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Intravesical idarubicin - a phase-I study

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Abstract In the scope of a pharmacokinetic and dose-finding study 33 patients received instillations of idarubicin in 11 different doses 1 h before scheduled transurethral resection of bladder cancer. The dose was increased continuously from 5 to 30 mg and the concentration from 0.25–1.5 mg/ml. Idarubicin uptake into tissue was measured along with the serum level. The results showed a clear correlation of the tissue levels with dose and concentration. A significantly higher concentration of idarubicin was measured in the tumor in comparison with the mucosa. Absorption into the muscle was minimal and serum levels were low. Systemic toxicity was not observed, but there were signs of local toxicity in 50% of the subjects. Cytotoxic concentrations in the mucosa were reached at doses of over 15 mg and concentrations of over 0.5 mg/ml. A phase-II study is in preparation.

Key words Idarubicin · Intravesical chemotherapy · Tissue concentration · Plasma uptake · Toxicity

Intravesical adjuvant therapy with cytostatic or immunotherapeutic agents is an established component in the treatment of noninvasive transitional cell cancer of the bladder (TCC). The aim of adjuvant therapy is to lengthen recurrence-free times and to reduce the tendency towards progression [16, 18]. Commonly used substances include doxorubicin (DOX) [7], mitomycin C (MMC) [3] and bacillus Calmette-Guérin (BCG) [14]. In addition, the therapeutic instillation of epidoxorubicin (EPI) leaving a marker lesion has shown response rates of 60–70% [8]; for carcinoma in situ a 70% rate of complete remission has been reported [5].

The following properties are required of an ideal intravesical therapeutic drug: high therapeutic effectiveness; high molecular weight and hence low systemic uptake; marked lipophilia, which results in rapid absorption into the tissue; minimal local toxicity.

Idarubicin, a 4'demethoxydaunorubicin (DNX), is a new anthracycline agent. A dose-limiting factor is myelosuppression, which has been described in phase-I studies in patients treated with weekly i.v. instillations of 10–12.5 mg/m^2; the maximum tolerated dose (MTD) is 17.5 mg/m^2 by weekly i.v. administration [17].

The molecular weight of idarubicin is 533.97 Da. Owing to the high molecular weight a systemic uptake after intravesical topical application seems unlikely. The high degree of lipophilia, however, means that rapid mucosal uptake and tumor penetration can be expected [9, 19].

In vitro data have demonstrated that the therapeutic potency of idarubicin is 8 times that of daunorubicin and 5 times than that of DOX. Even in solid tumors (breast, sarcoma) its activity is far higher than that of other anthracyclines, especially after i.v. administration. This difference in effectiveness was also found in in vivo models. Furthermore, the LD_{50} level of idarubucin is only one sixth to one quarter that of DOX [1]. According to various studies, the drug has a cytotoxic effect on solid tumors at a concentration of 10 ng per mg tissue.

In view of the known therapeutic action of anthracylines in the treatment of superficial TCC of the bladder and of the available results of treatment of leukemia and certain solid tumors [2] in combination with the described molecular properties of the substance, idarubicin is expected to be of therapeutic value in noninvasive bladder cancer.

The theoretical disadvantage of intravesical chemotherapy, i.e. significant systemic uptake of the drug, must be ruled out before treatment is initiated. A concentration gradient of 1:40,000 between bladder lumen and plasma compartment has been measured for both DOX [4, 12]

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Table 1 Patient characteristics

Clinical examination	
Patients	$n = 33$ (19 men, 14 women)
Age (years)	$\bar{x} = 67.0$ years (median 69.6, range 29.1–87.5 years)
Recurrent tumors	$n = 12$ (4 men, 8 women)
Previous instillation therapy	$n = 5$ [6 therapies: BCG (3), DOX (2), MMC (1)]
Urinary tract infection in the last 4 weeks	$n = 8$
Histology	
Metaplasia, dysplasia granulomatous urocystitis	$n = 5$
Leiomyoma, glandular hyperplasia	$n = 1$
Papilloma TaG0	$n = 1$
Cis (+ 5 concomitant Cis)	$n = 2$
Papillary tumor TaG1 + G2	$n = 9$
Invasive urothelial cancer T1G2 + G3	$n = 8$
Invasive urothelial cancer (> T2G3)	$n = 7$

Table 2 Patient selection

Inclusion criteria	Exclusion criteria
Confirmed or (endoscopically suspected bladder) cancer (regardless of T stage)	Known allergy to anthracyclines
Previous chemotherapy or hormone therapy admissible	Previous irradiation of the bladder
Adequately treated urinary infection	Current untreated urinary tract infection

Table 3 Dose levels of idarubicinol (IDA)

Dose level	Dose (mg)	Vol NaCl (ml)	Concentration IDA mg/ml NaCl
1	5	20	0.25
2	10	40	0.25
3	5	15	0.33
4	10	30	0.33
5	15	45	0.33
6	20	60	0.33
7	15	30	0.5
8	20	40	0.5
9	20	20	1.0
10	30	30	1.0
11	30	20	1.5

and EPI [10]. Inflammation of the bladder and radiotherapy can affect drug absorption, so that these factors should be used as exclusion criteria for instillation therapy. In a combination pharmacokinetic and dose-finding study the following parameters were investigated after a single intravesical application of idarubicin: tissue concentration of idarubicin/idarubicinol in the tumor, in the normal bladder mucosa, and in the tunica muscularis; systemic uptake of idarubicin through the bladder wall; local tolerance (side effects) and toxicity (mucosa biopsy) and also systemic toxicity.

Materials and methods

Between October 1989 and October 1991, a total of 33 patients (19 men, 14 women) with endoscopically confirmed or suspected bladder neoplasia each received a single instillation of idarubicin to the bladder (see Table 1 for demographic data). The inclusion and exclusion criteria are listed in Table 2. Increasing doses of 5, 10, 15, 20, and 30 mg, as well as concentrations of 0.25, 0.33, 0.5, 1.0, and 1.5 mg/ml idarubicin at a total of 11 dose levels (Table 3) were chosen. Each dose level was studied in three subjects. The substance was dissolved in 20–60 ml sterile saline. The solution was drawn up into a sterile disposable catheter and instilled into the emptied bladder. After exactly 60 min, the contents of the bladder were removed with an endoscope or, if the operation was delayed, through a second disposable catheter.

During transurethral resection, samples were taken from the tumor (area suspected of tumor involvement), from mucosa of normal appearance and from the tunica muscularis to measure the uptake of idarubicin and its main metabolite idarubicinol. Histological examination confirmed correct sample removal. In addition, a sample from apparently normal mucosa was examined histologically to evaluate the acute toxicity of idarubicin in the different layers of the bladder. All samples were immediately stored at −80 °C. Blood samples were taken prior to instillation and 0.5, 1, 2, 4, and 24 h after intravesical application. The blood was immediately centrifuged at 4000 rpm for 10 min. The serum was stored in polypropylene tubes at −80 °C until HPLC assays.

Tissue concentration

Once thawed, the tissue samples were washed in 0.9% NaCl. To rinse the idarubicin from the cell surface the tissue was homogenized and weighed after being frozen in liquid nitrogen. The anthracycline agent was then extracted and measured by means of HPLC. Measurements were performed in the laboratory headed by K. Mross. The technique has recently been published elsewhere [6].

Serum concentrations

The serum concentrations of idarubicin and its main metabolite idarubicinol were also measured in K. Mross's laboratory by means of HPLC assay [11]. The detection limit is 0.1 ng/ml, while the interassay variance is 12% at the detection limit and 7% at a concentration of 2.5 ng/ml.

Statistical methods

The measured tissue concentrations of idarubicin/idarubicinol were grouped according to the respective dose level and assessed with descriptive statistical methods (mean value, standard deviation). The serum concentrations are presented in the tables, serum levels of under 0.1 µg/ml being considered negative.

Results

Tissue concentration

Tissue absorption (Table 4) shows a dependency on the dose as well as on the idarubicin concentration. Muscle uptake was lowest, only in one case was a concentration of 10.5 ng/ml tissue (average 1.8 + 2.2 ng/mg) measured. In contrast, significant idarubicin concentrations dependent

Table 4 Tissue concentration of IDA/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)	02. (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 (ng/mg tissue) represent mean values (SD)

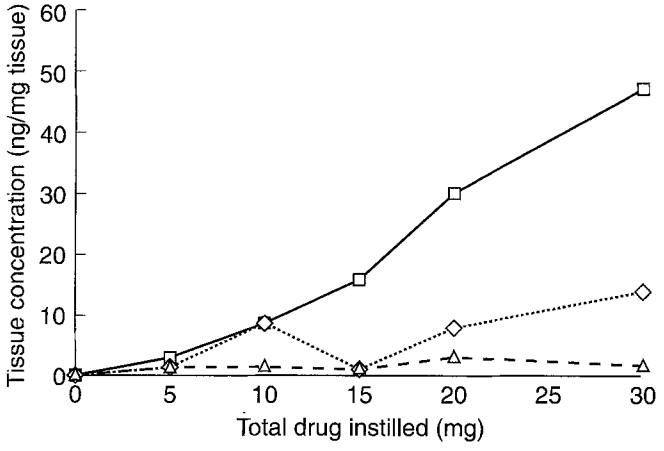

Fig. 1 Tissue concentrations of idarubicin in tumor (—□—), mucosa (····◇····), and muscle (––△––) against total dose instilled

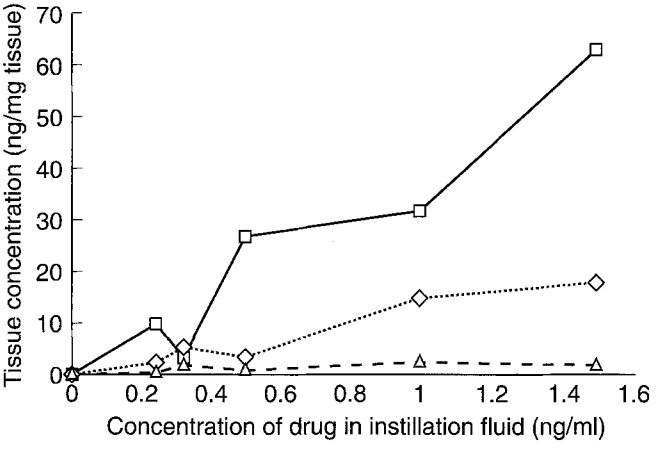

Fig. 2 Tissue concentrations of idarubicin in tumor (—□—), mucosa (····◇····), and muscle (––△––) against concentration of idarubicin in instillation fluid

on dose and concentration were determined in the mucosa and tumor (Figs. 1, 2). Comparable data were obtained for idarubicinol; the concentrations, however, were far lower than for idarubicin.

Serum concentration

Systemic uptake of idarubicin after an intravesical single instillation of the drug is low. Only in patients who received at least 20 mg of idarubicin were plasma levels between 0.1 and 0.6 ng/ml measured (5 of the 33 patients). The same applied for idarubicinol (Table 5) although the serum levels are considerably higher than for idarubicin.

Toxicity – histological examination

All of the samples from normal mucosa were assessed by the same pathologist to evaluate the acute toxic activity at the transitional cell epithelium. In over 50% the following findings were recorded: isolated beginning loss of the covering epithelium; normal stratification with no epitheliolysis, but subepithelial edema; edematous loosening of the subepithelial stroma with round-cell infiltration; abundance of highly ectatic vessels, some filled with granulocytes.

These findings are clear evidence of idarubicin's local toxic activity on healthy transitional cell epithelium. After resection, however, no local symptoms were observed. Nor was a correlation established with the total dose instilled or concentration of the drug instilled.

Table 5 Serum levels of idarubicin/idarubinol (IDA/IDAol) Three patients at each dose level (– not detectable, + detectable but not quanitifiable at detection limit)

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
	–	–	–	–	–	–	–	–	–	–	–	+

In no case was systemic toxicity according to WHO criteria detected, nor were any changes recorded in the documented laboratory parameters (blood count, electrolytes, creatinine clearance, urea, serum glutamate oxaloacetic and pyruvic transaminases, alkaline phosphatase, lactic dehydrogenase, bilirubin). This finding, along with the low serum levels, indicates that idarubicin/idarubicinol is not systemically toxic after intravesical instillation.

Discussion

The findings show a notable dose and concentration-dependent absorption of idarubicin into the mucosa, which seems particularly important in the treatment of carcinoma in situ, and to an even greater degree into the tumor. Idarubicin passively penetrates the cell membrane and exhibits a binding affinity to DNA comparable with that of DOX and stronger than that of DNX. Moreover, idarubicin shows a stronger inhibitory influence on nuclein acid polymerases [13, 20]. The influence of idarubicin and DNX on the DNA synthesis rate is comparable, while RNA synthesis is much more strongly inhibited by idarubicin.

While idarubicin's molecular weight (534 Da), which is relevant for the systemic uptake, is almost identical to that of DOX (580), idarubicin is considerably more lipophilic than DOX or DNX (distribution coefficient 32.3, 16.2, and 6.4).

In vitro studies [15] of 8-anthracycline derivatives have shown that idarubicin's IC_{50} level (the extracellular concentration that results in a 50% reduction of the surviving cells) is 0.010 μg/ml and hence much lower than that of DOX (0.076) or EPI (0.123 μg/ml). In contrast, intracellular anthracycline concentrations of 0.070 (IDA), 0.21

(DOX) and 0.24 μg/mg protein, respectively, were measured. This means that the same cytotoxic effect is reached at lower intracellular concentrations with idarubicin as with DOX or EPI. Under identical conditions the cellular uptake of idarubicin is 2–3 times that of other anthracyclines.

The increased cellular absorption at lower cytotoxic thresholds makes idarubicin suitable for topical treatment. In view of the properties discussed above an equivalent effect can be expected with roughly one-fifth and one-tenth of the DOX and EPI levels respectively.

In the present study the cellular uptake was measured, particularly in dependence on the total dose and the concentration of the instilled drug. The highest tissue concentrations in the tumor (63 + 12 ng/mg tissue) and in the tunica mucosa (18 + 17 ng/ml) were measured at the highest doses (30 mg) and concentrations (1.5 mg/ml).

There are a number of explanations for the large fluctuations. One unknown variable is the dilution factor resulting from the urine produced in the 1-h instillation period, despite the fact that the patients received no fluids for 12 h prior to the scheduled operation under anesthesia. Another explanation for the variable absorption is the heterogeneity of the patient population in terms of tumor type. It is obvious that absorption in a papillary tumor with a very extensive surface is going to be higher than in a solid tumor or a carcinoma in situ growing in the epithelium. The data obtained for cellular uptake show, however, that this effect is limited.

What is certain is that the cytotoxic intracellular idarubicin concentration of 10 ng/mg (= 0.010 μg/mg) known from preclinical data can be attained with idarubicin doses of 15 mg or more and concentrations of 0.5 mg/ml or more. Comparable findings were determined with DOX at a far higher dose (50 mg) and concentration (1.7 mg/ml) [12]. The highest concentrations measured in the tumor were 25 + 7 ng/ml for DOX. They are 2.5–3.6 times higher than in the mucosa, similar results were obtained for EPI [15].

It is evident that despite lower single doses (30 vs 50 mg) and concentrations (1.5 vs 1.7 mg/ml) of idarubicin than of DOX, the tissue concentrations for idarubicin were susbstantially higher than for DOX. As was predicted, the higher distribution coefficiency and the lower pKa level resulted in a faster cellular uptake and higher intracellular level of action.

The idarubicin/idarubicinol levels measured in the tunica muscularis were extremely low in comparison with mucosa and tumor. This indicates that only small amounts of idarubicin penetrate the basal membrane, a fact that is also supported by the idarubicin serum levels. Interestingly, the idarubicinol levels were higher than the idarubicin levels. This, however, correlates with the known pharmacokinetic and metabolizing data, according to which idarubicin is reduced to idarubicinol primarily in the liver and erythrocytes by means of an aldo-keto-reductase system.

All of the concentrations measured, however, were below 1 ng/ml, which rules out the risk of bone marrow involvement. Other types of systemic side effects can also be excluded with the serum levels measured.

The local toxicity, however, must be taken seriously. The fact that epitheliolysis, subepithelial edema, and incipient unspecific inflammatory changes are observed after a single 1-h instillation indicates that serious alterations in the urothelium with clinical symptoms of chemocystitis must be feared after repeated instillation with longer retention times. Repeated instillations of idarubicin must be measured against the rates of chemocystitis known for DOX (6.5%) [3] and EPI (13%) [5].

Nevertheless, idarubicin doses of over 15 mg and concentrations of over 0.5 mg/ml are suitable for patients with superficial bladder cancer in the scope of a phase-II study. In order to reach reliable mucosa levels for carcinoma in situ of at least 10 ng/ml tissue, we have selected a dose of 20 mg per 20 ml NaCl (1 mg/ml) for the beginning of a phase-II study.

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