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# Effects of intralesional injection of cisplatin dissolved in urografin and lipiodol on Ehrlich ascites tumor and normal tissues of CD-1 mice

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**Abstract.** The response of Ehrlich ascites tumor and the effect on normal tissues (kidney and small intestine) of CD-1 mice were evaluated after intralesional (i.l.) injection of cisplatin dissolved in urografin and lipiodol, which is henceforth termed CUL suspension. The results obtained were compared with the effects of i.p. and i.l. injections of cisplatin dissolved in sterile distilled water. Each of these treatment modalities involves the injection of 10 mg/kg cisplatin. The tumor response was evaluated by tumor growth-delay studies as well as by determining the percentage of cells in the S phase. Toxicity studies were accomplished by evaluation of the change in the body weight of mice and also by S-phase studies. S-phase fraction analyses were done with the use of the Cell Proliferation Kit. This commercial kit was used to measure bromodeoxyuridine (BrdU), a thymidine analogue that is incorporated into cells synthesizing DNA. Tumor, kidney, and small-intestine platinum concentrations were determined by measurement with a flameless atomic absorption spectrophotometer. The results of the tumor growth-delay studies showed that i.p. injection, with water being the drug carrier, produced the weakest antitumor effect, whereas i.l. injection of cisplatin, with lipiodol being the drug carrier, evoked the most enhanced effect. This finding was substantiated by BrdU-uptake analysis of tumor cells, wherein i.p. injections yielded the highest S-phase fraction and CUL treatment gave the lowest. Toxicity studies showed that a very significant decrease in body weight occurred in mice receiving i.p. treatment. No significant decrease in body weight was noted after i.l. treatment. BrdU analysis revealed that DNA synthesis in kidney cells and crypt cells of the small intestine was depressed after i.p. treatment. On the other hand, no significant effect was observed in the kidney or small intestine of CUL-treated mice. A correlation between the effects of the various treatment modalities (on tumors, kidney, and small intestine) and the retention of cisplatin was found.

**Key words:** Cisplatin — Iodized oil — Ehrlich-ascites tumor

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## Introduction

Since the disclosure by Rosenberg et al. [36] of its antitumor activity, cisplatin has achieved recognition in the clinical management of various malignant neoplasms, notably those of the ovary [7], the testis [8], and the head and neck [4, 42]. However, it has been shown that the most serious toxic side effects in animals and humans involve damage to the renal tubules [10, 40] and impairment of the gastrointestinal epithelium [5, 24].

In efforts to dispense with these toxic effects while delivering sufficiently high concentrations of the drug to the tumor, different methods of application have been attempted. A group of investigators attempted effective delivery by treating tumor-bearing animals with cisplatin bound to tumor-specific antibody [37]. Some authors attempted to make use of liposomes [39], purified bovine collagen [15], and polymer-coated albumin microspheres [41] as drug-carrier systems for cisplatin in efforts to reduce the drug's nephrotoxicity while preserving its antitumor activity. More recent reports revealed the use of iodized oil [1, 18, 20, 21], a lipid lymphographic contrast medium, as a drug carrier. Another means of preserving the activity of a drug while limiting its diffusion into the systemic circulation is intralesional (i.l.) injection. There are several reports of at least partial success in the treatment of various forms of human and animal cancers with i.l. injection of cisplatin [3, 15, 22, 44].

As an alkylating agent, cisplatin shows cytotoxic activity against a variety of tumors and normal tissues in humans and animals by inhibiting the synthesis of DNA in

cells [16, 33, 35, 45]. Traditionally, monitoring of DNA synthesis in individual cells has involved the use of [$^3$H]-thymidine and autoradiography for the identification of proliferating cells or cell populations. More recently, an alternative rapid, nonradioactive, less technologically dependent method has been developed in which 5-bromo-2′-deoxyuridine (BrdU), a pyrimidine analogue of thymidine, is incorporated into replicating DNA and is subsequently localized using a specific monoclonal antibody [11]. By staining for BrdU incorporation, quick and reliable measurement of the percentage of cells in the S phase can be demonstrated by immunocytochemical techniques [9, 30, 31]. The BrdU labeling index (LI) obtained provides the means of assessing the proliferative activity and therapeutic response of tumors and normal tissues [12, 19]. A commercial kit (Cell Proliferation Kit, Amersham International plc, UK) for BrdU labeling is now available that facilitates the incorporation of this method into routine pathology by providing standardized reagents and instructions.

The objective of this paper is to describe the results of in vivo studies on the effects of i.l. injection of cisplatin dissolved in urografin and lipiodol on Ehrlich ascites tumors and normal tissues (kidney and small intestine) of CD-1 mice. The results of the studies were compared with the effects of i.l. and i.p. injection of cisplatin dissolved in sterile distilled water. The therapeutic effect was assessed by observed changes in tumor volume as well as losses in mouse body weight. The effect of the drug on DNA synthesis in the tumor, kidney, and small intestine was also investigated with the use of a commercial kit for BrdU labeling, and attempts were made to correlate the observed effects with the uptake and retention of cisplatin in the various tissues studied. Tissue platinum concentrations were determined by atomic absorption spectrophotometry, a technique that has high sensitivity and specificity [3, 6, 15, 22, 24, 25, 27].

Lipiodol was chosen as a drug carrier for these studies not only because it has been found to remain selectively in the tumor for extended periods but also because it does not cause serious complications, especially when given at very minimal doses [1, 18, 20, 21].

## Materials and methods

*Tumor and mice.* The Ehrlich ascites tumor, a transplantable undifferentiated carcinoma of mice, was grown in 6-week-old male CD-1 mice (Charles River, Japan). The growth and the cytokinetic properties of the Ehrlich ascites cells used have been described elsewhere [34]. In vivo, ascites tumors were maintained by i.p. transplantation of about $5\times10^6$ cells from mouse to mouse every 7 days. The tumor was implanted in the gastrocnemius muscle of the right hindleg of each mouse by inoculation of $2\times10^6$ cells in 0.05 ml phosphate-buffered saline (PBS). The mice were kept in transparent plastic boxes with dry, soft wood shavings, were fed the normal diet, and had access to tap water ad libitum. They were housed at four to six mice per cage and maintained in an air-conditioned room with controlled temperature (23° C) and light (12 h; from 8:00 a.m. to 8:00 p.m.). All treatments were initiated 6 days after tumor implantation. For determination of the tumor size, the injection volume was taken into account such that the relative size of the tumor could be established.

*Reagents.* Cisplatin (Randa, Nihon Kayaku, Tokyo, Japan) was supplied in powder form. The aqueous contrast medium (Urografin 60%, Japan Schering, Osaka, Japan) and oily contrast medium (Lipiodol Ultra-Fluide, Laboratoire Guerbet, France) were used to dissolve cisplatin and produced a water-in-oil suspension (CUL).

*Preparation of the CUL suspension.* The methods of Araki et al. [1] and Kanematsu et al. [18] were partly adopted to mix cisplatin (a water-soluble antitumor agent) with urografin and then mix this combination with lipiodol to produce the CUL suspension. For this purpose, the cisplatin crystals were placed in an agate mortar. Urografin was added and ground well with a pestle. The solution was then mixed with lipiodol and ground again, and the final mixture was subjected to ultrasonic treatment for at least 3 min. Usually, 5 mg cisplatin was dissolved in 0.29 ml urografin and 1.14 ml lipiodol (for determination of tissue platinum concentrations, 2.5 mg cisplatin was mixed with the same volumes of urografin and lipiodol used before). The suspension showed a yellowish homogeneous color and remained so even on standing.

For preparation of the *aqueous suspension*, cisplatin was mixed with sterile distilled water using a mortar and pestle and the mixture was subjected to ultrasonic treatment for at least 3 min. All drug preparations were done immediately prior to use.

*Drug treatments.* Cisplatin, urografin, and lipiodol were admixed in appropriate concentrations such that the derived doses were delivered in an injection volume of 0.10 ml (i.l. and i.p. injections of aqueous suspension were also delivered in a volume of 0.10 ml for purposes of direct comparison). Calculations of the cisplatin dose in milligrams of drug per kilogram of body weight were based on the average weight of the mice used in the experiment.

Drug delivery was performed with a 26-gauge needle on a 1.0-ml syringe. For each i.l. injection a "fan" pattern was used, whereby the suspension was expressed as the needle was slowly withdrawn, which facilitated drug distribution throughout the tumor. The needle was left in place for at least 30 s to limit backflow of the drug from the injection sites.

*Tumor growth-delay assay.* These animals were divided into seven groups and were classified according to the therapeutic modality applied: the control (no treatment) group, the i.p. (distilled water only) group, the i.l. (distilled water only) group, the i.l. (urografin and lipiodol only) group, the i.p. (aqueous suspension) group, the i.l. (aqueous suspension) group, and the i.l. (CUL suspension) group. From the day of treatment (day 0) until day 20, tumor volume measurements were accomplished. The growth of tumors was determined by the measurement of tumors with vernier calipers every other day. The tumor volumes were calculated using the following formula: volume = length × width × thickness (mm) × $\pi/6$. The time in days required for the tumors to grow to 3 times their volume at day 0 was used as a measure of treatment effectiveness.

*Effects of each treatment modality on the body weight of mice.* The animals used in the tumor growth-delay assay were also used to determine the effects of each treatment on body weight. From the day of treatment (day 0) until day 20, the body weight of mice was measured every other day. The observed increase or decrease in body weight was determined in relation to the weight recorded at the start of treatment (the weight of mice at day $n$ minus the weight of mice at day 0).

*Determination of platinum concentrations in tumor, kidney, and small intestine.* Tumor-bearing mice were randomized into three groups: the i.p. (aqueous suspension) group, the i.l. (aqueous suspension) group, and the i.l. (CUL suspension) group. Each mouse received a dose of 5 mg/kg cisplatin.

At selected times (5, 15, and 30 min; 1, 2, 4, 8, 16, and 24 h; and 3, 6, and 12 days) after treatment, three mice per group were killed and tumor, kidney, and small-intestine specimens were collected for platinum analysis. Each sample was weighed [typical samples taken weighed 0.3–1.0 g (wet wt.)], minced with scissors, and transferred to a Pyrex tube. It was then liquefied and partly digested by heating (80° C, 5 h) with 3 ml $HNO_3$. Glass tubes were allowed to cool, and the clear, plate-yellow aqueous portion of the hydrolysate was separated

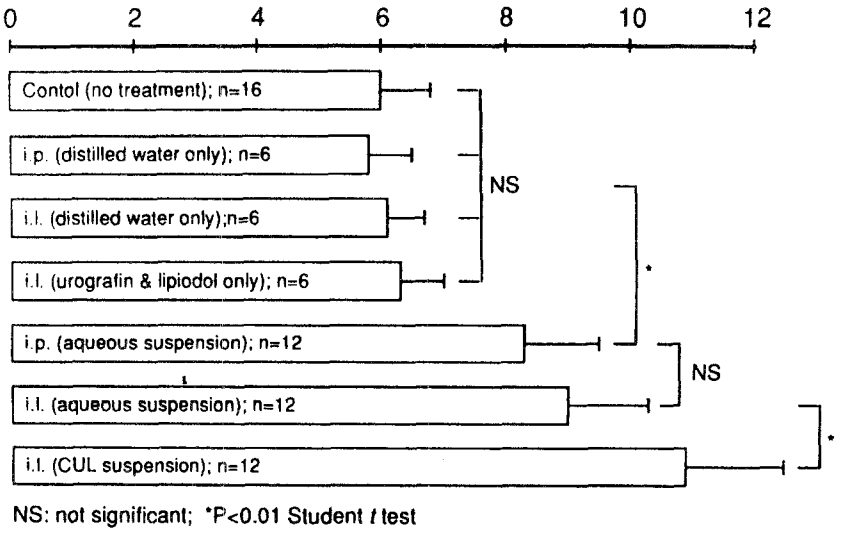


**Fig. 1.** Number of days required for Ehrlich ascites tumors to reach 3 times the size measured at initiation of treatment with different modalities

from a fatty phase that hardened into a light-colored wax-like solid upon cooling. This fatty phase (residual lipid) was discarded. Then, 3.5 ml NaOH (40%) and 2.0 ml $Na_2CO_3$ (25%) were added, the combination was mixed, and the pH was adjusted to 7 ~ 9. Next, 300 mg $(C_2H_5)_2NCS_2Na \cdot 3H_2O$ was added, heating was resumed (80° C, 1 h), and the mixture was then allowed to cool. The sample solution was transferred to a volumetric flask, after which 3 ml of chloroform was added, the flask was shaken, and the chloroform was retrieved and placed in an evaporating flask. The addition and retrieval of chloroform was done three times. The chloroform was then evaporated to dryness (using a rotary evaporator). The residue was dissolved in a small volume of 0.5 ml methanol, and 10 µl of the solution was injected into a Hitachi (Model 170-70) Zeeman-effect atomic absorption spectrophotometer. The equipment was operated under standard conditions and met the manufacturer's specification.

*BrdU assay.* The Cell Proliferation Kit includes the following: (1) labeling reagent (a concentrated aqueous solution of BrdU and 5-fluoro-2′-deoxyuridine; 10:1 ratio), (2) nuclease, (3) anti-BrdU monoclonal antibody (of murine origin, subclass IgG2a), (4) peroxidase anti-mouse IgG2a (concentrate), (5) peroxidase anti-mouse IgG2a (diluent, of caprine origin), (6) substrate/intensifier [containing an aqueous solution of hydrogen peroxide required for the peroxidase-catalyzed polymerization of DAB (see below) together with cobalt chloride and nickel chloride to intensify and increase the contrast of the resulting stain], and (7) 3,3′-diaminobenzidine tetrahydrochloride (DAB).

Mice were divided into four groups: the control (no treatment) group, the i.p. (aqueous suspension) group, the i.l. (aqueous suspension) group, and the i.l. (CUL suspension) group. At 24 h after the administration of the different treatment modalities, 1 ml labeling reagent/100 g body weight (equivalent to about 30 mg/kg) was injected i.p. into treated and control mice. After 1 h the mice were killed by cervical dislocation. The tumor, kidney, and small intestine were removed and fixed in 70% ethanol for at least 6 h (but no longer than 12 h), after which they were processed according to standard pathology methods. The tissues were then embedded in paraffin and cut in 4-µm-thick sections, which were mounted on poly-L-lysine-coated glass slides and then dried overnight at 37° C (to improve adhesion of the sections to the slides). After deparaffinization with xylene, to inactivate endogenous peroxidase the sections were immersed in 1% $H_2O_2$ in methanol for 30 min and washed three times with phosphate buffer. After inactivation of endogenous peroxidase the sections were stained by an immunocytochemical procedure. In brief, specimens were covered with anti-BrdU antibody, incubated for 1 h, and washed three times with PBS. Next, sufficient peroxidase anti-mouse IgG2a to cover each specimen was applied for 30 min, the specimens were again washed three times with PBS, and the slides were immersed in DAB staining solution for 10 min. Finally, the slides were lightly counterstained with hematoxylin. All of these procedures were done at room temperature.

To ensure confidence in interpretation of the experimental results, "negative" controls consisting of the following were included: (1) immunocytochemical staining of tissues (tumor, kidney, and small intestine) of mice that had not been exposed to BrdU and (2) immunocytochemical staining of tissue sections of mice that had not been treated with cisplatin but had received i.p. injections of the BrdU labeling reagent; in these cases the anti-BrdU monoclonal antibody had been omitted and substituted with PBS.

An Argus-10 Image Processor (Hamamatsu Photonics K.K., Hamamatsu City, Japan) was used for cell counting. This equipment includes analog contrast enhancement, simultaneous background subtraction with averaging, and digital contrast manipulation among its many applications, which makes it the ideal system for use as a stand-alone processor or a preprocessor for quantitative image analysis. Counting of BrdU-labeled and unlabeled cells was easily accomplished by use of a computer mouse.

All counts were performed at ×400 magnification and the BrdU labeling index (LI) was expressed as the ratio of the total number of BrdU-labeled cells to the total number of cells counted and was recorded as a percentage. In the calculation of the mean BrdU LI for the various tissues, viable-appearing cells (cells with intact cellular and nuclear membranes) were counted in three to five microscopic fields from well-labeled areas. In the corresponding calculation for tumors, approximately 1000 tumor cells were counted. In the kidney, the proportion of labeled to unlabeled cells was determined for each animal on samples of approximately 1000 ~ 3000 cells from each of two regions, the renal corpuscle and the remainig part of the nephron tubule. For the small intestine, only crypts cut through the longitudinal axis were considered, and the LI was calculated as the ratio of the number of BrdU-labeled cells to the total number of cells in ten crypts.

*Statistical analysis.* Differences between groups were evaluated using Student's *t*-test. The differences were considered significant at $P$ values of $<0.05$.

## Results

### *Tumor growth-delay assay*

The results of the tumor growth-delay assay are summarized in Fig. 1. The differences among the control groups

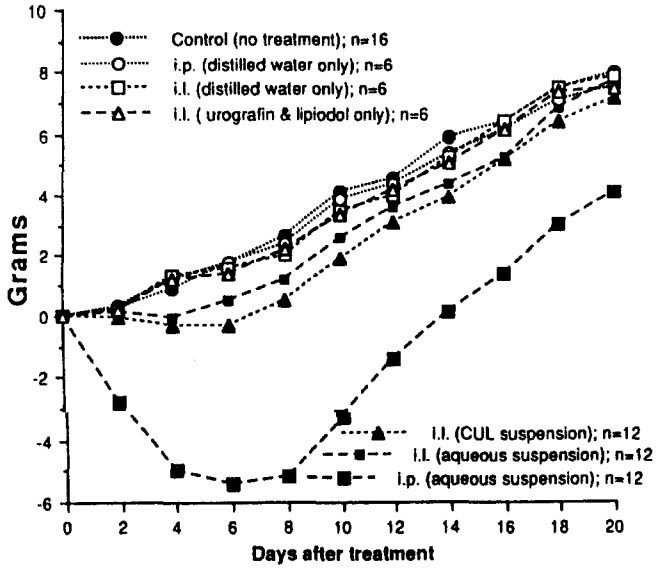


Fig. 2. Mean increase or decrease observed in the body weight of CD-1 mice after bolus injection of 10 mg/kg cisplatin by different treatment modalities

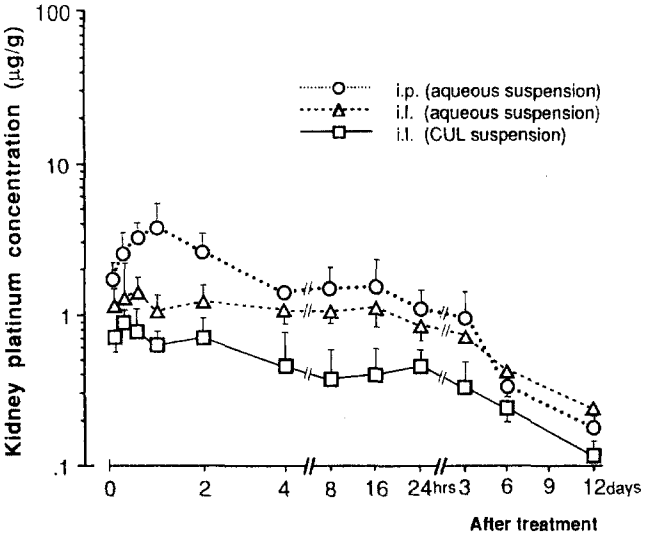


Fig. 4. Mean total platinum concentrations measured in kidneys of Cd-1 mice after bolus injection of 5 mg/kg cisplatin by different treatment modalities. Each point represents the mean value for 3 samples

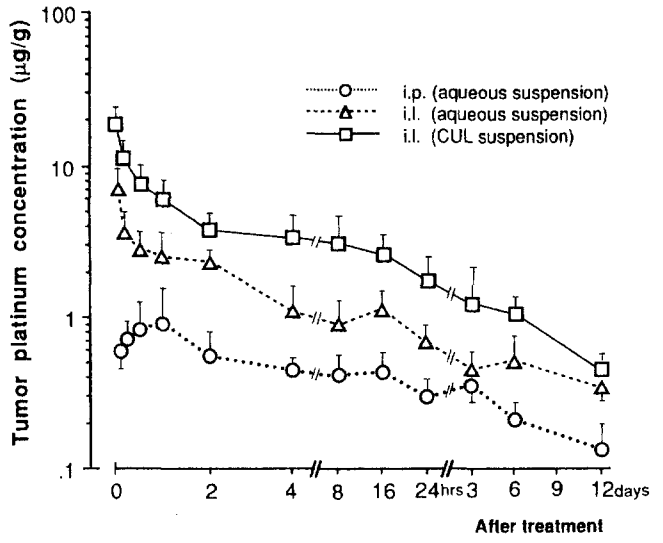


Fig. 3. Mean total platinum concentrations measured in Ehrlich ascites tumor tissues after bolus injection of 5 mg/kg cisplatin by different treatment modalities. Each point represents the mean value for 3 samples

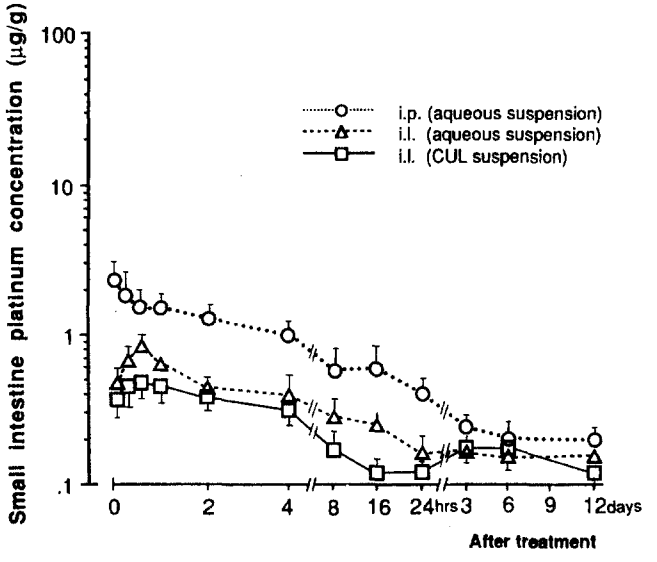


Fig. 5. Mean total platinum concentrations measured in small intestines of CD-1 mice after bolus injection of 5 mg/kg cisplatin by different treatment modalities. Each point represents the mean value for 3 samples

were not significant. Slight enhancement of the tumor response was observed for the i.p. (aqueous suspension) group. However, no significant difference was noted between the i.p. and i.l. (aqueous suspension) groups. Treatment with the CUL suspension caused the longest growth delay.

*Body weight loss*

No loss in body weight was observed among the control groups (Fig. 2). Only a slight decrease in the body weight of mice was noted at 2~4 days after i.l. injection of either aqueous or CUL suspension. However, after i.p. dosing an accelerated decrease in weight occurred that lasted for up to 6 days and showed signs of recovery on days 8~20.

*Analysis of platinum concentrations in tumor, kidney, and small intestine*

The mean platinum concentration measured in Ehrlich ascites tumors at 1 h after i.l. injection of the CUL suspension was about 3 times higher than that measured in the i.l. (aqueous suspension) group and about 7 times higher than that detected in the i.p. treated group (Fig. 3). After 12

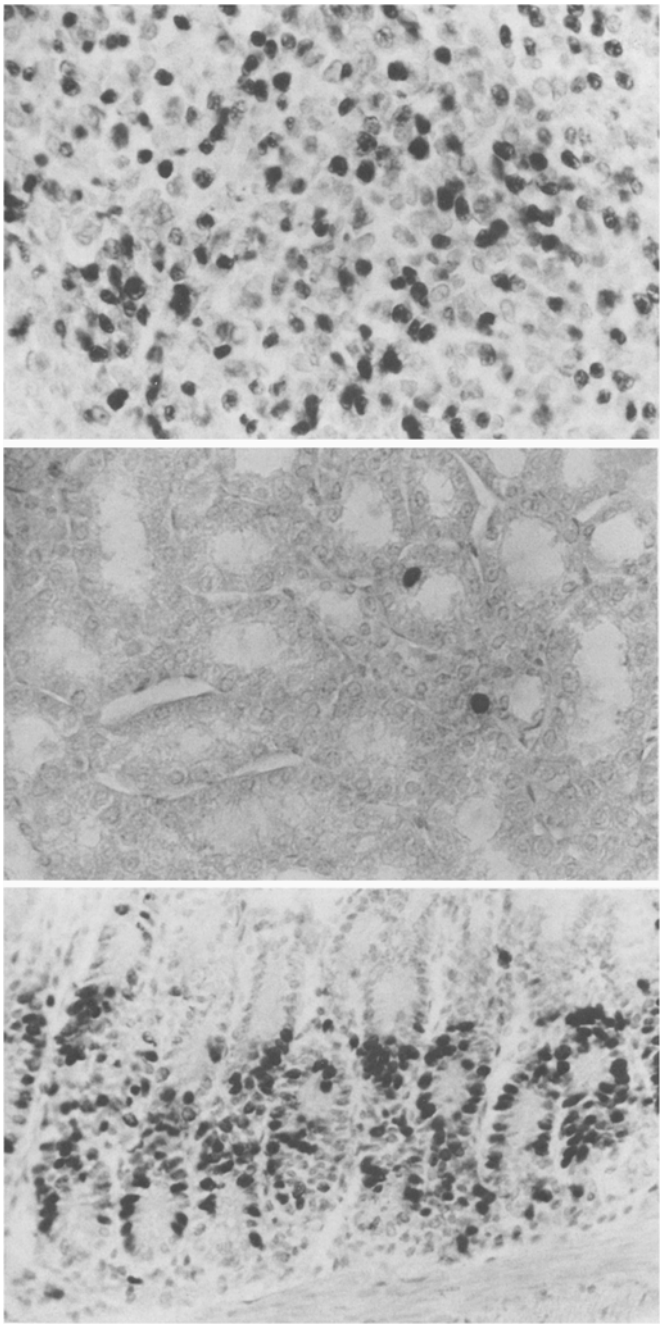

**Fig. 6A–C.** Photomicrographs of immunocytochemically stained sections of **A** Ehrlich ascites tumor, **B** kidney tissue, and **C** small-intestine tissue taken from untreated (control) CD-1 mice

days, the mean platinum concentration measured in CUL-treated tumors was 1.38 and 3.33 times higher than that detected in the i.p. and i.l. (aqueous suspension) group, respectively. The difference between the tumoral platinum concentration detected in CUL-treated mice versus the i.p. and i.l. (aqueous suspension) groups was always statistically significant.

For kidney (Fig. 4) and small-intestine (Fig. 5) platinum concentrations, the results showed that the levels measured in the i.p. (aqueous suspension) group were almost always higher than those detected in the other groups, the exception being the kidney concentrations recorded at 6 and 12 days after i.l. injection of the aqueous suspension. On the other hand, the concentrations observed in CUL-treated mice were always lower than those recorded for the i.p. group and were also usually lower than those measured in the i.l. (aqueous suspension) group, the exception being the levels measured in the small intestine on days 3 and 6 after treatment.

**Table 1.** BrdU LI for Ehrlich ascites tumors according to treatment modality

| Group | Treatment modality | Mice ($n$) | BrdU LI (mean ± SD) |
|---|---|---|---|
| I | Control (no treatment) | 6 | 32.80% ± 3.78% |
| II | i.p. (Aqueous suspension) | 4 | 18.71% ± 1.69% |
| III | i.l. (Aqueous suspension) | 4 | 15.69% ± 2.78% |
| IV | i.l. (CUL suspension) | 4 | 11.02% ± 2.66% |

All differences are significant except for that between groups II and III

**Table 2.** BrdU LI for kidneys of CD-1 mice according to treatment modality

| Group | Treatment modality | Mice ($n$) | BrdU LI (mean ± SD) |
|---|---|---|---|
| I | Control (no treatment) | 6 | 0.30% ± 0.05% |
| II | i.p. (Aqueous suspension) | 4 | 0.09% ± 0.05% |
| III | i.l. (Aqueous suspension) | 4 | 0.17% ± 0.05% |
| IV | i.l. (CUL suspension) | 4 | 0.23% ± 0.06% |

Differences are not significant except for those between groups I and II and groups II and IV

**Table 3.** BrdU LI for small intestines of CD-1 mice according to treatment modality

| Group | Treatment modality | Mice ($n$) | BrdU LI (mean ± SD) |
|---|---|---|---|
| I | Control (no treatment) | 6 | 47.00% ± 8.72% |
| II | i.p. (Aqueous suspension) | 4 | 18.89% ± 7.90% |
| III | i.l. (Aqueous suspension) | 4 | 30.25% ± 7.91% |
| IV | i.l. (CUL suspension) | 4 | 37.95% ± 3.25% |

All differences are significant except for those between groups I and IV, groups II and III, and groups III and IV

*BrdU assay*

BrdU-labeled cells were easily distinguished from unlabeled cells in the tumor, kidney, and small intestine (Fig. 6). The mean tumor BrdU LI was 32.80% ± 3.78% for the untreated control group (Table 1). There was no significant difference between i.p. and i.l. treatments with the aqueous suspension. Tumors treated with the CUL suspension showed the lowest percentage of BrdU-labeled cells.

The effects of the various treatment modalities on DNA synthesis in the kidney and small intestine of CD-1 mice are shown in Tables 3 and 4, respectively. In the kidney, the most pronounced effect was seen after i.p. treatment, where the BrdU LI was reduced to about one-third of the control level. After i.l. injection of aqueous suspension, the BrdU

LI was reduced to approximately one-half of the control value. In contrast, the difference between the BrdU LIs recorded for the control group and the CUL-treated group was not significant.

In the small intestine, no significant difference was noted between the control group and the CUL-treated group or between the CUL-treated group and the i.l. (aqueous suspension) group. However, similar to the findings in the kidney, the lowest BrdU LI was seen after i.p. treatment.

## Discussion

The rationale for direct i.l. injection of cisplatin is to increase the effective concentration of cisplatin in the tumor tissue while decreasing the exposure of normal tissue [3, 22, 24]. The rationale for the use of lipiodol as a delivery vehicle is to delay cisplatin resorption and prolong the period during which the target cells are exposed to cisplatin [1, 18, 20, 21]. The rationale for the use of urografin is that it serves as an intermediate between cisplatin and lipiodol [18]. As the specific gravities of lipiodol (1.275–1.290) and urografin (1.328–1.332) at 20° C are much the same, these compounds mix well in suspension.

In this work, it was shown that i.l. (local) treatment more effectively caused a tumor-growth delay. The least effective result was obtained by i.p. (systemic) administration. Other studies have indicated that local treatment of a tumor may be more beneficial than systemic treatment. Penn et al. [32] showed that when even a minimal drug dose was given i.l., a significant increase in the survival of rats with gliosarcoma was achieved, whereas systemic treatment showed no effect. Likewise, Lipmann et al. [26] showed that Lewis lung carcinoma could be successfully treated in three of ten mice by intratumoral injection of macromycin B, whereas no animal treated i.p. survived.

Obviously, i.p. administration cannot raise the concentration of cisplatin in the tumor tissue to levels such as those attained by i.l. administration. The platinum concentration detected in tumors of the groups treated i.l. with both aqueous and suspensions was always higher than that measured in the i.p. group at time points ranging from 5 min to 12 days after the injection of 5 mg/kg cisplatin. Since a higher concentration occurred in the i.l. groups, it is quite reasonable that i.l. injection of cisplatin produced a better response in the experiments.

Several factors could be considered as an explanation for the effectiveness of i.l. treatment with the CUL suspension. The most obvious is the delivery to the tumor cells of sufficient amounts of the agent at a dose level that is systemically nontoxic. No severe inflammatory reaction or necrosis of the skin at the site of injection was observed in any animal; thus, local toxicity was regarded as negligible. A second important factor is the sustained retention of the drug in tumors. As a lymphographic agent, lipiodol is known to be recovered selectively via the lymphatic system; however, since tumors have a poorly developed lymphatic system, if any, drainage of lipiodol and the drug incorporated into it is inadequately accomplished and these agents, are therefore retained for longer periods [1, 18, 20, 21].

Our findings also showed that the kidney and small intestine contained relatively higher and longer-persisting levels of platinum (or accumulation of drug) following i.p. treatment. On the basis of this observation, we supposed that the marked loss of body weight noted in i.p. treated mice was probably due to higher concentrations of cisplatin in the kidney and small intestine.

Generally, the study of cell proliferation has involved the use of [$^3$H]-thymidine to allow the monitoring of DNA synthesis in individual cells by autoradiography and, hence, the identification of proliferating cells or cell populations. However, this technique is time- and energy-consuming and requires the use of radioactive materials, which may be hazardous if not handled properly.

BrdU was first used for the treatment of different malignancies [2, 13, 29]. It has been used in vivo and in vitro to label replicating DNA in patients with gastric cancers [17, 34], central nervous system tumors [14, 43], and prostrate adenocarcinoma [30]. Even at the elevated dose of 1000 mg BrdU/body [31], adverse reactions to BrdU infusions were not observed.

The availability of a commercial kit with standardized reagents and methods was influential in our decision to evaluate in vivo the effects of the various treatment modalities described herein on DNA synthesis in tumors, kidneys, and small intestines. Since the most serious toxic side effects of cisplatin involve damage to the renal tubules and impairment of the gastrointestinal epithelium, it was of considerable interest to obtain further information concerning the comparable effects of the various treatment modalities on events related to cell proliferation in the tumor and the critical and normal tissues.

With this kit, measurement of BrdU LIs in the Ehrlich ascites tumor, kidney, and small intestine of mice was accomplished. The results obtained using this method support the findings reported in other publications. Langer et al. [23] undertook autoradiography and BrdU-uptake studies in Ehrlich ascites tumor cells 6 days after the cells had been inoculated into the peritoneum of mice and revealed an LI of 34.9% and 34.6%, respectively. This finding is in close agreement with the results we obtained in untreated (control) CD-1 mice, for which the mean BrdU LI was $32\% \pm 3.78\%$. The outcome of earlier research involving the kidney and small intestine of mice also correlates with the results of the present study. Using [$^3$H]-thymidine, Litvak and Baserga [28] disclosed a mean LI of about 0.35% in 2-month-old male $CAF_1$ mice. The mean BrdU LI determined for untreated (control) CD-1 mice in the present experiment was $0.30\% \pm 0.05\%$. Similarly, a study of the uptake of BrdU and [$^3$H]-BrdU [38] in the small intestine of BALB/c mice showed that at 1 h after the injection of 50 mg/kg BrdU, the mean BrdU LI was $34.1\% \pm 13.5\%$, and after the injection of 25 μCi [$^3$H]-BrdU, the mean [$^3$H]-BrdU LI was $38.9\% \pm 18.4\%$. These results are not significantly different from those obtained in the present experiment, which showed a mean BrdU LI of $47.00\% \pm 8.72\%$ for untreated (control) CD-1 mice. These findings may prove that in vivo BrdU labeling with the use of the Cell Proliferation Kit to determine the fraction of cells in the S phase was relatively accurate.

The effects of the various treatment modalities on the S-phase fraction appear to show a close relationship to the amount of drug retained in the various tissues. In the tumor, kidney, and small intestine, the percentage of cells in the DNA-synthesis phase decreases as the retention of the drug increases.

The BrdU assessment of cell cycling appeared to be an early reliable measure of chemotherapeutic efficacy. It was shown that the BrdU LI for Ehrlich ascites tumors was significantly diminished following i.l. injection of the CUL suspension as compared with i.l. and i.p. injection of the aqueous suspension, with no substantial loss of body weight being observed in the mice. The most pronounced effect on the tumor was produced by i.l. injection of the CUL suspension: at 24 h after treatment, the BrdU LI was reduced to less than one-third of the control level. In normal tissues, the most pronounced effect was caused by i.p. treatment. In the intestine, the BrdU LI was reduced to about 40% of the control value in CD-1 mice. Moreover, the BrdU LI for the kidney was reduced to about 45% of that found for CD-1 mice after i.p. treatment.

The CUL suspension appears to be an excellent candidate for i.l. administration because of the anticancer effect of cisplatin, the high concentrations of drug maintained in the tumor for extended periods, and the low concentrations occuring in the kidney and small intestine. It is hoped that this study will lead to a better utilization of i.l. therapy of human cancer.

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