Optimising Mitomycin C Activity During Intravesical Instillation

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Summary. Walker 256 carcinosarcoma was shown to be sensitive to mitomycin C in vitro. In order to make practical recommendations for the clinical intravesical use of mitomycin C, this cell line was used to evaluate the activity of mitomycin C under different conditions. Mitomycin C loses its antitumour activity rapidly at pH's below 6. At higher pH's (up to 10) mitomycin C is stable and suitable for intravesical application. To secure a sufficiently high pH in the bladder during intravesical treatment, phosphate buffer 0.05 M with a pH of 7.4 is recommended. Constituents of urine very little decrease the activity of buffered mitomycin C during a 2 h application. Prednisolone, which has been suggested to prevent the harmful chemical cystitis, has no inhibitory effect on the activity of mitomycin C.

Key words: Mitomycin C, Instillation therapy, Superficial bladder cancer, Antitumour activity, Urinary pH

Introduction

Mitomycin C is an aminoquinone antibiotic isolated from Streptomyces caesporotus in 1956. It is effective against many human malignancies [1]. For 15 years mitomycin C has been used as local instillation therapy for superficial bladder cancers [7]. In 1977 Kato showed that in a solution containing 0.02-1 μg of mitomycin C per ml half of the T-24 cells (line isolated from human bladder transitional cell carcinoma) died in 2 h [7].

Adenosine triphosphate is the basic energy source of living cells and not found in dead cells [4]. In our study we have measured the ATP content of Walker 256 carcinosarcoma cells in experimental conditions after mitomycin C incubations to evaluate the sensitivity of the cell line in these conditions.

The pH of human urine may vary between 4.6 and 8.2 [9]. It is presumed that the extreme pH's may affect the stability of the cytostatic drug during instillation.

Materials and Methods

Luminometer 1250 (LKB-Wallac, Turku, Finland) equipped with LKB 2210 potentiometric recorder was used for the determination of ATP.

ATP monitoring reagent R (LKB-Wallac, Turku, Finland), containing purified firefly luciferase and Tris-HCl buffer (0.25 M, pH 7.75) were needed for light reaction in bioluminescence assay. TCA (1%) was used to release the intracellular ATP from the cells. Mitomycin C was obtained from Bristol-Myers Company, and prednisolone from Sigma Chemical Company.

The following buffers (reagents obtained from Baker or Merck) were used: citrate-HCl (pH 2.0, 4.0, 5.0 and 6.0), Mg(OH)2-NaHCO3 (pH 6.0 and 7.4), citric acid-phosphate (pH 6.0 and 7.0), phosphate buffer (KH2PO4-Na2HPO4) (pH 7.0, 7.4 and 8.0), glycine-NaOH (pH 10.0) and phosphate - buffered saline (pH 7.4). The buffers were prepared according to Stauf and Jaenicke [10].

The cell line used in this study was rat Walker 256 carcinosarcoma. A solid tumour was removed from the subcutaneous space, cut into small pieces with a scalpel on a petri dish and spread out in the medium. The cell line was routinely maintained on RPMI1640 (with 10% foetal calf serum L-glutamine 292 mg/l, and penicillin and streptomycin 100 IU/ml and 100 μg/ml, respectively).

Determination of Mitomycin C Activity

The sensitivity of Walker carcinosarcoma 256 to mitomycin C was studied in vitro by incubating cells in the presence of different concentrations of mitomycin C (0, 0.01, 0.1, 1.0 and 5.0 μg/ml) in microtitre plates in the incubator (5% CO2 + 95% air, 37 °C) for 24 and 48 h. Cell viability was determined at each time by bioluminescence, i.e. by measuring adenosinetriphosphate, the basic energy source of the cells. The method has been described in detail in a
MITOMYCIN C; concentrations 0–5.0 µg/ml + BUFFER; pH 2–10

ingubation at 37°C
0.2 or 4 h

WALKER 256 CELL SUSPENSION

+ 1% TCA

Quantitation of living cells by bioluminescence

Fig. 1. Experimental design for testing mitomycin C stability

控制, no mitomycin C. On the shaded area are situated all results with buffers pH 6, 7, 7.4, 8 and 10

forthcoming paper [4] and is shortly as follows: cell suspension on microtitre plates is extracted for ATP by 1% TCA (100 µl suspension + 100 µl TCA solution) and mixed well with Finnpipette. 100 µl of the mixture is pipetted into measuring cuvettes containing buffered ATP monitoring reagent (400 µl). Bioluminescence is read directly after a short mixing by luminometer.

Testing of Mitomycin C Stability at Different pH's

The experimental procedure for testing mitomycin C stability is shown in Fig. 1.

Results

The sensitivity of Walker 256 carcinosarcoma to mitomycin C is illustrated in Fig. 2. It is evident that mitomycin C decreases cell viability in a dose- and time-dependent manner.

Cytostatic activity of mitomycin C after incubation for 0, 2 and 4 h in buffers with different pH (2–10) is presented in Fig. 3. At pH equal to or higher than 6, mitomycin C is stable at least for 4 h. In acidic conditions mitomycin C rapidly loses its cell-killing activity.

Search for a clinically useful buffer is presented in Table 1 and Fig. 4. The ideal buffer does not inactivate mitomycin C, may be instilled without bladder irritation, and has adequate buffering capacity to keep a sufficiently alkaline pH in the bladder during treatment. Table 1 shows that many buffers might be clinically useful (best perhaps Mg(OH)₂-NaHCO₃ buffer). It also shows that the activity of mitomycin C is not diminished in the presence of urine (10%). The final selection of the buffer results from Fig. 4: if titrated with human urine (pH 5.26), phosphate buffers can most effectively withstand the decrease of pH and are therefore recommended.