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Tissue disposition and plasma concentrations of idarubicin after intravesical therapy in patients with bladder tumors*

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Summary. We studied single doses of intravesical idarubicin (IDA) given as 1-h instillations to 33 patients with bladder tumors. The dose was escalated from 5 to 30 mg and the concentration, from 0.25 to 1.5 mg/ml for evaluation of the importance of both the total amount of drug and the drug concentration on the levels of IDA found in different tissues (tumor, mucosa and muscle). Additionally, plasma uptake over 24 h was studied. The results demonstrated that (1) the levels of IDA in extracts of bladder tumors were significantly higher than those in normal bladder tissue, (2) the incorporation of IDA into tumors depended on the total amount of drug instilled and on the concentration of drug in the instillation fluid, (3) cytotoxic concentrations of IDA were noted in all tumors when the total amount of drug instilled was >15 mg and the drug concentration in the instillation fluid was >0.33 mg/ml, and (4) plasma levels of IDA were negligible.

Introduction

Most bladder cancer patients (75%–85%) present with superficial lesions that include papillary tumors involving only the mucosa (Ta) or the submucosa (T1) and flat carcinoma in situ (Tis). Carcinoma in situ uncommonly presents alone and is most often recognized in cases of previous or concomitant papillary tumors. In the treatment of superficial bladder tumors, the objectives are to eradicate existing disease, to avert the occurrence of new tumors and to prevent progression to muscle invasion, metastasis, or refractory local disease that requires cystectomy. Intravesical therapy following a transurethral resection may be cytotoxic to residual overt or occult carcinomas and premalignant mucosal lesions. Intravesical therapy has been widely adopted for use as definitive therapy and as prophylaxis against recurrent superficial bladder tumors, particularly in patients considered to be at high risk for recurrence (high disease stage and grade, multifocality, size, previous bladder cancer). Similarly, patients with carcinoma in situ represent a high-risk group.

Several anticancer agents have been used to treat bladder cancer; these can be divided into chemotherapeutic substances (thiotepa [8], doxorubicin [5], epirubicin [6], and mitomycin [3]) and immunotherapeutic agents (interferon [2] and bacille Calmette-Guérin, BCG [18]). Theoretically, one disadvantage of intravesical chemotherapy with anticancer agents may be the occurrence of systemic side effects due to drug uptake through the bladder wall and its capillary plexus into the circulation. Using radiolabeled doxorubicin (DOX), it has been shown that the concentration gradient between the bladder lumen and the plasma compartment is approximately 40,000 [4]. The limiting factor for intensive intravesical chemotherapy with DOX appears to be chemically induced cystitis, which has been found in about 25% of cases [17].

In the present study, patients with proven bladder cancer were treated intravesically with IDA. This daunorubicin (DNR) analogue exhibits an antitumor spectrum similar to that of DNR [1], but considerably more clinical research has been carried out in solid tumors using IDA as compared with DNR, mainly because the former can be given orally. The physicochemical characteristics of IDA differ from those of DNR with respect to the pKa and the partition coefficient; moreover, the former drug is much more lipophilic than the latter and is thus capable of faster passage through cell membranes. The purpose of the present study was to examine both the disposition of IDA in different bladder tissues and its penetration into the plasma compartment after intravesical administration.

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Table 1. Patients' characteristics

Patients	$n = 33$ (19 men, 14 women)
Age (years)	$\bar{x} = 67.0$ (range, 29.1–87.5; median, 69.6)
Recurrent tumor	$n = 12$ (4 men, 8 women)
Previous instillations	$n = 5$ [6 therapies: BCG (3), ADM (2), MMC (1)]
UTI of <4 weeks	$n = 8$
Histology:	
Metaplasia, dysplasia, granulomatous urocystitis	$n = 5$
Leiomyoma, glandular hyperplasia	$n = 1$
Papilloma Ta Go	$n = 1$
Cis (+5 concomitant Cis)	$n = 2$
Papillary tumor Ta G1 + G2	$n = 9$
Invasive urothelial cancer T1 G2 + G3	$n = 8$
Invasive urothelial cancer (>T2 G3)	$n = 7$

UTI, Urinary tract infection

Patients and methods

Patients and tissue/plasma samples. A total of 33 patients (19 men and 14 women) with bladder cancer were treated with 5, 10, 15, 20 and 30 mg IDA given intravesically; their characteristics are summarized in Table 1. The instillation volume varied, and the drug concentrations were 0.25, 0.33, 0.5, 1.0 and 1.5 mg/ml. Each dose and concentration level was monitored in three subjects. The drug was dissolved in 20–60 ml sterile saline using a closed system. The solution was drawn into a 50-ml syringe and inoculated through a catheter into the empty bladder. The catheter was clamped for 1 h, after which the content of the bladder was recovered. The bladder was filled with saline to remove the drug. After this procedure, resection of the tumor was carried out by transurethral resection (TUR), during which samples of mucosa and muscle were taken. All samples were divided into two sections: one for histological examination and the other for determination of IDA and idarubicinol (IDAol) concentrations. The samples were stored deep-frozen at –70° C. Additionally, blood samples were taken before the intravesical administration at 0.5, 1, 2, 4 and 24 h after instillation. They were immediately centrifuged at 3,000 *g* for 10 min, and the plasma was transferred into polypropylene tubes. Plasma and tissue samples were stored at –70° C until the HPLC assay was carried out.

Tissue-extraction procedure. The procedure for sample preparation was recently described [9] and slightly modified. After the tissue samples had thawed, 750 µl cold (4° C) 0.9% saline was added. This preparation was vortexed for 10 min to remove weakly adsorbed IDA from the surface and was then centrifuged at 4° C and 2,000 *g* for 4 min. The fluid was discarded and the weight of the "washed" tissue was determined, after which the tissue was deep-frozen with fluid nitrogen (77 K). The tissue samples were homogenized by dismembration for 1 min at 77 K using a Mikrodismembrator II (Braun, Melsungen, FRG) and suspended in 3 ml tetrahydrofuran/methanol/acetonitril (1 : 1 : 1, by vol.; Merck, Darmstadt, FRG) plus 300 µl 3 M silver nitrate in water. The anthracyclines were extracted by shaking for 10 min. Phase separation was obtained by centrifugation for 10 min at 2,000 *g*. The supernatant was transferred to a tube and evaporated to dryness at 40° C under a stream of nitrogen. The residue was dissolved by vortexing in 500 µl 0.02 M sodium dihydrogenphosphate-acetonitrile (pH 3.0; 3 : 2, v/v), and 25–50 µl was injected onto the HPLC column.

Plasma-extraction procedure. IDA and IDAol were extracted from human plasma samples using Bond Elut C-18 columns (ICT, Frankfurt, FRG) that had been pretreated with 5 ml methanol and 5 ml distilled water. Plasma was introduced onto the extraction column and subsequently purged with 4 ml buffer (0.02 M NaH_2PO_4, pH 3.0), dried with a flow of air, and eluted with 4 ml chloroform/methanol (1 : 1, v/v). The eluate was evaporated to dryness at 50° C under a stream of nitrogen. The residue was redissolved in 100 µl buffer, vortexed and centrifuged (1 min, 15,000 *g*, 20° C), and 50 µl was injected onto the analytical HPLC column. The internal standard was DNR (50 ng), which was added to all plasma samples prior to extraction. All samples were prepared in duplicate, and a full calibration line was included for each series (40 analyses).

HPLC-analysis system. IDA and IDAol as well as DNR (internal standard) were separated and detected using an HPLC system described elsewhere [12], consisting of a reversed-phase column (3 µm C-18 MicroSpher; length, 200 mm; inside diameter, 4.6 mm; Chrompack, Frankfurt, FRG) equipped with a guard column (5 µm C-18; length, 4 mm; inside diameter, 4 mm) and fluorescent detection (excitation wavelength, 480 nm; emission wavelength, 550 nm). Elution was performed under isocratic conditions with 0.02 M sodium dihydrogenphosphate-acetonitrile (pH 3.0; 3 : 2, v/v) at a flow rate of 0.8 ml/min. The lower limit of detection in this assay was 0.1 ng/ml (for 1-ml plasma samples). The

Table 2. Plasma concentrations of IDA and IDAol

Dose level	IDA Time (h) after drug instillation						IDAol Time (h) after drug instillation					
	0	0.5	1	2	4	24	0	0.5	1	2	4	24
5 mg:												
0.25 mg/ml	–	–	–	–	–	–	–	–	–	+	–	–
	–	–	–	–	–	–	–	–	–	+	–	–
	–	–	–	–	–	–	–	–	–	+	–	–
0.33 mg/ml	–	–	–	–	–	–	–	+	+	–	+	–
	–	–	–	–	–	–	–	+	0.1	+	–	–
	–	–	–	–	–	–	–	–	+	–	–	–
10 mg:												
0.25 mg/ml	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	+	–	–
	–	–	–	–	–	–	–	–	+	–	–	–
0.33 mg/ml	–	–	–	–	–	–	–	–	–	–	–	0.2
	–	–	–	–	–	–	–	–	–	–	–	+
	–	–	–	–	–	–	–	–	–	–	–	+
15 mg:												
0.33 mg/ml	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	+	+	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–
0.5 mg/ml	–	–	–	–	–	–	–	–	–	–	–	+
	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–
20 mg:												
0.33 mg/ml	–	–	–	–	–	–	–	–	+	+	0.9	+
	–	–	–	–	–	–	–	–	–	+	+	+
	–	–	–	–	–	–	–	–	–	–	+	+
0.5 mg/ml	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–
1.0 mg/ml	–	+	+	+	+	–	–	+	+	0.1	0.2	0.1
	–	+	0.2	0.1	+	–	–	+	0.2	0.1	0.2	0.2
	–	–	–	–	–	–	–	–	–	+	+	+
30 mg:												
1.0 mg/ml	–	–	0.4	0.2	+	–	–	–	0.5	0.5	0.4	0.5
	–	–	0.2	0.2	0.3	–	–	0.7	0.5	0.6	0.6	0.5
	–	+	0.6	0.4	+	–	–	0.5	0.8	0.5	0.6	0.7
1.5 mg/ml	–	–	–	–	–	–	–	+	0.2	–	–	0.2
	–	–	–	–	–	–	–	–	–	0.2	0.2	0.3
	–	–	–	–	–	–	–	–	–	–	–	+

Two different concentrations were studied at all dose level (5–30 mg) except 20 mg, at which three different concentrations were investigated. Three patients were evaluated at each concentration. –, Not detectable; +, detectable but too close to the detection limit to quantify

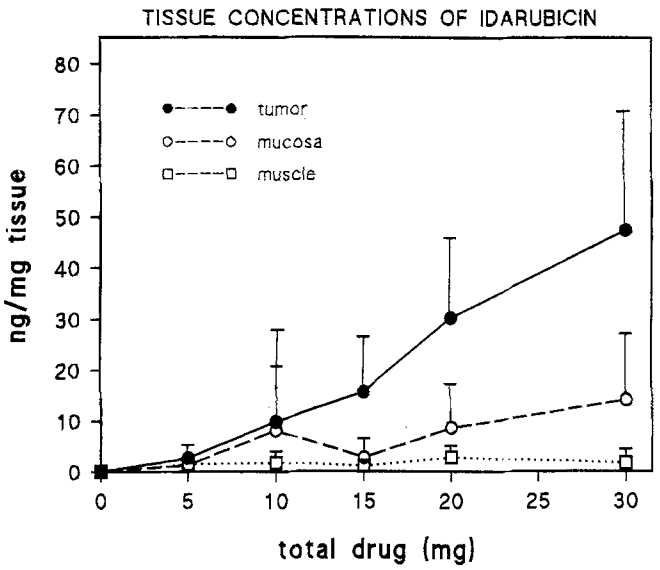

Fig. 1. IDA concentrations in muscle, mucosa and tumor in relation to the total amount of drug instilled

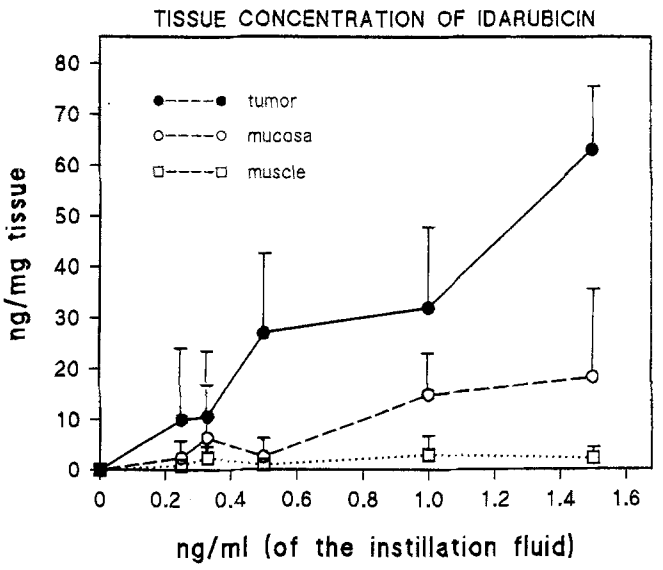

Fig. 2. IDA concentrations in muscle, mucosa and tumor in relation to the concentration of drug in the instillation fluid

Table 3. Tissue disposition of IDA and IDAol

Dose level	IDA			IDAol		
	Muscle	Mucosa	Tumor	Muscle	Mucosa	Tumor
5 mg:						
0.25 mg/ml	0.1 (0.1)	1.3 (1.4)	2.7 (1.3)	0.1 (0.1)	0.2 (0.1)	0.5 (0.6)
0.33 mg/ml	2.4 (2.5)	1.3 (1.4)	3.4 (2.7)	0.3 (0.5)	0.1 (0.1)	0.3 (0.3)
10 mg:						
0.25 mg/ml	1.8 (1.9)	4.7 (3.3)	14.7 (18.6)	0.2 (0.3)	0.4 (0.6)	1.8 (2.9)
0.33 mg/ml	1.8 (1.7)	11.5 (15.2)	2.6 (1.3)	0.2 (0.2)	1.2 (1.8)	0.5 (0.3
15 mg:						
0.25 mg/ml	0.6 (0.5)	0.5 (0.3)	13.0 (13.6)	0.1 (0.1)	0.1 (0.1)	0.9 (0.8)
0.50 mg/ml	1.5 (1.9)	4.2 (4.3)	18.4 (6.4)	0.1 (0.2)	0.5 (0.1)	1.5 (0.4)
20 mg:						
0.33 mg/ml	3.4 (1.1)	4.1 (2.7)	23.0 (15.5)	0.4 (0.4)	0.5 (0.6)	1.9 (1.9)
0.50 mg/ml	0.6 (0.2)	2.2 (1.8)	35.6 (17.9)	–	0.1 (0.0)	5.8 (4.3)
1.00 mg/ml	4.6 (5.1)	19.8 (2.9)	32.1 (14.3)	0.8 (0.4)	2.6 (0.9)	2.0 (1.7)
30 mg:						
1.00 mg/ml	1.2 (0.4)	10.1 (9.2)	31.9 (20.5)	0.1 (0.1)	1.5 (1.6)	7.3 (5.8)
1.50 mg/ml	2.2 (1.8)	18.2 (16.8)	63.0 (12.2)	0.8 (0.0)	4.8 (5.9)	12.4 (1.3)

Data represent mean values (±SD) expressed in ng/mg tissue

interassay precision was 12% at the detection limit and 7% at a concentration of 2.5 ng/ml.

Results

Penetration of IDA into the blood during and following intravesical instillations was low. Only in patients treated with 20 and 30 mg IDA were low amounts of IDA detectable (in 5 of 33 patients). The plasma levels ranged from 0.1 to 0.6 ng/ml. IDAol, the major metabolite of IDA, was detectable in 24 of 33 subjects, also in very low amounts ranging from 0.1 to 0.9 ng/ml (Table 2). The distribution of IDA in tissue samples was different (Table 3). The lowest amount of IDA was found in muscle, ranging from undetectable levels up to 10.5 ng/mg (mean, 1.8±2.2 ng/mg). No significant trend towards higher tissue concentrations with increasing drug doses was seen. In mucosa and tumor-tissue samples significant drug concentrations were detected; for both tissue types, a clear relationship between the tissue concentration measured and the total amount of drug instilled could be established as depicted in Fig. 1. In addition, a close correlation between the measured tissue concentrations and the concentration of drug in the instillation fluid could be found (Fig. 2). Similar results were obtained for IDAol (see Table 2). The concentrations of IDAol were always lower than those of IDA.

Discussion

The appreciable concentration of IDA found in bladder tumor tissue following intravesical administration is interesting. The cellular uptake of this anthracyline is considered to be caused by its passive permeation of the cell membrane and its subsequent binding to important cell constituents such as DNA, RNA and several other proteins. Differences in the physicochemical characteristics of DOX, epirubicin, DNR and IDA have been established for the partition coefficient, which plays a crucial role in the absorption of substances through a cell membrane, for the pKa and for the molecular weight [10]. The latter property differs only slightly from 534 Da for IDA to 580 Da for DOX. Moreover, IDA is much more lipophilic than DOX or DNR (partition coefficients: 32.3, 16.2 and 6.4, respectively), and it inhibits tumor cell growth at lower concentrations (IDA is about 5 times more potent than DNR).

In the present study, the influence of both the total amount of drug instilled and the concentration of drug in the instillation fluid on the disposition of IDA in different tissues from human bladders was studied. The highest levels were found in mucosa and in tumor tissue at the highest dose level (30 mg) and the highest concentration (1.5 mg/ml), reaching levels of 63 ± 12 ng/mg in the tumor and 18 ± 17 ng/mg in the mucosa. There may be several reasons for these rather large variations. The dilution factor for the instilled drug due to urine production during the 1-h instillation period is unknown but is assumed to be highly variable. All patients had stopped their fluid intake 12 h previously to minimize urine production. The absorption of drug by the tumor and bladder tissue is considered to be a very fast process that displays saturation kinetics. Nevertheless, the drug-uptake kinetics in these various tissues (mucosa, muscle and tumor) is unknown. It is known that urinary infection, chemical cystitis caused by frequent instillations, and recent transurethral procedures as well as previous radiotherapy may enhance mucosal and transmucosal absorption [16]. The heterogeneity of the tumor stages and the different tumor types may explain the large variation. Exophytic papillary tumors exhibit a large surface area in relation to their tissue volume.

On the basis of the present results, if IDA is instilled at doses of ≥ 15 mg and at concentrations of ≥ 0.5 mg/ml, drug levels previously found to be cytotoxic in in vitro studies (>10 ng/mg) will be reached. Similar results have been obtained after the instillation of a total dose of 50 mg DOX at a concentration of 1.7 mg/ml [13]. In that study, the highest concentrations were found in tumor tissues, which contained 25 ± 7 ng/mg, these concentrations were 2.5- to 3.6-fold those measured in histologically normal bladder tissue [13]. Similar findings have also been obtained using epirubicin, whereby the highest concentration was found in tumor tissue and half of that value was measured in the bladder wall [7]. The results of the present study were generally in agreement with these observations. The major difference was that although the total dose and the concentration of DOX used in the previous study (50 mg and 1.7 mg/ml) [13] were greater than the highest dose and concentration of IDA tested in the present investigation (30 mg and 1.5 mg/ml), the tumor tissue concentrations after DOX exposure were lower than after IDA exposure. As has correctly been predicted [10], a drug displaying a higher partition coefficient and a lower pKa can more readily penetrate cell membranes, resulting in higher intracellular drug levels at lower total drug doses and lower drug concentrations. Exactly this proved to be the case.

The concentrations of IDA and IDAol in the muscle were very low as compared with the drug levels measured in the mucosa and in the tumor, suggesting that only a minor part of the total amount of drug delivered intravesically reaches deeper parts of the bladder wall. This finding is in line with the results of the plasma determinations. Only trace amounts of IDA and IDAol were found in plasma samples, which, interestingly, more often contained IDAol than IDA, a phenomenon that is related to the very well-known pharmacokinetics and metabolism of IDA in plasma [15]. IDA is extensively metabolized by an aldo-keto reductase system to the reduced form, IDAol; this takes place mainly in the liver, but also in erythrocytes. The concentrations were always <1 ng/ml, which is much lower than that required to cause a myelosuppressive effect. Obviously, only a very minor part reached the capillary plexus and then the circulation. These findings are similar to the results previously obtained using epirubicin [11]. As the plasma levels of IDA and IDAol measured after intravesical chemotherapy were negligible, a cardiac risk for this treatment schedule did not exist. These low plasma concentrations led to the prediction of a lack of hematological toxicity.

A detailed description of the patients' characteristics, the histological findings and the toxicity data will be published elsewhere [14]. The results of the present study suggest that a dose of at least 15 mg IDA at a concentration of 0.5 mg/ml be used in the intravesical treatment of patients participating in phase II studies. The question as to whether local tumor control can be achieved using this combination or whether higher doses and concentrations might be more effective requires further investigation.

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