or 5 min (IA-5)

| | Concentration of Bisantrene (μg/g) Route and rate of infusion | | | |
|---|---|---|---|---|
| Tissue | IV-60 | IA-60 | IA-10 | IA-5 |
| Kidney | 128.6 | 157.6 | 160.9 | 209.5 |
| Sigmoid colon | 10.0 | 9.4 | 4.5 | 21.3 |
| Rectus muscle | 1.3 | 1.5 | 5.2 | 15.6 |
| Bladder (right side) | 2.3 | 6.9 | 2294 | 9399 |
| Bladder (left side) | 3.5 | 3.2 | 488 | 326 |
| Bladder (neck) | – | 3.7 | 116 | 753 |

gan not receiving blood from the internal iliac artery, was not altered by changes in the rate and route of drug injection (Table 2).

We expected that the greater clearance of drug associated with rapid IA injection and the high concentrations of drug within tissues perfused by the injected artery reflected intravascular precipitation of Bisantrene. The distribution of the orange fluorescent drug in the bladder was clearly demarcated under visible light and under ultraviolet light. To determine the microscopic sites of precipitation, we studied histological sections from multiple portions of the bladder obtained 2 h after administration of 260 mg/m² Bisantrene over 5 min into the right internal iliac artery. Unfixed bladder wall but not formalin-fixed bladder contained extensive deposits of highly fluorescent Bisantrene lining the endothelial walls of most small arterioles, capillaries, and venules (Fig. 1).

To look for evidence of cytotoxicity to normal structures, autopsies were performed on four calves 7, 10, 21, and 28 days following rapid unilateral internal iliac artery injection of 260 mg/m² of Bisantrene. One of these animals had weakness of the leg on the injected side upon recovering from anesthesia and subsequently developed an 8-cm ulceration of the skin over the ipsilateral gluteal region. Seven days later the right internal iliac artery was found to be occluded and there was extensive fibrosis of the right bladder wall. We could not determine whether occlusion of internal iliac artery was secondary to the trauma of cannulation of the vessel or to effects of the drug. Similarly, the scarred region of the bladder may have resulted from necrosis secondary to drug or secondary to ischemia caused by the vascular occlusion. The three other animals given identical doses of Bisantrene into one internal iliac artery had patent vessels and only mild thinning and scarring of the bladder wall. The only other pathologic abnormalities present in all animals were rare foci of renal tubular scarring.

## Discussion

Injection of drugs into arteries supplying specific regions of the body has been attempted for many years as a means of controlling the growth of a variety of human cancers, particularly those located in the extremities or in the pelvis [5, 6]. Recent efforts have focused upon enhancing the efficiency of uptake of drug in the target tissue by infusion drug at low concentrations for prolonged periods by means of portable pumps [5–7]. Alternatively, concentrated solutions of drug have been injected over short periods of time to achieve high, albeit transient, concentrations of drug in the vasculature of tumors. Regional perfusion has been combined with maneuvers designed to slow passage of drug, such as partial occlusion of the capillary bed with inert or drug-carrying microspheres [8, 9].

Regardless of the approach, the magnitude of pharmacologic advantage of IA drug delivery depends entirely upon the extent to which drug is cleared during the first pass through the target region [5, 6]. To our knowledge, the intentional precipitation of drugs within vascular channels to enhance drug clearance to specific tissues has not been used for therapeutic purposes. We believe our demonstration that high concentrations of an active anticancer drug, Bisantrene, can be achieved simply, rapidly, and without unacceptable toxicity in tissues supplied by the internal iliac artery in calves justifies evaluation of this approach in the management of some locally advanced human cancers confined to the pelvis and refractory to conventional treatment with surgery or radiotherapy. Delivery of drug by percutaneous angiographic access and rapid IA injection via one or both internal iliac arteries in humans is technically straightforward.

We believe several other anticancer drugs, beside Bisantrene, can be selectively deposited in specific organs by IA injection. Of particular interest are drugs such as hexamethylmelamine which, like Bisantrene, is poorly soluble at physiological pH but, unlike Bisantrene, is lipid-soluble, a property likely to facilitate transport form depots of precipitate across vascular walls [8, 10]. Furthermore, it may be possible to modify the solubility of other drugs so that they too can be evaluated for effectiveness when given by *directed intravascular precipitation.*

*Acknowledgements.* This work was supported by NCI grant CA 15083 D2 and The Helen C. Levitt Fund, Mayo Foundation. We thank K. E. Holley, Department of Pathology, Mayo Clinic and Mayo Foundation for the fluorescent photomicrographs and the Medical Research Division, American Cyanamid Company, Pearl River, NY for supplying Bisantrene. The current addresses of T. Tsukamoto and A. Odegaard are Department of Urology, Sapporo Medical College, Sapporo, Japan and Department of Pathology, University of Trondheim, Trondheim, Norway, respectively.

*Note added in proof.* Eight move calves were examined for toxicity 2 to 4 weeks after injection of 260 mg/m² of Bisantrene into an internal iliac artery over 10 min. Six calves had evidence of some necrosis of ladder tissue. Lower doses are being studied.

## References

1. Alberts DS, Mackel C, Pocelinko R, Salmon SE (1982) Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis [(4,5-dihydro-1H-imidazol-2-yl) hydrazone]dihydrochloride with correlative in vitro human tumor clonogenic assay. Cancer Res 42: 1170
2. Ames MM, Kovach JS (1982) Parenteral formulation of hexamethylmelamine potentially suitable for use in man. Cancer Treat Rep 66: 1579
3. Bachur NR, Moore AL, Bernstein JG, Liu A (1970) Tissue distribution and disposition of daunomycin (NSC-82151) in mice, fluorometric and isotopic methods. Cancer Chemother Rep 54: 89

4. Buck M, Kovach JS (1985) Blood and tissue concentration of Bisantrene measured by a simple fluormetric assay. Cancer Chemother Pharmacol (in press)
5. Collins JM (1984) Pharmacologic rationale for regional drug delivery. J Clin Oncol 2: 498
6. Ensminger WD, Gyves JW (1984) Regional cancer chemotherapy. Cancer Treat Rep 66: 1173
7. Ensminger W, Niederhuber J, Dakhil S, Thrall J, Wheeler R (1981) Totally implanted drug delivery system for hepatic arterial chemotherapy. Cancer Treat Rep 65: 393
8. Kato T (1984) Radiation, local and systemic chemotherapy, and new treatment modalities: In: Bladder cancer, part B. Allen R. Liss, New York
9. Kato T, Nemoto R, Mori H, Takahashi M, Tamakawa Y, Harada M (1984) Arterial chemoembolization with microencapsulated anticancer drug. JAMA 245: 1123
10. Kovach JS (1983) Pharmacokinetic studies of anticancer agents during phase I trials: In: Pharmacokinetics of anticancer agents in humans. Elsevier Science, Amsterdam, p 433
11. Peng YM, Davis TP, Alberts DS (1981) High performance liquid chromatography of a new anticancer drug, ADCA-physiochemical properties and pharmacokinetics. Life Sci 29: 361
12. Powis G (1981) Reversed-phase high-performance liquid chromatographic assay for the antienoplastic agent 9,10-anthracenedicarboxaldehyde bis (4,5-dihydro-1H-imidazol-2-yl hydrazone) dihydrochloride. J Chromatogr 226: 514
13. Powis G, Kovach JS (1983) Disposition of Bisantrene in humans and rabbits: evidence for intravascular deposition of drug as a cause of phlebitis. Cancer Res 43: 925
14. Spiegel RJ, Blum RH, Levin M, Pinto CA, Wernz JC, Speyer JL, Hoffman KS, Muggia FM (1982) Phase I clinical trial of 9,10-anthracene dicarboxaldehyde (Bisantrene) administered in a five-day schedule. Cancer Res 42: 354
15. Von Hoff DD, Myers JW, Kuhn J, Sandbach JF, Pocelinko R, Clark G, Coltman CA Jr (1981) Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis [4,5-dihydro-1H-imidazol-2-yl)hydrazone]dihydrochloride(CL216, 942). Cancer Res 41: 3118
16. Wu WH, Nicolau G (1983) Disposition and metabolic profile of a new antitumor agent: Cl 216, 942 (Bisantrene) in laboratory animals. Cancer Treat Rep 66: 1173
17. Yap BS, Yap HY, Blumenschein, GR, Bedikian AY, Pocelinko R, Bodey GP (1982) Phase I clinical evaluation of 9,10-anthracenedicarboxy-aldehyde[bis 4,5-dihydro-1H-imidazol-2-yl)hydrazone]dihydrochloride (Bisantrene). Cancer Treat Rep 66: 1517

Received January 31, 1985/Accepted March 25, 1985