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Intravesical Cytostatics: pH-Dependence of Antitumour Activity

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Summary. The effects of pH on the antitumour activity of six cytostatics – cisplatin, doxorubicin, ethoglucid, mitomycin C, thiotepa and VM-26 – used in intravesical instillations were tested in vitro. The activity of cytostatics was determined on the Walker 256 carcinosarcoma cell line by using ATP-bioluminescence method. Cytostatics were incubated at different pH's (5.0–7.4) for 2 and 4 h. The activity of most of the cytostatics was not affected by pH, but mitomycin C had somewhat lower activity at pH 5.0 at 4 h and cisplatin at pH 6–7 at 2 and 4 h. Because the pH of normal urine is within the range used in this test, the use of a buffer solution during the intravesical instillation therapy is not necessary. However, according to our clinical experience, buffering is recommended to avoid chemical cystitis.

Key words: Instillation therapy, Superficial bladder cancer, Antitumor activity, Cytotoxic effect, Urinary pH.

Introduction

The pH of urine varies normally between 5 and 8, but it may exceptionally be as low as 4.6 or as high as 8.2 [3]. It has been shown that phosphate buffered physiological saline solution with a pH of 7.4 provides optimal conditions for intravesical mitomycin C therapy; acidic conditions may decrease the antitumour activity of mitomycin C [2]. The purpose of this work was to investigate the antitumour activity of other intravesically administered cytostatics in the physiological pH range of urine.

Materials and Methods

The method for determination of the antitumor activities has been described in a previous publication [2]. Since the intracellular ATP concentration indicates the amount of viable cells in a suspension, measurements of ATP were used to assay the cytostatic effect of the drugs.

The cell line used in this study was rat Walker 256 carcinosarcoma.

A luminometer (type 1250, LKB-Wallac, Turku, Finland) equipped with LKB 2210 potentiometric recorder was used for the determination of ATP. The ATP Monitoring reagent® (LKB-Wallac, Turku, Finland) containing purified firefly luciferase and Tris-HCl buffer (0.25 M, pH 7.75) was needed for light reaction in the bioluminescence assay. Trichloracetic acid (1%) was used to release the intracellular ATP from the cells.

The following buffers were used (obtained from Baker or Merck): citrate-HCl (pH 5.0 and 6.0); phosphate buffer (KH_2PO_4 – Na_2HPO_4), pH 7.0; and phosphate buffered saline (pH 7.4). The buffers were prepared as described by Stauff and Jaenicke [4].

The cytostatics used were: cisplatin (Platinol®), doxorubicin (Adriamycin®), ethoglucid (Epodyl®), mitomycin C (Mutamycin®), thiotepa (Thio-tepa®) and VM-26 (Vumon®). Approximate IC-50 concentrations were determined for each drug. These concentrations appear in Table 1.

Results

The ATP levels measured by bioluminescence are presented in Table 1. The cytostatic activities at different pH's as per cent of control are shown in Fig. 1.

The activity of most of the cytostatics tested was not affected by the pH of the growth media. Mitomycin C had a somewhat lower activity at a pH of 5.0 but only after 4 h incubation. Cisplatin showed a slight reduction of cytostatic activity at pH of 6–7 at 2 and at 4 h as compared to the antitumour activity at other pH-values.

Discussion

ATP is a good indicator of the amount of living cells in the experimental setting described in this work [2]. By using bioluminescence, it is possible to determine the amount of ATP in the cell suspensions conveniently and rapidly. The ATP bioluminescence method may thus be employed to determine optimal pH conditions for cytostatic solutions.

Table 1. Number of living Walker 256 cells x 10^6/1 ml cell suspension (mean ± sd) after 24 h growth in vitro in the presence of cytostatic drugs, which have been preincubated at indicated pH's for 0.2 or 4 h

Drug and pH	*n*	Preincubation period (h)		
		0	2	4
Cisplatin 3 μg/ml				
pH 5.0	3	1.8 ± 0.1	2.1 ± 0.1	2.6 ± 0.4
pH 6.0	3	2.2 ± 1.0	3.1 ± 0.3	3.5 ± 0.2
pH 7.0	3	2.6 ± 0.1	3.7 ± 0.6	3.9 ± 0.2
pH 7.4	3	2.0 ± 0.03	1.8 ± 0.2	1.8 ± 0.2
Controls	6	6.1 ± 0.3		
Doxorubicin 5 μg/ml				
pH 5.0	3	1.3 ± 0.1	2.0 ± 0.7	1.5 ± 0.1
pH 6.0	3	2.4 ± 0.4	2.1 ± 0.1	2.1 ± 0.3
pH 7.0	3	1.8 ± 0.2	1.9 ± 0.1	2.1 ± 0.1
pH 7.4	3	1.5 ± 0.0	1.9 ± 0.1	1.4 ± 0.2
Controls	12	5.2 ± 0.3		
Ethoglucid 400 μg/ml				
pH 5.0	3	1.1 ± 0.1	1.3 ± 0.2	1.3 ± 0.2
pH 6.0	3	1.3 ± 0.1	1.0 ± 0.1	1.4 ± 0.2
pH 7.0	3	1.2 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
pH 7.4	3	1.8 ± 0.2	2.0 ± 0.1	2.0 ± 0.1
Controls	12	5.1 ± 0.4		
Mitomycin C 0.5 μg/ml				
pH 5.0	3	0.17 ± 0.00	0.21 ± 0.02	0.50 ± 0.14
pH 6.0	6	0.21 ± 0.04	0.24 ± 0.06	0.28 ± 0.05
pH 7.0	6	0.19 ± 0.05	0.22 ± 0.04	0.22 ± 0.03
pH 7.4	6	0.22 ± 0.02	0.21 ± 0.04	0.22 ± 0.04
Controls	3	0.82 ± 0.14		
Thiotepa 15 μg/ml				
pH 5.0	3	2.9 ± 0.2	2.9 ± 0.2	2.8 ± 0.3
pH 6.0	3	3.0 ± 0.1	2.8 ± 0.03	2.7 ± 0.03
pH 7.0	3	3.0 ± 0.2	2.8 ± 0.1	2.8 ± 0.2
pH 7.4	3	2.5 ± 0.1	2.6 ± 0.1	2.7 ± 0.3
Controls	6	4.0 ± 0.1		
VM-26 1 μg/ml				
pH 5.0	3	2.0 ± 0.0	1.9 ± 0.1	2.0 ± 0.1
pH 6.0	3	2.9 ± 0.2	2.7 ± 0.4	2.8 ± 0.5
pH 7.0	3	2.9 ± 0.03	2.9 ± 0.1	2.8 ± 0.5
pH 7.4	3	2.5 ± 0.2	2.5 ± 0.1	2.6 ± 0.3
Controls	11	5.0 ± 0.3		

The effect of urinary pH on the activity of intravesically administered cytostatics is not well documented, with the exception of mitomycin C [2]. The effect of pH on the concentration of doxorubicin has been tested by Eksborg et al. [1], who found that the concentration of doxorubicin rapidly decreased in alkaline solution (pH about 10). The levels of doxorubicin in acidic solutions were not tested.

In this study we found that the commonly used intravesical cytostatics retain their activity at the physiological urinary pH's. The use of a buffer solution in clinical intravesical cytostatic administration is not mandatory. However, according to our clinical experience, buffering with or without glucocorticoids is recommended to avoid chemical cystitis.

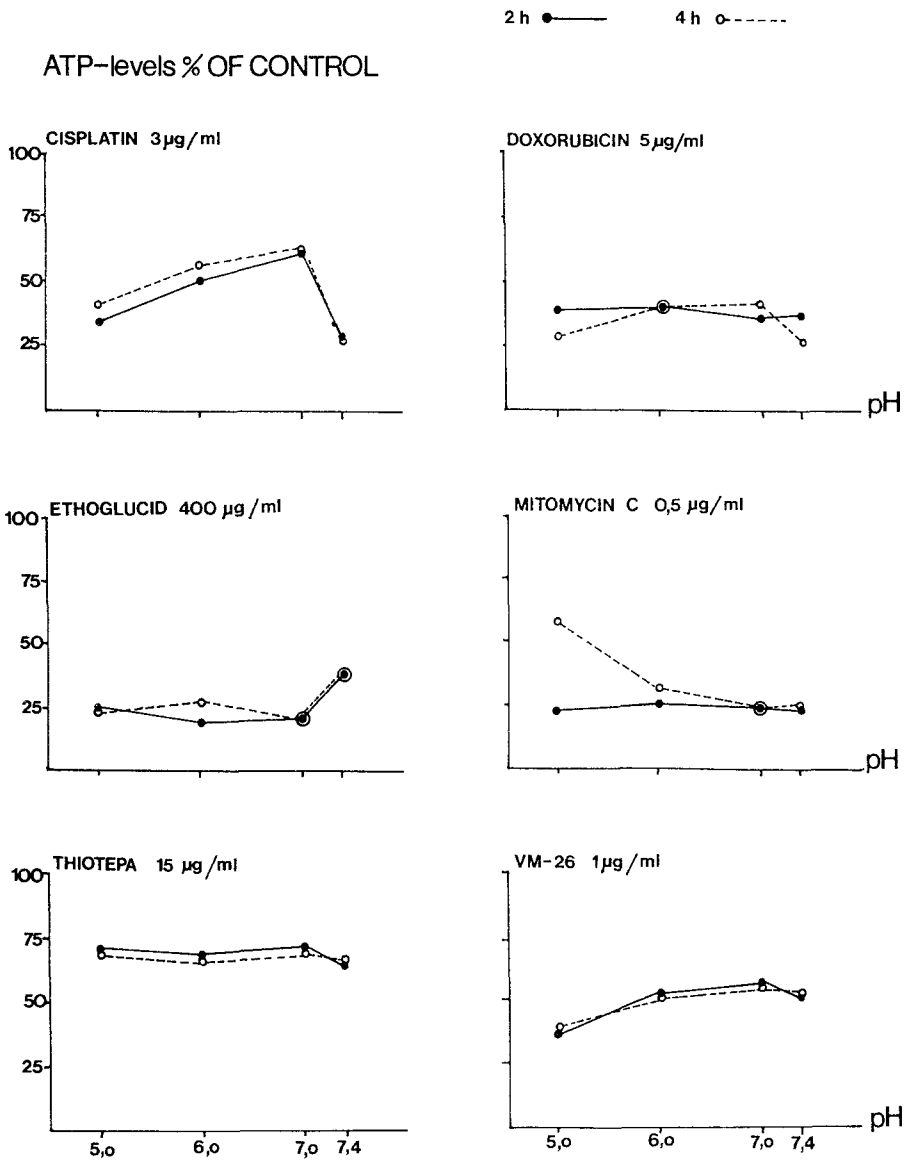

Fig. 1. The effect of pH on the activity of six cytostatics

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