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URINATION FREQUENCY AND CYSTIC PRESSURE RESISTANCE AFTER FRACTIONATED WHOLE OR PARTIAL IRRADIATION OF THE RABBIT URINARY BLADDER

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Urination frequency and cystic pressure resistance have been used as end-points to assess x-ray-induced changes of bladder function. Whole or half bladders of adult male rabbits were irradiated, caudally or cranially. The absorbed dose was 33 Gy, 36 Gy or 39 Gy, given in 5 daily fractions. Animals which received a whole bladder dose of 39 Gy or 36 Gy showed increased urination frequency and enhanced bladder pressure resistance during the whole follow-up time of 100 weeks, compared with the sham-irradiated controls. At half bladder irradiation, only the highest doses (39 Gy to the cranial part of the bladder and 39 Gy or 36 Gy to the caudal part) gave rise to a slight increase in frequency at about 20 weeks after exposure.

New qualities of ionizing radiations together with improved application techniques raised the possibility for a tumor conforming precision therapy (e.g. 1, 2). The advantage of such a treatment would mainly be a reduction of the healthy tissue target volume, for example by application of dynamic spot scan techniques in irradiation of bladder carcinomas (3).

To obtain the best results with new radiation modalities, data on the tolerance dose as a function of the field size are required. In the present pilot study, the short- and long-term effects of x-rays on normal bladder were therefore investigated in a rabbit model. The aim of this study was to investigate the influence of the tissue volume irradiated on bladder function in assays similar to those introduced for the mouse model by Stewart et al. (4).

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Material and Methods

Adult male New Zealand rabbits weighing 2.8–3.5 kg were kept in individual cages and had free access to water and rabbit pellets (Veterinaria). The animals were anesthesized 10 min prior to irradiation or sham-irradiation, with 6 mg/kg Rompun (Bayer) and 60 mg/kg Ketalar (Bayer) injected i.m. Catheterization of the bladder was made with 2.7 mm baby balloon catheters (Euromedical) and, after voiding, the bladder was filled with 40 ml contrast medium Urografin (Schering) 76% in physiological NaCl, 1:20. Groups of three animals were irradiated according to different protocols.

The anesthesized animals were placed in a specially designed plexiglass holder with lead windows that permitted directing the radiation field on the whole or half bladder with little exposure of surrounding tissue. To confirm the position of the bladder, low-power x-ray pictures (9.0 mA, 4.5 s, 50 kV) were made before each fraction of irradiation. Three animals per treatment group received a total dose of 0 Gy, 33 Gy, 36 Gy or 39 Gy in 5 fractions on 5 successive days. The bladders were irradiated with 300 kV x-rays (Isovolt 3002 from Seifert; filtration: 1.0 mm Al) through two opposed lateral fields at a source-to-skin distance of 50 cm. The dose rate was

0.8 Gy min^{-1}. The radiation fields were defined by adaptable lead windows, 8 mm thick. The follow-up period was 100 weeks. One animal from the group '36 Gy, whole bladder' and one from the group '39 Gy, whole bladder' had to be killed 74 and 75 weeks after treatment respectively due to heavy loss of weight.

The assay was based on a system for convenient measurement of radiation-induced bladder impairment in mice (4). The urination frequency was measured every three weeks over 24-h-test periods by placing the animals in metabolism cages. The excreted urine dropped onto a bimetal thermometer and the signal, caused by a rise in temperature, was transmitted to a multi-channel chart recorder and registered as a single pulse for each micturition event. The urination frequency is given as the number of pulses per 24 h. The average volume of urine per micturition event was also calculated (amount of urine/number of micturitions per 24 h). Cystometry was performed once every 8-week period by measuring the intracystic pressure at 40, 30, 20, and 10 ml volume. The animals were anesthesized and the bladders were completely voided through a sterile catheter. The bladders were then filled in steps of 10 ml with isotonic saline kept at 22°C. Measurement was performed one min after each such an instillation step.

The animals were weighed weekly. Fresh urine samples were taken by cystometry for urine test stripes (Combur-9-test from Boehringer) to ascertain healthy conditions.

Based on the definition of Rubin & Casarett (5), a modified definition of an experimental tolerance dose, $TD_{66.7/100}$, was introduced. It is defined as the dose at which two-thirds of the animals develop a urination frequency three-fold the mean value of the control animals, 100 days p.i.

Results

Generally, no signs of untoward damage related to the urinary bladder were seen. The urine test was uniformly normal during the whole test period of 100 weeks. The weight increase was essentially the same for irradiated as for sham-irradiated animals. A few animals which expressed very strong changes in bladder function lost some weight near the end of the follow-up period and two of them had to be killed due to heavy weight-loss at week 74 and 75 respectively.

The sham-irradiated animals had a constant and similar urination frequency (8 ± 2 per 24 h) during the entire period of observation. All animals in the '39 Gy, whole bladder' group developed a rapidly increasing frequency one week after irradiation but the situation had always returned to control levels at week 10. After this acute phase, a progressively increased frequency was again observed after a follow-up period of 20 weeks until the end of the experiment after 100 weeks (Fig. 1). The '36 Gy, whole bladder' group also showed an enhanced frequency compared with that of the control. The effect here was less pronounced but it was detectable during the whole period after week 10 (Fig. 2). In the '33 Gy, whole bladder' group only one single animal developed an increased urination frequency, around week 22 (Fig. 3).

All animals but one (in the group '33 Gy, caudal bladder') that had received a 'half bladder' irradiation expressed less obvious reactions. The groups in which the caudal part of the urinary bladder had been given 39 Gy and 36 Gy, and those in which the cranial part of the bladder had been irradiated at 39 Gy, expressed a slight enhancement of the mean urination frequency around week 20. The increased frequency returned to the control level after week 30. Mean

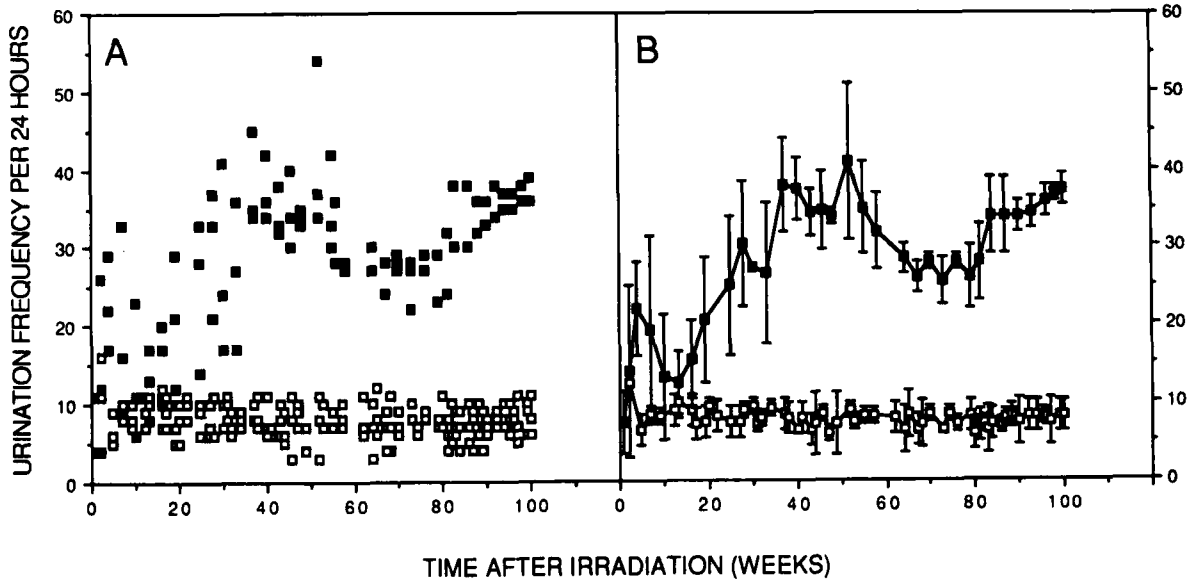

Fig. 1. Urination frequency after whole bladder exposure with 39 Gy. A) Urination frequency of individual animals. B) Means of all animals per group. Bars indicate standard errors. □ = sham-irradiated control rabbits. ■ = irradiated animals.

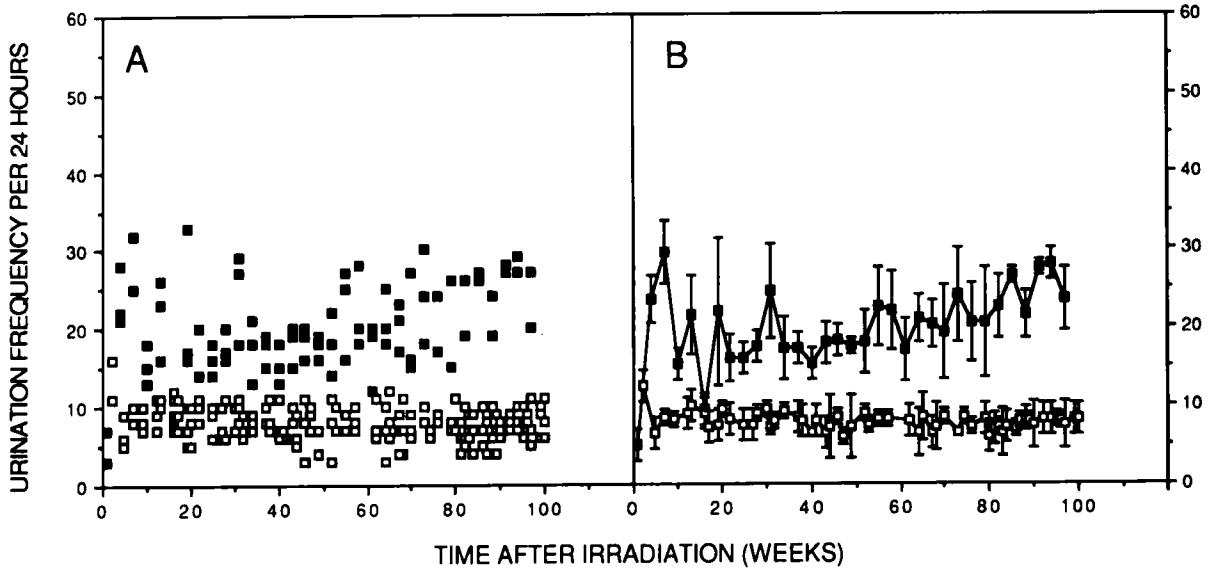

Fig. 2. Urination frequency after whole bladder exposure with 36 Gy. See legend to Fig. 1.

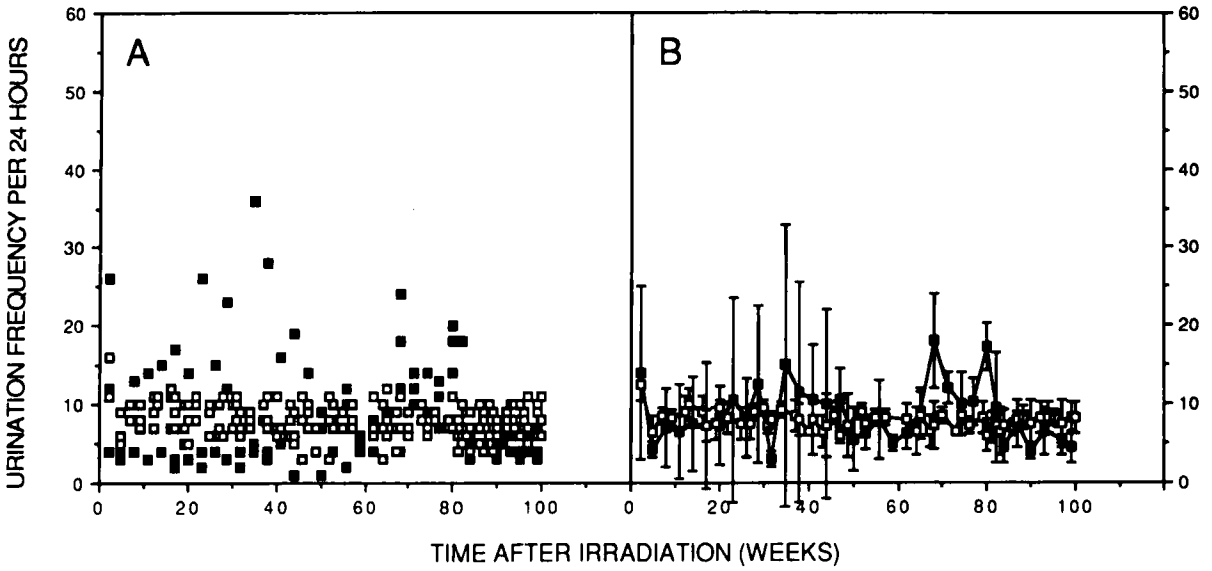

Fig. 3. Urination frequency after whole bladder exposure with 33 Gy. See legend to Fig. 1.

frequency values of the '33 Gy, caudal bladder' animals were similarly increased in the period 5 to 30 weeks and one animal had a very high frequency from 60 to 70 weeks (data not shown). Thus, a clear dose–response correlation was seen only after irradiation of the whole bladder.

To exclude a possible influence of the amount of urine excretion, the amounts of urine per micturition event and per 24-h-period were calculated as described above (Table). In the control animals the mean urine volume per micturition event was 16.2 ml and the mean urine volume per 24 h was 162 ml. The amount of excreted urine per micturition event correlated inversely with increased urination frequency but values for urine per 24 h remained constant for most irradiated animals (Table). In one rabbit from the '33 Gy, caudal bladder' group, reacting with very high urination frequency, there was a high total excretion of urine. The amount of urine per event was within control range.

An increased pressure profile was seen in whole-bladder-treated rabbits as compared with the controls. With the exception of the '33 Gy, whole bladder' group, the whole bladder-irradiated animals developed more conspicuous changes than did the caudal bladder-treated groups. Animals exposed over the cranial bladder did not show significant changes in the pressure profile after any of the doses tested. These results are in agreement with the urination frequency measurements. The onset of a detectable increase of the bladder pressure was around week 14, after

Table

Excreted urine volume per micturition event and per 24 h

	Treatment		
	Weeks 1–5	Weeks 20–30	Weeks 60–70
Excreted urine volume per micturition event (ml)			
Control	16.0 ± 2.9	15.9 ± 0.8	16.7 ± 0.7
Whole bladder			
39 Gy	5.6 ± 0.9	3.3 ± 0.4	4.7 ± 0.3
36 Gy	7.8 ± 1.5	7.0 ± 0.4	7.7 ± 1.8
33 Gy	11.9 ± 3.7	10.1 ± 1.8	10.5 ± 2.6
Caudal part			
39 Gy	10.4 ± 1.3	12.3 ± 2.6	16.7 ± 2.5
36 Gy	14.2 ± 3.2	13.4 ± 3.6	16.1 ± 2.3
33 Gy	12.6 ± 0.9	13.4 ± 1.8	15.9 ± 3.1
Cranial part			
39 Gy	9.0 ± 1.6	10.0 ± 2.4	16.7 ± 4.8
36 Gy	12.4 ± 1.9	15.4 ± 2.3	13.4 ± 1.8
33 Gy	13.9 ± 2.3	14.7 ± 3.4	18.1 ± 1.3
Excreted urine volume per 24 h (ml)			
Control	174 ± 33	151 ± 25	161 ± 21
Whole bladder			
39 Gy	154 ± 20	120 ± 20	141 ± 12
36 Gy	154 ± 27	152 ± 27	146 ± 20
33 Gy	158 ± 20	157 ± 23	153 ± 20
Caudal part			
39 Gy	156 ± 18	165 ± 30	156 ± 17
36 Gy	151 ± 19	172 ± 29	178 ± 27
33 Gy	206 ± 47	153 ± 19	217 ± 59
Cranial part			
39 Gy	147 ± 31	140 ± 14	154 ± 17
36 Gy	146 ± 6	168 ± 29	149 ± 13
33 Gy	154 ± 20	156 ± 9	172 ± 30

Values are means ± standard errors.

whole bladder or caudal bladder irradiation. Results of this assay showed no dose–response relationship.

Discussion

The urination frequency test and cystometry were used as functional assays for radiation response. These assays permitted single animals to be followed sequentionally throughout the entire period of study when reactions could occur. The experiments were designed to elucidate the importance of irradiated tissue volume in experiments on rabbits simulating therapeutic irradiation of bladder carcinoma.

Our findings at whole bladder irradiation are in agreement with results of Stewart et al. (4, 6, 7) in experiments on mice. After an acute reaction during the first 10 weeks, which was not reported for the murine system, urination frequency returned to normal values. The onset of permanent urination frequency changes was seen around weeks 20–30 after exposure, especially in animals with a whole bladder treatment. Half-organ irradiated animals showed no significant enhancement of urination frequency even after the highest delivered doses.

Results of cystometrical analysis did not show an increase of response with increased dose, the determining factor seemed instead to be the irradiated normal tissue volume. Data obtained from frequency measurements may be more reliable, since these tests were made over a 24-h-period to eliminate diurnal variations in contrast to cystometry, which was performed in 15–20 min per animal and testing.

In our study, a modified definition of the term tolerance dose, introduced by Rubin & Casarett (5), was selected. The $TD_{66.7/100}$ was regarded to be reached if, 100 weeks after exposition, two out of three rabbits of a particular treatment group developed a three-fold increased urination frequency compared with the mean control values.

Based on the above-mentioned definition the tolerance dose for the whole bladder was 36 Gy total dose given in 5 daily fractions. If half of the tissue volume was irradiated the tolerance was higher than 39 Gy. Apparently the dose levels were too low for a measurable effect in half bladder irradiation and an accurate estimate of the dose modifying factor for the tissue volume is impossible in this pilot study. However, our studies demonstrate that the tissue tolerance dose was increased at partial treatment of the bladder in the order of 10% or more compared to the whole-organ irradiation.

Our model can be used to obtain important preclinical information on bladder tolerance for new radiation modalities in therapy which allow a better conformation of field size and tumor shape (pions, protons, light ions conformation therapy). The results also indicate that objective recordings of micturition frequency in patients could help to understand the value of a reduced target volume and should therefore be addressed very accurately.

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REFERENCES

1. Kummermehr J, Schraube H, Ries G, et al. Biological effectiveness of neutrons and pi-mesons in gut, bone, and transplantable tumors. Strahlenther Onkol 1989; 165: 276–82.
2. van der Kogel AJ, Martin JC, Smith AR, Raju MR. Late effects of fractionated pi-mesons compared to X-rays on mouse lung. Int J Radiat Oncol Biol Phys 1988; 14: 1175–84.

3. Studer UE, von Essen CF, Enderli J-B, Bodendörfer G, Zingg EJ. Preliminary results of a phase I/II study with pi-meson (pion) treatment for bladder cancer. Cancer 1985; 56: 1943–52.
4. Stewart FA, Michael BD, Denekamp J. Late radiation damage in the mouse bladder as measured by increased urination frequency. Radiat Res 1978; 75: 649–59.
5. Rubin P, Casarett GW. A direction for clinical radiation pathology. Front Radiat Ther Onc 1972; 6: 1–16.
6. Stewart FA, Randhawa VS, Michael BD, Denekamp J. Repair during fractionated irradiation of the mouse bladder. Br J Radiol 1981; 54: 799–804.
7. Stewart FA, Randhawa VS, Michael BD. Multifraction irradiation of mouse bladders. Radiother Oncol 1984; 2: 131–40.