Pharmacokinetics of Adriamycin and Tissue Penetration in Murine Ovarian Cancer

Robert F. Ozols,1 Gershon Y. Locker, James H. Doroshow, Karen R. Grotzinger, Charles E. Myers, and Robert C. Young

INTRODUCTION

The penetration of Adriamycin into tumor cells and the pharmacokinetics of Adriamycin i.v. and i.p. were examined in C3HHeB/FeJ mice with a transplantable ovarian cancer. Adriamycin was previously demonstrated to be effective therapy in this model only via the i.p. route. Adriamycin-specific intranuclear fluorescence was detected in ascites tumor cells within 1 min following an i.p. dose but was undetectable at any time following an equitoxic i.v. dose. Adriamycin-specific fluorescence was observed after an i.p. dose in all the tumor cells coating the diaphragm. After an i.v. dose, there was only faint and patchy intranuclear fluorescence in tumor cells coating the diaphragm. Adriamycin i.p. penetrated only into the outermost 4 to 6 cell layers of intraabdominal tumors, whereas after the i.v. route Adriamycin-specific fluorescence was observed in a patchy distribution throughout the tumor.

The visual observations were confirmed by measurement of tissue Adriamycin levels. Over a 48-hr period, the concentration of Adriamycin and its fluorescent metabolites after i.p. administration exceeded by at least 1 log the i.v. drug level in ascites fluid, with the peak i.p. level (7.5 μg/ml) almost 30 times higher than the peak i.v. level (0.26 μg/ml). In the tumor cells, the peak level was 50 times higher after an i.p. dose, and at least a 1-log differential between i.v. and i.p. levels was maintained throughout the 48 hr studied. In contrast, the peak drug levels in heart, liver, and kidney were markedly higher after the i.v. route than after the i.p. route, with ratios of 3:1 for heart and kidney and 8:1 for liver. The differential in tissue concentrations rapidly decreased, so that by 24 hr there was no difference in drug concentration between the routes in any of the three organs. These observations explain the increased efficacy of i.p. over i.v. Adriamycin therapy in murine ovarian cancer and suggest further evaluation of this mode of therapy in suitable patients with ovarian cancer.

ABSTRACT

The penetration of Adriamycin into tumor cells and the pharmacokinetics of Adriamycin i.v. and i.p. were examined in C3HHeB/FeJ mice with a transplantable ovarian cancer. Adriamycin was previously demonstrated to be effective therapy in this model only via the i.p. route. Adriamycin-specific intranuclear fluorescence was detected in ascites tumor cells within 1 min following an i.p. dose but was undetectable at any time following an equitoxic i.v. dose. Adriamycin-specific fluorescence was observed after an i.p. dose in all the tumor cells coating the diaphragm. After an i.v. dose, there was only faint and patchy intranuclear fluorescence in tumor cells coating the diaphragm. Adriamycin i.p. penetrated only into the outermost 4 to 6 cell layers of intraabdominal tumors, whereas after the i.v. route Adriamycin-specific fluorescence was observed in a patchy distribution throughout the tumor.

The visual observations were confirmed by measurement of tissue Adriamycin levels. Over a 48-hr period, the concentration of Adriamycin and its fluorescent metabolites after i.p. administration exceeded by at least 1 log the i.v. drug level in ascites fluid, with the peak i.p. level (7.5 μg/ml) almost 30 times higher than the peak i.v. level (0.26 μg/ml). In the tumor cells, the peak level was 50 times higher after an i.p. dose, and at least a 1-log differential between i.v. and i.p. levels was maintained throughout the 48 hr studied. In contrast, the peak drug levels in heart, liver, and kidney were markedly higher after the i.v. route than after the i.p. route, with ratios of 3:1 for heart and kidney and 8:1 for liver. The differential in tissue concentrations rapidly decreased, so that by 24 hr there was no difference in drug concentration between the routes in any of the three organs. These observations explain the increased efficacy of i.p. over i.v. Adriamycin therapy in murine ovarian cancer and suggest further evaluation of this mode of therapy in suitable patients with ovarian cancer.

INTRODUCTION

Systemic chemotherapy in patients with advanced ovarian cancer has produced objective response rates of 26 to 85% (11). However, most of these patients do not achieve a complete remission and as a consequence do not have a significant increase in survival. Careful restaging procedures frequently document residual i.p. tumor deposits often in the form of plaque-like surface peritoneal implants which are refractory to continued chemotherapy. This has led to alternate methods of administering active agents. The feasibility of methotrexate administered i.p. via a semipermanent Tenckhoff dialysis catheter in patients with refractory ovarian cancer has been demonstrated (15).

Adriamycin is an antineoplastic agent which might be suitable for i.p. administration since it is among the most active of agents against ovarian cancer (11). Its effectiveness i.p. in a murine ovarian cancer has been described recently (16). Adriamycin was the most active agent studied in this tumor, with 28 of 40 long-term survivors resulting from a single i.p. injection 2 days after inoculation of 10^6 tumor cells into the peritoneal cavity. Only the i.p. route was effective; Adriamycin i.v. produced neither an increase in median survival time nor any long-term survivors. We used the intrinsic fluorescence of Adriamycin as a probe to investigate the penetration of Adriamycin into free-floating ascites cells, subdiaphragmatic tumor deposits, and solid intraabdominal masses. In addition, Adriamycin levels in ascites and tumor cells were measured after i.p. and i.v. administration, as were tissue levels (heart, liver, kidney), to determine if higher intraabdominal drug levels could be obtained and if systemic toxicity could be potentially diminished by the i.p. route.

MATERIALS AND METHODS

Drugs. Adriamycin (Adria Laboratories, Wilmington, Del.) was obtained from the Investigational Drug Branch, Division of Cancer Therapy, National Cancer Institute. It was reconstituted immediately prior to use with 0.9% NaCl solution such that the volume injected was 0.01 ml/g body weight.

Murine Ovarian Cancer. The murine ovarian tumor used in this study originated from a spontaneous mouse teratoma (10) which has been maintained by serial i.p. inoculation of 10^6 cells into C3HeB/FeJ mice (The Jackson Laboratory, Bar Harbor, Maine). The growth characteristics, pattern of metastases, and response to chemotherapy have been described previously (9, 16). After i.p. transfer of tumor cells, ascites is evident within 4 to 6 days and is followed by the development of subdiaphragmatic tumor deposits and intraabdominal carcinoma leading to death within 22 to 25 days.

Tissue Preparation for Fluorescent Studies. C3HeB/FeJ mice were used 8 days after i.p. inoculation of 10^6 tumor cells to study the penetration of Adriamycin into malignant ascites cells in vivo. The i.p. injections were of an LD_{10} dose (5 mg/kg). Adriamycin i.v. was injected via the tail vein at both an LD_{10} dose (10 mg/kg) and at the 50% lethal dose for immediate toxicity (50 mg/kg). At specific times ranging from 15 sec to 48 hr following the injection of Adriamycin, 1.0 ml of ascites...
was withdrawn with a 25-gauge needle and diluted to 10 ml with iced 0.85% NaCl solution, pH 7.4 (phosphate-buffered saline). The cells were centrifuged at 1500 rpm for 15 min, and the supernatant was discarded. After 2 additional washes, the cells were suspended in 0.5 ml of buffer, and aliquots were smeared on glass slides.

To investigate the penetration of Adriamycin into solid tumor masses, animals inoculated with 10\(^6\) tumor cells underwent paracentesis at 2 and 3 weeks after transplantation. This procedure prolonged survival and allowed for the growth of larger intraabdominal masses.

The tumors varied from plaque-like deposits subdiaphragmatically to 1- to 2-mm solid tumor masses. Occasionally, the entire ovary was replaced with a solid tumor mass, up to 10 mm in diameter. In addition, at the site of the paracentesis, the left lower quadrant of the abdomen, there often occurred s.c., extravertoneal tumor masses presumably resulting from tumor cells migrating from the peritoneal cavity via the needle tract.

Animals with solid tumor deposits were given injections of LD\(_{10}\) i.v. and i.p. doses of Adriamycin. The tumors were rapidly excised and processed by one of 2 methods at specific times after drug injection. In an effort to instantaneously stop any diffusion of Adriamycin and to see if it could be detected outside the nucleus, the tissues were plunged into a beaker containing 2-methylbutane (Eastman Chemical Co., Rochester, N. Y.) which itself was immersed in liquid nitrogen (2). The tissues were then lyophilized, and the freeze-dried samples were fixed in fluorescent-free plastic (American Histolabs, Silver Spring, Md.); 6 \(\mu\)m-thick sections were then cut on a microtome. Alternatively, the tissues were rapidly frozen in a dry ice-acetone bath, and 6-\(\mu\)m sections were cut on a cryostat (8).

The slides were then examined for Adriamycin-specific fluorescence using a Zeiss fluorescence microscope with a mercury lamp light source (8, 13). A Zeiss Excitor Filter No. 1 and a Barrier Filter 53/44 were used. A x125 Zeiss objective was used for all photographs taken with Ektachrome Ed 135 film (Eastman Kodak, Rochester, N. Y.) pushed to an ASA of 400.

**Tissue Preparation for Adriamycin Levels.** C3HeB/FeJ mice were inoculated i.p. with 10\(^6\) tumor cells. After 10 days, at which time there were 4 to 6 ml ascites per animal, the mice were separated into 2 groups and Adriamycin (10 mg/kg) was administered i.p. or i.v. At 15 min, 1 hr, 6 hr, 24 hr, and 48 hr after Adriamycin administration, 3 animals from each drug group were sacrificed by cervical dislocation and compared to controls. The ascites was withdrawn with a Pasteur pipet. The heart, liver, and kidneys were removed and washed free of blood with 0.85% NaCl solution and then with glass-distilled water. Tissues were stored on ice prior to drug extraction. The ascites was centrifuged at 1500 rpm for 5 min, removed, and stored protected from light at 4\(^\circ\). The ascites cytocrit was 64.7 ± 1.4 (S.D.). The tumor cells were washed 3 times in iced 0.85% NaCl solution and twice in distilled water prior to assay.

**Adriamycin Assays.** Total tissue and ascites cell levels of Adriamycin and its fluorescent metabolites were assayed by the Donelli modification (7) of the method of Schwartz (18). While it is known that Adriamycin is metabolized in the body (4), published assays for anthracycline metabolites in tissue suffer from conditions too gentle to strip drug from tissue (1, 17) or too vigorous to maintain the metabolites in their original form (5). We therefore chose an assay for total Adriamycin and fluorescent metabolites which has been shown to reflect total drug content in tissue (18) rather than an assay for tissue metabolite(s) for which relevance to the true in vivo situation is unclear.

Individual organs to be assayed for drug content were blotted dry, weighed, and then homogenized for 20 sec in 3 ml of glass-distilled water using a Brinkmann Model PCU-2-110 Polytron. Ascites cells were suspended in 3 to 4 ml of glass-distilled water and similarly homogenized. An aliquot of the cell homogenate was set aside for protein determination by the method of Lowry et al. (14).

To 1 ml of the organ or cell homogenate, 0.3 ml of 33% AgNO\(_3\) and 0.25 ml of distilled water were added. The mixture was protected from light and shaken vigorously for 1 hr in a Dubnoff shaking water bath at 4\(^\circ\). The mixture was then centrifuged at 1500 rpm in a refrigerated centrifuge. One ml of the supernatant was then added to 3 ml of 1-butanol, shaken for 1 min, and then centrifuged for 3 min at 1500 rpm.

An aliquot of the organic phase was then removed for measurement of fluorescent intensity at 470 nm excitation and 560 nm emission in a Perkin Elmer Model MPF-44A spectrofluorometer equipped with a Houston Instrument Series 2000 omnigraph recorder. Readings were converted to Adriamycin concentration by comparison with a standard curve of Adriamycin in butanol (5 to 5000 ng/ml). Organ content of drug was expressed as ng of Adriamycin fluorescent equivalents per g of organ wet weight. Ascites cell content was expressed as ng Adriamycin equivalent per mg cell protein. In all determinations, background organ or cell fluorescence, as determined in control animals, was converted to equivalent drug levels and subtracted from the experimental results. The lower level of sensitivity of the assay was 50 ng per g organ weight or 2 ng drug per mg cell protein.

The necessity for 1 to 2 ml of mouse plasma made determinations of total Adriamycin and fluorescent metabolites in individual animals technically difficult, but ascites supernatant levels could be determined by the method of Benjamin et al. (4). Two ml of the ascites supernatant were added to 4 ml of 55% absolute ethanol in 0.45 N HCl and vortexed for 1 min. The resulting suspension was protected from light and refrigerated for 24 hr. The mixture was then centrifuged in a Beckman L5-50 ultracentrifuge for 15 min at 50,000 \(\times\) g. The supernatant was decanted and read fluorimetrically at 470 nm excitation and 560 nm emission. Readings were converted to ng of drug per ml supernatant by comparison to a standard curve of Adriamycin in acid:alcohol. Background fluorescence in ascites from control animals was converted to drug equivalents and subtracted from the experimental results. The sensitivity threshold of the assay was 50 ng drug per ml ascites.

**RESULTS**

**Fluorescence Localization Studies.** Under the conditions described, Adriamycin fluoresces yellow-orange. Tissues examined after frozen sectioning compared to after freezing in liquid nitrogen, lyophilization, and fixation in plastic had a greater distortion of underlying histology. However, with both methods, only intranuclear fluorescence was detected, and thus for routine processing the standard frozen section was adequate, since even more rapid freezing with a quenching process prolonged survival and allowed for the growth of larger intraabdominal masses. Adriamycin Assays. Total tissue and ascites cell levels of Adriamycin and its fluorescent metabolites were assayed by the Donelli modification (7) of the method of Schwartz (18). While it is known that Adriamycin is metabolized in the body (4), published assays for anthracycline metabolites in tissue suffer from conditions too gentle to strip drug from tissue (1, 17) or too vigorous to maintain the metabolites in their original form (5). We therefore chose an assay for total Adriamycin and fluorescent metabolites which has been shown to reflect total drug content in tissue (18) rather than an assay for tissue metabolite(s) for which relevance to the true in vivo situation is unclear.

Individual organs to be assayed for drug content were blotted dry, weighed, and then homogenized for 20 sec in 3 ml of glass-distilled water using a Brinkmann Model PCU-2-110 Polytron. Ascites cells were suspended in 3 to 4 ml of glass-distilled water and similarly homogenized. An aliquot of the cell homogenate was set aside for protein determination by the method of Lowry et al. (14).

To 1 ml of the organ or cell homogenate, 0.3 ml of 33% AgNO\(_3\) and 0.25 ml of distilled water were added. The mixture was protected from light and shaken vigorously for 1 hr in a Dubnoff shaking water bath at 4°. The mixture was then centrifuged at 1500 rpm in a refrigerated centrifuge. One ml of the supernatant was then added to 3 ml of 1-butanol, shaken for 1 min, and then centrifuged for 3 min at 1500 rpm.

An aliquot of the organic phase was then removed for measurement of fluorescent intensity at 470 nm excitation and 560 nm emission in a Perkin Elmer Model MPF-44A spectrofluorometer equipped with a Houston Instrument Series 2000 omnigraph recorder. Readings were converted to Adriamycin concentration by comparison with a standard curve of Adriamycin in butanol (5 to 5000 ng/ml). Organ content of drug was expressed as ng of Adriamycin fluorescent equivalents per g of organ wet weight. Ascites cell content was expressed as ng Adriamycin equivalent per mg cell protein. In all determinations, background organ or cell fluorescence, as determined in control animals, was converted to equivalent drug levels and subtracted from the experimental results. The lower level of sensitivity of the assay was 50 ng per g organ weight or 2 ng drug per mg cell protein.

The necessity for 1 to 2 ml of mouse plasma made determinations of total Adriamycin and fluorescent metabolites in individual animals technically difficult, but ascites supernatant levels could be determined by the method of Benjamin et al. (4). Two ml of the ascites supernatant were added to 4 ml of 55% absolute ethanol in 0.45 N HCl and vortexed for 1 min. The resulting suspension was protected from light and refrigerated for 24 hr. The mixture was then centrifuged in a Beckman L5-50 ultracentrifuge for 15 min at 50,000 \(\times\) g. The supernatant was decanted and read fluorimetrically at 470 nm excitation and 560 nm emission. Readings were converted to ng of drug per ml supernatant by comparison to a standard curve of Adriamycin in acid:alcohol. Background fluorescence in ascites from control animals was converted to drug equivalents and subtracted from the experimental results. The sensitivity threshold of the assay was 50 ng drug per ml ascites.

**RESULTS**

**Fluorescence Localization Studies.** Under the conditions described, Adriamycin fluoresces yellow-orange. Tissues examined after frozen sectioning compared to after freezing in liquid nitrogen, lyophilization, and fixation in plastic had a greater distortion of underlying histology. However, with both methods, only intranuclear fluorescence was detected, and thus for routine processing the standard frozen section was adequate, since even more rapid freezing with a quenching...
agent was unable to localize Adriamycin in an extranuclear cellular compartment in the tissues studied.

In the free-floating ascites cells, Adriamycin-specific fluorescence was detected in the nuclei 60 sec after a 5-mg/kg i.p. dose. By 3 hr (Fig. 1), the fluorescence was maximum and persisted for 48 hr. After an equitoxic i.v. dose (10 mg/kg), intranuclear fluorescence was not detected at any time point up to 48 hr. Only with a massive i.v. dose (50 mg/kg), which was an acute 50% lethal dose, could Adriamycin-specific intranuclear fluorescence be observed.

The localization of Adriamycin into solid metastatic ovarian tumors in 24 mice was then studied. The diaphragms of 10 mice were examined at various times (ranging from 15 min to 48 hr) after being injected with equitoxic i.v. and i.p. doses of Adriamycin. Fig. 2 depicts a diaphragm coated with tumor cells 30 min after an i.p. dose of Adriamycin. All of the cells coating the diaphragm, as well as some of the tumor cells which have penetrated between the muscle cells via the lymphatics, have intense Adriamycin-specific fluorescence in the nuclei. The nuclei of the muscle cells themselves also have intranuclear Adriamycin-specific fluorescence, but it is of less intensity than that in tumor nuclei.

This is in contrast to the fluorescence observed after an equitoxic i.v. dose (Fig. 3). Here, there is only faint Adriamycin-specific fluorescence in the nuclei of tumor cells adherent to the diaphragm, while the fluorescence in the nuclei of the muscle cells is of greater intensity. The minimal and patchy fluorescence in tumor cells coating the diaphragm was observed at all times (15 min, 1 hr, 3 hr, 24 hr, and 48 hr) after an i.v. injection.

In contrast to the uniform distribution of Adriamycin into the tumor nuclei coating the diaphragms, only the outermost layers of intraabdominal tumors in 6 mice demonstrated intense Adriamycin-specific fluorescence after an i.p. dose. Fig. 4 depicts the penetration of Adriamycin into the outermost 4 to 6 cell layers of a 0.25-cm tumor attached to the mesentery 4 hr after an LD_{10} i.p. dose. After an i.v. dose (Fig. 5), fluorescence was observed in a patchy distribution within the core of similar intraabdominal tumors; however, the intensity of fluorescence after Adriamycin i.v. was not as great as observed in the outermost cell layers of tumors following Adriamycin i.p.

In extraperitoneal tumors, arising at the site of paracentesis, faint, patchy Adriamycin-specific fluorescence was observed after Adriamycin i.v. (Fig. 6). There was less observable fluorescence in similar extraperitoneal tumors examined after an equitoxic i.p. dose of Adriamycin.

In contrast to the weak and patchy distribution of intranuclear fluorescence in intraabdominal and extraperitoneal tumors following Adriamycin i.v., there was uniform and intense fluorescence in all heart muscle nuclei examined at the same time as the tumors (Fig. 7). An equitoxic i.p. dose (which was one-half the i.v. dose) resulted in markedly decreased intranuclear fluorescence in heart muscle nuclei examined in the first 24 hr after injection.

Adriamycin Levels. Chart 1 shows the concentration of Adriamycin and its fluorescent metabolites in ascites fluid after 10 mg/kg administered either i.p. or i.v. For every time point, the concentration after i.p. administration exceeds by at least 1 log the i.v. drug levels in ascites fluid. The peak i.p. drug level of 7.5 μg/ml is almost 30 times higher than the peak i.v. level (0.28 μg/ml). For both modes of administration, there was a sharp initial drop in drug concentration over the first 6 hr, with a suggestion of a plateau phase thereafter.

The difference in Adriamycin concentration in the ascites tumor cells between i.p. and i.v. administration was also great (Chart 2); peak levels after i.p. administration were 50 times greater than after the i.v. dose, and at no point measured within the first 48 hr was the difference any less than 1 log. There was no suggestion of a late plateau of drug levels in the cells after i.p. administration; drug concentration in tumor cells decreased steadily after the first 6 hr.

In marked contrast to the situation in ascites and tumor cells are the differences found in drug levels between i.v. and i.p. administration in heart, liver, or kidney. In the 3 organs examined, peak drug levels were markedly higher after the i.v. route than after the i.p. route with ratios of 3:1 for heart (Chart 3).
and kidney (Chart 4) and 8:1 for liver (Chart 5). While this differential in tissue concentrations was maintained at early time points, by 24 hr there was no difference in drug concentration between the routes in any of the 3 organs. The decline of the concentration differential with time seems to result from a steady drop off of tissue levels after i.v. administration while the levels after i.p. administration are either rising or plateauing.

**DISCUSSION**

Factors which determine the responsiveness of many human ovarian cancers to Adriamycin i.v. are not known. In a previous study, it was demonstrated that 70% of C3HeB/FeJ mice treated with a single LD<sub>0</sub> i.p. dose of Adriamycin 2 days after i.p. inoculation with 10<sup>6</sup> tumor cells could be cured whereas Adriamycin i.v. had no effect on survival (16). That observation suggested that the failure to respond may be a function of the local drug concentration. The present study compared the penetration of Adriamycin into the nuclei of tumor cells following i.v. and i.p. administration. The pharmacokinetics of i.v. and i.p. Adriamycin, the latter being administered into a large volume (4 to 5 ml) of ascites fluid, were also studied. Such large-volume i.p. administration (either in ascites already present or in 2 to 3 liters of dialysis fluid) has been suggested as the optimal means of administering drugs to treat i.p. cancers in humans to ensure maximal exposure to high concentrations of agent (6).

The results of our studies reveal that, after i.p. administration, Adriamycin can be rapidly localized in the nuclei of free-floating ascites cells, in the 4- to 6-cell-layer-thick tumor deposits coating the diaphragms, and in the outermost layers of solid i.p. tumor masses. After equitoxic i.v. administration, on the other hand, only faint fluorescence was visualized in the solid tumor masses, and none was seen in the ascites cells. These visual observations were confirmed by the actual measurement of Adriamycin levels in the tissues. There were markedly higher concentrations of drug in tumor cells and ascites fluid following i.p. administration. The greater than 10-fold difference in tumor cell concentration between i.p. and i.v. drug is constant over 48 hr and suggests a therapeutic advantage to the i.p. route whether it is drug concentration or (concentration x time) drug levels which correlate with tumor kill. It was also demonstrated previously that Adriamycin i.p. produced a greater suppression of DNA synthesis in murine ovarian tumor cells than did the i.v. drug (16). Those findings coupled with the actual Adriamycin drug levels and intranuclear localization studies provide a reasonable explanation for the greater efficacy of Adriamycin i.p. in murine ovarian cancer.

The lack of intense intranuclear fluorescence past the outermost 4 to 6 cell layers of solid intraabdominal ovarian tumor masses in C3HeB/FeJ mice following i.p. administration of Adriamycin suggests that this mode of therapy may be more beneficial if bulky abdominal disease is not present. It is probable, however, that multiple factors including vascularity and extent of tumor necrosis, as well as tumor size and drug diffusion, will determine the optimal mode for the delivery of drug to ovarian cancer cells.

A second rationale for the i.p. administration of Adriamycin rests on the assumption that the drug levels attained in normal
organs would be lower than after i.v. administration, and such lower levels would lead to lessened toxicity. The current study shows a marked difference in peak tissue levels between the 2 routes of administration, but little or no difference by 24 hr. Thus, the i.p. route is likely to lead to lessened host toxicity if peak rather than sustained low drug concentrations are an important determinant of toxicity. There is evidence that in the bone marrow of rabbits toxicity may correlate with high initial drug concentrations (12). Furthermore, in humans there are now reports of lessened cardiotoxicity of i.v. drug when given in frequent low doses rather than in intermittent high bolus doses (19). Frequent low-dose regimens have also been associated with lower peak cardiac drug levels in animal models.

The usefulness of Adriamycin i.p. in humans may be limited by a potentially significant problem, local toxicity (acute peritonitis or chronic peritoneal fibrosis). Extravasation of Adriamycin into soft tissues can lead to a severe cellulitis and necrosis. Such a reaction in the peritoneum would make i.p. Adriamycin into soft tissues can lead to a severe cellulitis and necrosis. Such a reaction in the peritoneum would make i.p. administration of the drug impossible in humans. Although the mice in our studies had no apparent acute local toxicity, this does not preclude the possibility of later side effects. In humans, not all tissues seem equally sensitive to the drug; Adriamycin has safely been instilled in the bladder with minimal toxicity (3). It is not yet known whether the peritoneum will react to local high concentrations of Adriamycin in a manner analogous to that of soft tissue or of the bladder.

The results of our studies reveal that the i.p. administration of Adriamycin has certain pharmacokinetic advantages over the i.v. route and so may be a reasonable candidate for therapeutic instillation into the abdomen. Such instillations have been shown to be practical in humans (15) using methotrexate, an agent with less efficacy against ovarian cancer than Adriamycin has (11). If resistance to anthracyclines is concentration dependent and Adriamycin can be practically administered i.p. to humans, then another useful modality will have been added to the treatment of a difficult tumor.

REFERENCES


Figs. 1 to 7. Fluorescent photomicrographs of ascites cells and frozen sections of tissues from Adriamycin-treated C3HeB/FeJ mice with ovarian cancer. All magnifications, X 250.

Fig. 1. Ascites tumor cells 3 hr after Adriamycin (5 mg/kg i.p.)

Fig. 2. Penetration of Adriamycin into the plaque-like tumor deposits (arrow) coating the diaphragm 30 min after a 5-mg/kg i.p. dose of Adriamycin. The muscle cells of the diaphragm, below the tumor deposit, have an intrinsic greenish yellow fluorescence.

Fig. 3. Same as Fig. 2 except the Adriamycin was given i.v. There is minimal fluorescence in the nuclei of tumor cells coating the diaphragm while the nuclei of the muscle cells (arrow) have bright intranuclear fluorescence.

Fig. 4. Solid intraabdominal tumor 1 hr after Adriamycin i.p. The fluorescence is only in the outermost 4 to 6 cell layers (arrow). The bulk of the tumor (right lower corner) has no Adriamycin-specific intranuclear fluorescence.

Fig. 5. Solid intraabdominal tumor 30 min after Adriamycin i.v. There is Adriamycin-specific fluorescence in the core of the tumor.

Fig. 6. Extrapitoneal tumor 30 min after Adriamycin i.v. The intranuclear fluorescence is weak and patchy.

Fig. 7. Heart muscle nuclei demonstrating bright intranuclear fluorescence 15 min after an i.v. dose of Adriamycin.
Pharmacokinetics of Adriamycin and Tissue Penetration in Murine Ovarian Cancer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/39/8/3209

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.