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Permeability of the Bladder Mucosa to Thiotepa, Adriamycin, and Daunomycin in Men and Rabbits

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Summary. The permeability of the bladder mucosa to thiotepa and to the anthraquinonic antibiotics, adriamycin and daunomycin, was investigated both in humans and in experimental animals. Instillations in rabbits were performed either in intact males or in animals with ligated ureters. Absorption of thiotepa was significantly higher than that of the antibiotics both in men and in rabbits. Furthermore a qualitative difference was observed in rabbits in relation to time and with regard to fixation to vesical tissues. In man, absorption was highest after transurethral surgery. It was also increased in cases with extensive anaplastic tumours or in the presence of acute inflammatory reactions.

Key words: Cancer chemotherapy, Vesical absorption, Adriamycin, Thiotepa, Daunomycin.

The literature on vesical absorption of chemotherapeutic agents is sparse, although it is well known that intravesical instillations of cytotoxic drugs can be followed by severe myelotoxicity. Some aspects of this problem are discussed elsewhere (3).

The present research was undertaken with the following aims:

a) to compare the absorption of thiotepa from the bladder with that of other chemotherapeutic agents (adriamycin, daunomycin);

b) to study the absorption of these drugs across the human bladder in various clinical conditions;

c) to investigate, in the rabbit, the modalities of drug absorption in relation to time, dosage and dilution (ureters were tied in a group of animals, in order to maintain constant concentration throughout the experiment).

Daunomycin and adriamycin were employed, in addition to thiotepa, as they can be easily determined quantitatively by spectrophotofluorometry.

In the various experiments, the amount of the drug under investigation that could be recovered from the bladder at different time intervals was subtracted from the total initial dose. The difference between the two values was considered equivalent to the absorbed dose, although the loss of small amounts of the drug by fixation to the tissues of the bladder cannot be excluded. In order to clarify this point, some quantitative determinations of tissue fixation of the drug were also performed.

MATERIALS AND METHODS

A) In Humans

40 patients were divided in various groups as follows:

Group 1: Patients with normal bladders, although previously treated transurethrally for single papillary tumours with an interval of at least a month from the surgical procedure,
Group 2: Patients with papillary well differentiated transitional cell carcinoma or papilloma,
Group 3: Patients with solid anaplastic carcinoma,
Group 4: Patients undergoing transurethral surgery less than one week prior to the study,
Group 5: Patients with acute cystitis.

One half of the patients in each group was treated with thiotepa, the other half was treated

with adriamycin. Each group consisted of at least 5 patients. Only occasional patients were treated with instillations of daunomycin.

Thiotepa was employed in a dose of 50 mg in 30 ml of sterile water. The catheter was usually removed following the instillation and the patient was left free to walk. After one hour a catheter was inserted again, and the bladder was drained and rinsed with sterile water up to a total volume of 500 ml.

The same technique was applied to the patients treated with adriamycin and daunomycin, which were employed respectively at the dose of 10 and 20 mg. In a few patients specimens were removed from the bladders at 15, 30 and 45 minutes after the instillations.

B) In Rabbits

Groups of at least 4 male rabbits were employed, weighing 2 Kg ± 0,5. The drugs under test were employed in dosages of 1 or 2 mg/kg, diluted in distilled water to 2 ml/Kg body weight, and instilled into the bladder by catheterisation. At 30, 60, 90, and 120 min the bladder content was aspirated for volume measurement, removed of an aliquot of 0,1 ml for the assay, and then replaced intravesically through the catheter. Some experiments were carried out following bilateral ligation of the ureters in order to evaluate the effect of accumulation of urine in the bladder during the experiment. Ureteral ligation was performed under general anaesthesia with ethyl-urethane (750 mg/Kg).

Blood samples were obtained and some animals were sacrificed at the end of the experiment so that the drug under test could be determined quantitatively in the blood or in the tissue of the bladder.

Adriamycin and daunomycin were measured by the spectrophotofluorometric assay described by Dusonchet et al. (1). Thiotepa was determined by a colorimetric method (4).

As the solutions of thiotepa are unstable and undergo decay with time, all the measurements were carried out employing as a standard a solution having the same titre of that used for the instillation which had been kept at 37° throughout the duration of the experiment.

RESULTS

A) Human Studies

The results obtained with thiotepa and adriamycin are indicated in Tables 1-3. The results obtained in 5 cases using daunomycin are shown in Table 4. Fig. 1 shows the absorption of thiotepa from the human bladder in a patient with multiple papillary tumours.

Table 1. Absorption of thiotepa and adriamycin in men after 60' of intravesical treatment

Drug	Mean (all groups)	Group (mean values) 1	2	3	4	5
Thiotepa	47,8 %	10,5 %	28 %	63 %	73 %	21 %
Adriamycin	18,36 %	2,2 %	20 %	31,4 %	62 %	9,9 %

Table 2. Absorption of thiotepa (50 mg) in men after 60' of intravesical treatment

	No. of cases	Mean absorption	Range
Group 1	5	10,5 %	5,7 -23,4 %
Group 2	10	28 %	7,3 -40,9 %
Group 3	10	63 %	12,05-90 %
Group 4	12	73 %	39,5 -97,3 %
Group 5	3	21 %	18,3 -24,8 %
Total	40	47,8 %	

Table 3. Absorption of adriamycin (10 mg) in men after 60' of intravesical treatment

	No. of cases	Mean absorption	Range
Group 1	10	2,2 %	0,88-77,3 %
Group 2	10	20,0 %	1,5 -40 %
Group 3	5	31,4 %	15 -53,2 %
Group 4	3	62 %	42 -74 %
Group 5	5	9,9 %	0 -23 %
Total	33	18,36%	

Table 4. Absorption of daunomycin (20 mg) in men after 60' of intravesical treatment

Case No.	Bladder condition	% of absorbed drug
1	Normal (3 months after loop resection)	11,4 %
2	Large, non infiltrating papillary tumour	1,5 %
3	Normal (6 months after diathermy)	24,5 %
4	Small non-infiltrating papillary fibroepithelioma	5,5 %
5	Anaplastic carcinoma	42 %

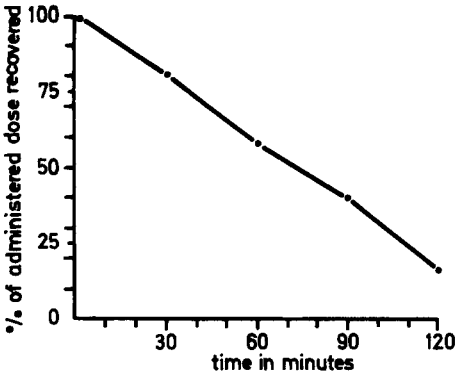

Fig. 1. Absorption of thiotepa from the human bladder in a patient with multiple papillary tumours

From the available data it can be observed that all the drugs tested, i. e. thiotepa, adriamycin and daunomycin, are absorbed through the bladder wall. The data are rather consistent, despite some variability in single cases and the wide range within each group. It is clearly shown that bladder permeability is greater under pathological conditions than in the normal organ.

The presence of a papillary tumour and especially a solid anaplastic carcinoma increases absorption, which is also enhanced by acute inflammation. Our results show that the greatest permeability is consistently observed following endoscopic surgical procedures which destroy the integrity of the urothelial layer. In this group absorption was higher than 50 % in all patients, reaching almost 100 % in some cases. In our experiments, the rate of absorption of thiotepa from normal bladders, as well as in cases of cystitis and of non-infiltrating papillary tumours, is relatively lower than reported by Lunglmayr and Czech (2). This may be due to some difference in the technique employed, such as the prolonged rinsing of the bladder after the contact period, that we have carried out routinely in each case, thereby improving recovery of the drug.

Bladder permeability to thiotepa is significantly greater than to adriamycin (Table 1). The data obtained with daunomycin are too few for a definite conclusion but its behaviour seems similar to that of adriamycin.

Absorption of thiotepa, in relation to time, appears to be constant and progressive for the first two hours, after which it becomes virtually complete, at least when the mucosal surface is enlarged by extensive papillary growths.

No blood levels of the drugs have been measured, but no reduction in leucocyte or platelet counts was observed in the patients treated with either adriamycin or daunomycin, whereas moderate but transient leucopenia was occasionally observed following thiotepa instillations.

B) Experiments in Rabbits

The results of the experiments carried out in rabbits are summarised in Table 5. As in humans, the individual results are variable within a wide range. From a comparison of the results obtained with 1/mg/Kg of the three compounds employed, (Fig. 2) a clear-cut difference can be observed between thiotepa and either adriamycin or daunomycin.

Thiotepa was absorbed to a greater extent and with a different pattern, i. e. absorption takes place at a relatively constant rate in relation to time, while adriamycin and daunomycin, after a rapid fall at 30', tend to be absorbed much more slowly subsequently. The difference at 120' is statistically significant.

In experiments where a higher concentration (2 mg/Kg) was used, the results did not differ significantly from those obtained with 1 mg/Kg (Fig. 3).

In rabbits with ligated ureters, all drugs were absorbed more quickly than in intact animals, presumably as a consequence of lack of progressive dilution (Fig. 4).

Daunomycin differs from thiotepa also with regard to tissue fixation, which does not occur with thiotepa but is detectable for daunomycin and adriamycin even if the bound aliquot does not exceed 5 % of the administered dose. Fixation is greater when a higher dose is instilled (Fig. 5).

Traces of thiotepa only were detected in peripheral blood, whereas daunomycin and adriamycin could never be demonstrated outside the bladder.

CONCLUSIONS

From the data obtained, the following conclusion can be reached: Absorption of drugs from the bladder varies considerably according to the nature of the administered compound. Such a difference seems to be only partly related to molecular weight, as variation in bladder permeability to different drugs is not only quantitative but also qualitative, as shown by the different patterns of absorption curves as related to time.

Integrity of the urothelial layer is of the utmost importance with regard to bladder permeability. This holds true not only after transurethral resection, where extensive areas of mucosal surface are removed and the underlying muscle exposed, but also for simple diathermy where the lining is altered by coagulation necrosis. Absorption after endoscopic procedures was on

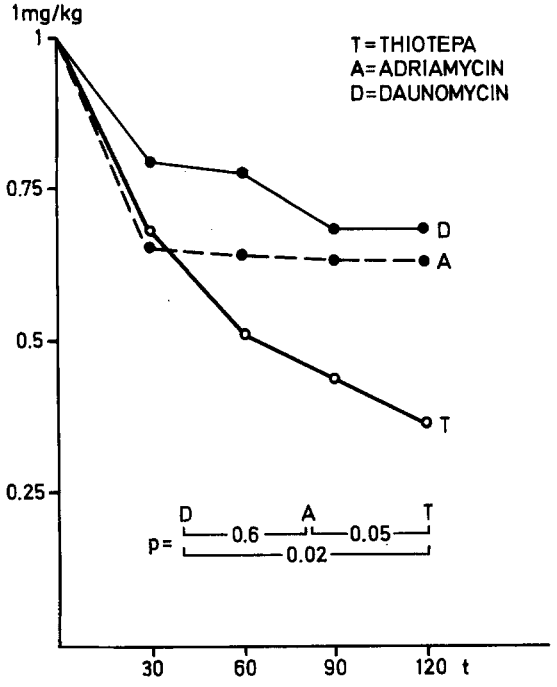

Fig. 2

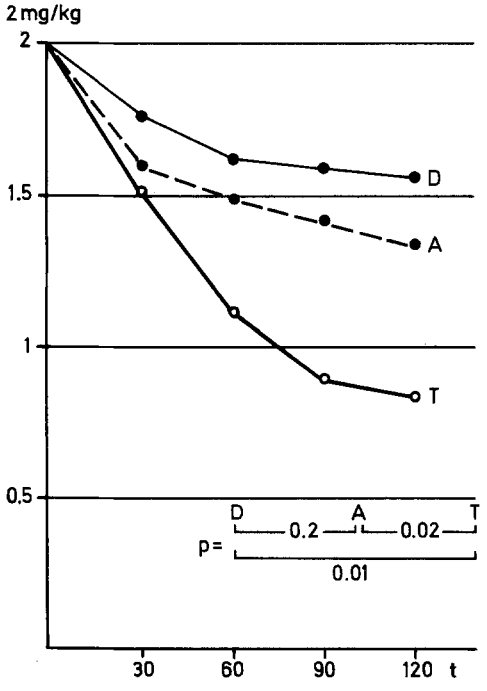

Fig. 3

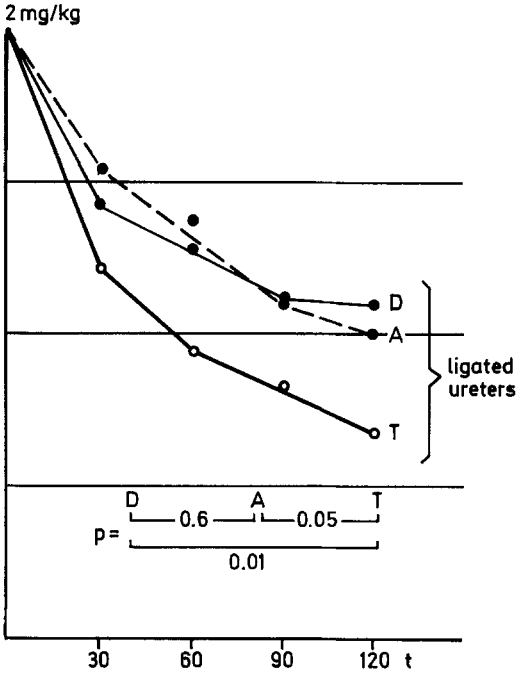

Fig. 4

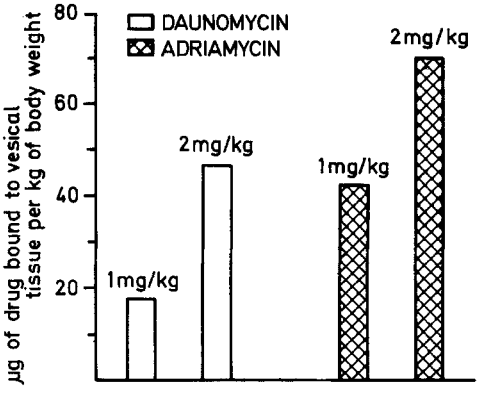

Fig. 5

Figs. 2, 3 and 4. Absorption of drugs from the rabbit bladder. All data are referred to the dose per kilogram of body weight. p-values apply to the final figures

Fig. 5. Adriamycin and Daunomycin fixation to the tissues of rabbit's bladder, 2 hours after instillation

Table 5. Absorption of drugs through the rabbit bladder

Drug	Dosage mg/Kg	No. of animals	Mean quantities (mg/Kg of body weight) of drugs remaining in bladder. All the per cent values refer to the quantities initially present in bladder											
			30'	±(S.D.)	%	60'	±(S.D.)	%	90'	±(S.D.)	%	120'	±(S.D.)	%
Daunomycin	1	7	0,79	0,158	21	0,77	0,134	23	0,68	0,151	32	0,68	0,151	32
Daunomycin	2	4	1,75	0,207	12,5	1,61	0,202	19,5	1,59	0,212	20,5	1,55	0,212	22,5
Daunomycin	2[a]	5	1,42	0,270	29	1,28	0,209	36	1,13	0,083	43	1,11	0,044	46
Adriamycin	1	5	0,65	0,137	35	0,64	0,151	36	0,63	0,161	37	0,63	0,161	37
Adriamycin	2	4	1,58	0,232	21	1,48	0,375	26	1,41	0,282	28,5	1,33	0,254	33,5
Adriamycin	2[a]	6	1,53	0,230	23,5	1,37	0,273	31,5	1,10	0,214	45	1,01	0,330	50
Thiotepa	1	5	0,68	0,194	32	0,51	0,219	49	0,44	0,202	56	0,36	0,173	64
Thiotepa	2	5	1,50	0,474	25	1,11	0,294	45	0,89	0,248	56	0,83	0,264	59
Thiotepa	2[a]	5	1,21	0,268	40	0,94	0,089	53	0,83	0,158	59	0,68	0,089	66

[a] These experiments were carried out on rabbits with ligated ureters
S. D. = standard deviation

average higher than in non operated bladders, even in cases where the mucosal surface was greatly increased by extensive papillary tumour. Solid anaplastic carcinoma produced a higher vesical permeability than well differentiated tumours, in spite of the fact that the latter often presented a greater mucosal surface area.

Intravesical chemotherapy in the presence of solid infiltrating bladder tumours is inappropriate and may also be dangerous. Topical chemotherapy for residual papillary lesions should be employed very cautiously in the early postoperative period.

REFERENCES

1. Dusonchet, L., Gebbia, N., Gerbasi, F.: Spectrophotofluorometric characterization of adriamycin, a new antitumour drug. Pharmacological Research Communication 3, 55 (1971)
2. Lunglmayr, G., Czech, K.: Absorption studies on intraluminal thio-tepa for topical cytostatic treatment of low-stage bladder tumors. Journal of Urology 106, 72 (1971)
3. Pavone-Macaluso, M., Gebbia, N.: Absorption of drugs from the bladder and intravesical chemotherapy. Urological Research 4, 1-3 (1976)
4. Yulin, L. Tan, Donald, R. Cole.: New method for determination of alkylating agents in biologic fluids. Clinical Chemistry 2, 58 (1965)

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