THE COMBINED USE OF DIMETHYL SULFOXIDE WITH CHEMOTHERAPEUTICS ON BLADDER CELL CULTURE

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ABSTRACT

Purpose : *In this experimental study, we investigated the effect of dimethyl sulfoxide (DMSO) on the in vitro cytotoxicity of chemotherapeutic agents doxorubicin (Dox.) and mitomycin-C (Mito.-C), which are used in the intravesical chemotherapy for the treatment and prophylaxis of superficial bladder cancer.* ***Materials and Methods :*** *R4909 bladder cancer cell line (Science 176: 1337, 1972) was maintained as monolayer cultures in 75 cm^2 flasks in RPMI-1640 medium supplemented with %10 fetal calf serum (FCS). After trypsinization, $2x10^5$ R4909 cells were distributed in sterile plates. On these prepared plates; DMSO, Mito.-C and Dox. was added at 1/2 dilutions separately and in combination with DMSO. After 72 hours incubation, 3-(4,5-dimethylthiazol-2)2,5 diphenyl tetrozolium bromide (MTT) was added to the plates. These plates were centrifuged at 3000 rpm for 6 minutes to be read under 550 nm ELISA reader in order to obtain the optic density (OD) value which shows the cell survival.* ***Results :*** *According to OD values, DMSO had minimal effect on cell survival at concentrations equal and less than 4%. No enhancement of tumor cell kill by the addition of DMSO was observed.* ***Conclusion :*** *Our data indicate that, the addition of DMSO to drug solutions for intravesical chemotherapy at the time of treatment is unlikely to enhance the cytotoxic effect of the intravesical chemotherapeutic agents studied.*

Key Words: *Dimethyl Sulfoxide, Intravesical Chemotherapy, Cytotoxicity, Bladder Cancer.*

INTRODUCTION

Superficial transitional cell carcinoma of the bladder comprises about 70-80% of cases at diagnosis and has a recurrence rate of between 30-90% in most series. Even though most recurrences tend to remain superficial, up to 30% will progress to disseminated superficial disease or infiltrative disease (1, 2). Hence, the treatment of superficial bladder cancer aims for the eradication of the primary tumor and then prevention of recurrences, some of which may invade deeper into the muscle layers. It is common urological practice to use intravesical therapy following transurethral resection (TUR) to lower the risk of recurrent disease.

Adjuvant intravesical chemotherapy or intravesical immunotherapy is indicated in patients who are at a high risk for tumor recurrence by virtue of having multiple tumors, recurrent tumors, high-grade tumors associated with urothelial atypia, or carcinoma in situ (3). To date, many chemotherapeutic agents have been used with different doses and schedules. However, it has been recently shown that

intravesical chemotherapy has not decreased the long-term incidence of tumor recurrence, progression and mortality (4). Attempts have been made to increase the efficacy and enhance anti-tumor activity of this treatment by promoting the penetration of drugs into urothelial cells by the addition of Tween 80, urokinase or dimethylsulfoxide (DMSO) (5-7). We previously showed that the concomitant addition of DMSO with intravesical chemotherapeutic agent enhances the absorption of the agent to the entire bladder wall in animal models (8).

The aim of the present experimental study was to determine the in vitro effect of DMSO on the cytotoxicity of two intravesical chemotherapeutic agents; Doxorubicin (Dox.) and mitomycin-C (Mito.-C) that are most frequently used.

MATERIALS AND METHODS

R4909 bladder cancer cell line (Science, 176: 1337, 1972) was maintained as monolayer cell cultures in 75 cm^2 flasks in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS). The cells were used over a restricted range of 10 passages, to minimise any changes resulting from long-term culture. For subculture, the cells were detached by 3-5 minutes incubation at 37°C in an aqueous solution of trypsin / EDTA (disodium salt of ethylene-diamine tetra-acetic acid) and seeded in fresh flasks at a split ratio of 1:10. After trypsinization, $2x10^5$ R4909 cells per well were distributed in 96-well flat-bottomed sterile plates and incubated for 24 hours at 37° C in a humidified atmosphere of 5% CO_2 in air. The culture fluid was then replaced with fresh medium alone or containing either DMSO, drug alone (Mito.-C versus Dox.) or in combination with DMSO, in triplicate. Standard pharmaceutical preparations of Dox. and Mito.-C were dissolved in sterile water and used at concentrations which reduced colony forming ability by approximately 50%. After 72 hours of incubation, 20 µl 3-(4,5-dimethylthiazol-2)2,5 diphenyl tetrozolium bromide (MTT) was added to the wells of the plates. In an effort to estimate the cell survival capacity, an in vitro assay was performed using a modification of the colorimetric MMT assay described by Mossman (9). These plates were then centrifuged at 1400 g for 6 minutes. After the aspiration of the fluid portion of the medium, 200 µl. isopropylalcohol was added and stored at +4° C overnight and then the optical density (OD) values were measured under the 550nm. ELISA reader to obtain the cell survival capacity.

The paired student's t test was used to determine statistical differences in different groups.

RESULTS

The dose response of R4909 cells exposed to a range of concentrations of DMSO is shown in Table-1. DMSO had a minimal effect on cell survival at concentrations equal and less than 4%.

The mean percentage clonogenic cell survival of R4909 cells exposed to the two drugs (Mito.C, Dox.) in the presence and absence of DMSO is shown in table-2. The cytotoxicity of Mito.-C and Dox. was reduced by 4% DMSO, although the data was not statistically significant. However, no enhancement of tumor cell kill by the addition of DMSO was observed.

Table - 1: Optical density values of R4909 cells after exposure to DMSO.

DMSO concentration (%)	Optic density	p value
1	.700	
2	.728	p>0.05*
3	.690	
4	**.644**	
5	.398	
10.	.371	p<0.05*
20	.270	

* Compared with optic density value at DMSO concentration of 4%.

Table - 2 : Optical density values after exposure of R4909 cells to each of two drugs, either alone or combined with 4% DMSO.

Cell treated with	Optical density	Standard error	p value
4%DMSO	.644	.024	
Mito.-C	.308	.030	>0.05
4%DMSO+Mito.-C	.399	.026	
Dox.	.402	.015	>0.05
4%DMSO+Dox.	.425	.012	

DISCUSSION

Bladder cancer is an important clinical problem, with an estimated 54,200 new cases and 12,100 deaths in the United States in 1999 (10). The magnitude of the problem is increased by the high prevalence of patients with superficial bladder cancer under active surveillance or treatment. Administration of adjuvant intravesical chemotherapy after transurethral resection of superficial bladder cancer has become common practice to try to reduce the high recurrence rate of these tumors and possibly to reduce progression towards invasive cancer. Most published studies have confirmed that intravesical treatment can reduce the short and intermediate term incidence of recurrence, but they have failed to demonstrate long-term reduction of the risk of progression towards invasive cancer (11,12). Intravesical Bacille Calmette-Guerin (BCG) therapy is the most effective intravesical therapy for the treatment and prophylaxis of superficial bladder cancer. Numerous clinical and laboratory studies during the last 20 years have focused on the optimal candidates for this treatment modality and the optimal regimen for BCG administration. Long-term follow-up results of BCG-treated patients are now reported (13).

Because intravesical chemotherapy prophylaxis has not decreased the long-term incidence of tumor recurrence, progression or mortality, several studies have been performed using different agents with varying doses and schedules and also using adjuvant agents. Tween 80 (trademark for polysorbate 80) is a surface-active detergent that has been reported to enhance the uptake of adriamycin into the urinary bladder wall and into the systemic circulation (14). It also has been reported to increase the cytotoxicity in vitro of drugs used for intravesical instillation therapy, although Tween 80 in itself is not cytotoxic (5).

Hyaluronidase is an enzyme that splits mucopolysaccharides containing hyaluronic acid. Experimental studies have shown that many tumor cells are surrounded by a halo containing hyaluronic acid, which prevents direct contact with the elements of cellular immune defense. In vitro and in vivo studies have demonstrated that adjuvant hyaluronidase increases the local response rate of malignant urothelial cells to chemotherapeutic agents (15,16). This effect presumably is due to improved diffusion into the bladder mucosa and enzymatic splitting of hyaluronic acid contained in a protective halo around malignant urothelial cells.

Dimethylsulfoxide is a dipolar solvent known to have various pharmacological activities, such as increasing the cytotoxicity of chemotherapeutic drugs, enhancement of membrane permeability and enhancement of the absorption of various drugs through the bladder (17). A concentration of 50% DMSO is also used clinically for the treatment of interstitial cystitis (18). We (8) and others (19) have also shown that absorption of intravesically instilled agents could be increased both throughout the bladder wall and tumoral lesions by using DMSO as an adjuvant to intravesical chemotherapeutic agents administration. In addition to its solvent properties, DMSO has been found to induce differentiation in tumor cell lines (20). Enhancement of cytotoxic drug effect in murine hepatocarcinoma cells (21) occurred after DMSO had been present in the medium for 48 hours prior to treatment, and was not seen on simultaneous exposure to drug and DMSO. Thus, it is possible that the effect of intravesical drugs on the human cell lines would be increased after a period of pre-treatment with DMSO.

It is important to know the advantages and disadvantages of these treatment modalities in order to be able to individualise treatment as much as possible. Dox. and Mito.-C have high molecular weights (580 and 329 Da respectively) so that systemic absorption of the drugs is very rare. It must be emphasised that, their systemic effects during treatment period can be increased when these drugs are used with additional agents that enhance their absorption through the bladder wall.

Our data indicate that, the addition of DMSO to drug solutions for intravesical chemotherapy at the time of treatment is unlikely to enhance the cytotoxic effect of the intravesical chemotherapeutic agents studied. The marked enhancement of drug absorption by DMSO may be helpful for the long-term results of intravesical chemotherapy for not only superficial tumors but also locally invasive bladder carcinoma.

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