Urol Res (1988) 16:329–331

Urological Research

Intravesical Chemotherapy: Combination with Dimethyl Sulfoxide Does not Enhance Cytotoxicity in Vitro

L. Walker, M. C. Walker, C. N. Parris, and J. R. W. Masters

Department of Pathology, Institute of Urology, University College London, St Paul's Hospital, London, United Kingdom

Accepted: March 10, 1988

Summary. There is evidence that dimethyl sulfoxide (DMSO) can increase the anticancer activity of chemotherapeutic drugs. As DMSO is instilled into the bladder for interstitial cystitis, it could be readily adopted in clinical practice if it was found to enhance the effectiveness of the drugs used for intravesical chemotherapy. The purpose of this study was to investigate, using a human bladder cancer cell line, the hypothesis that DMSO enhances the activity of these agents. However, the addition of 4% DMSO to the four drugs most frequently used for intravesical chemotherapy (adriamycin, epodyl, mitomycin-c, thiotepa) did not increase tumour cell kill in vitro.

Key words: Cytotoxic drugs – Dimethyl sulfoxide – Intravesical chemotherapy – Transitional cell carcinoma

Introduction

Between 75 and 85% of bladder cancers are confined to the superficial layers of the bladder on first diagnosis [1]. After conventional surgical treatment, either transurethral resection or diathermy, up to 82% of patients have a recurrence within one year [20]. Chemotherapeutic agents, instilled directly into the bladder, can delay tumour recurrence and invasion into muscle [8]. However, approximately one-third of the patients do not gain any benefit from this form of treatment [19], and more effective intravesical chemotherapy is needed.

Dimethyl sulfoxide (DMSO) is a dipolar solvent. In combination with chemotherapeutic drugs it increases antitumour activity in vivo in rats [22] and in vitro against mouse hepatocarcinoma cells [18]. With topical preparations, DMSO enhances uptake of acyclovir into the skin [15], and of salicylates [3] and cisplatin [14] through the bladder. The solvent is already used clinically as a bladder instillation for the treatment of interstitial cystitis [16]. We therefore studied in vitro the effect of DMSO on the cytotoxicity of the four intravesical chemotherapeutic agents that are most frequently used.

Materials and Methods

Drugs

Standard pharmaceutical preparations of Adriamycin (doxorubicin HCl, Farmitalia Carlo Erba, St Albans, England), Epodyl (ethoglucid, Imperial Chemical Industries, Macclesfield, England) Mitomycin-C (Kyowa Hakko Kogyo Co Ltd, Tokyo, Japan), and Thiotepa (triethylene thiophosphoramide, Lederle Laboratories, Gosport, England) were dissolved in sterile water (water for injection, BP, Beecham, Brentford, England) as stock solutions, and stored at −20 °C until use. The drugs were dissolved in tissue culture medium and used at concentrations which reduced colony-forming ability by approximately 50%: 400 ng/ml adriamycin, 50 μg/ml epodyl, 400 ng/ml mitomycin-c, and 15 μg/ml thiotepa.

Cell Line

RT112 is a continuous cell line, established in this laboratory in 1970. The line was derived from a moderately-differentiated human transitional cell carcinoma of the bladder [16]. The cells on xenotransplantation produce tumours histologically similar to the original biopsy [6, 9].

Cell Culture

RT112 cells were maintained as monolayer cultures in 25 cm^2 flasks (Nunc, Gibco, Paisley, Scotland) in RPMI 1640 medium (Gibco) supplemented with 5% heat-inactivated foetal bovine serum (Flow, Irvine, Scotland) and 1% L-glutamine (Gibco) in a humidified atmosphere of 5% CO_2 in air. 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 to 5 minutes' incubation in an aqueous solution of 0.05% trypsin (Difco 1:250, London, England) and 0.016% Versene (disodium salt of ethylene-diamine tetra-acetic acid; BDH Chemicals, Poole, England) and seeded in fresh flasks at a split ratio between 1:5 and 1:10.

Clonogenic Assay

Five hundred RT112 cells, with a plating efficiency of approximately 40%, were plated in 5 cm vented plastic petri dishes (Nunc, Gibco, Paisley, Scotland) and incubated for 48 hours at 36.5 °C in a humidified atmosphere of 5% CO_2 in air. The culture fluid was then replaced with fresh medium alone or containing either 4% DMSO, drug alone or in combination with 4% DMSO, in triplicate dishes. After exposure lasting one hour, the medium was removed and the cells washed three times with 5 ml aliquots of medium. Fresh medium was then added, and the cells incubated for 14 days at 36.5 °C in a humidified atmosphere of 5% CO_2 in air to permit colony growth. The colonies were fixed in methanol (BDH) and stained with 10% Giemsa (BDH). Colonies consisting of 50 or more cells were counted using a binocular dissecting microscope. The mean colony-forming ability was expressed as a percentage of the controls. The data are derived from a minimum of three separate experiments for each drug.

To control for the influence of pH on clonogenic cell survival, the pH of medium alone and containing 4% DMSO were compared after incubation for 24 hours in identical conditions. Measurements were made with a Dow Corning pH meter and a KCl electrode (Dow Corning, Halstead, England) at 36.5 °C.

Results

The dose-response of RT112 cells exposed for 1 hour to a range of concentrations of DMSO is shown in Table 1. DMSO had a minimal effect on colony forming ability at concentrations of less than 5%. The addition of DMSO to medium produced an increase in the mean value of the pH by 0.16 units at 36.5 °C.

The mean percentage clonogenic cell survival of RT112 cells exposed to the four drugs in the presence and absence of DMSO is shown in Table 2. The cytotoxicity of mitomycin-c was reduced by 4% DMSO, although the data were not statistically significant. However, no enhancement of tumour cell kill by the addition of DMSO was observed.

Discussion

As a potential adjuvant to intravesical treatment, DMSO has the advantage that a 50% solution is already used in the bladder for the treatment of interstitial cystitis [16] and positive findings could be applied directly in clinical studies. Previous findings in animals [17, 22] and in mouse tumour cell lines [18] prompted the hypothesis that DMSO might increase drug cytotoxicity in human cell lines. Clonogenic assay of human bladder and testicular cancer cells in monolayer culture provided data consistent with the clinical pattern of response to radiation and drug treatment of these tumours in patients [11, 21]. Previous observations in vitro on the enhancement of drug effect by combination with another solvent, Tween 80 [12], are supported by clinical studies of adriamycin combined with Tween 80 in the treatment of superficial bladder cancer [4]. However, in these studies, DMSO did not increase the effect of the intravesical agents tested.

In view of the range of chemical structures of the anticancer agents studied and their lack of effect, it was thought unlikely that DMSO had changed the structure or degree of ionisation of the drugs. The alteration of the pH of the solution resulting from the presence of DMSO is small, and would have different effects on the activities of these drugs [5]. Although the effect of mitomycin-c was reduced by the addition of DMSO, consistent with the rise in pH, the small increase in acidity would not account for the reduced cell

Table 1. Percentage clonogenic cell survival as a function of concentration of dimethyl sulfoxide concentration (vol/vol)

DMSO concentration	Mean % Clonogenic Cell Survival	Standard Error
1%	91.7	3.58
2%	88.6	12.7
3%	96.1	7.87
4%	93.4	5.30
5%	90.7	3.70
6%	82.0	1.71
8%	69.2	7.12
10%	59.1	3.03
20%	44.2	10.0

Table 2. Mean percentage clonogenic cell survival after a one-hour exposure of RT112 cells to each of four drugs, either alone or combined with 4% dimethyl sulfoxide

Cell Treatment	Mean % Clonogenic Cell Survival	Standard Error	t-value	Probability
4% DMSO alone	94.5	2.94		
Adriamycin	46.5	5.21		
Adriamycin + DMSO	49.3	8.13	0.2839	0.785
Mitomycin-C	44.9	1.97		
Mitomycin-C + DMSO	62.2	6.58	2.151	0.065
Epodyl	74.7	5.80		
Epodyl + DMSO	68.1	4.72	0.8849	0.397
Thiotepa	60.0	8.02		
Thiotepa + DMSO	59.9	6.80	0.0095	0.993

kill. No comparable reduction of effect was seen when DMSO was combined with thiotepa which, like mitomycin-c, is more active in a low pH environment.

The enhanced cytotoxicity observed with DMSO in the animal models and in vitro may represent a different phenomenon from that seen with Tween 80. The latter solvent is thought to act by increasing membrane permeability to drugs [4, 12]. This effect was most pronounced with relatively impermeable, ionised drugs, such as adriamycin. This finding confirmed in vitro data from other laboratories [13], and supported the view that Tween 80 enhanced clonogenic cell kill by facilitating the passage of ionised drugs through the cell membrane. Although it was a tenable hypothesis that DMSO might have a similar effect, the present data do not support this view, and enhancement by DMSO of the effects of anticancer agents observed in other model systems requires a different explanation.

In addition to its solvent properties, DMSO has been found to induce differentiation in tumour cell lines [2, 7, 10]. Enhancement of cytotoxic drug effect in murine hepatocarcinoma cells [18] occurred after the DMSO had been present in the medium for 48 hours prior to treatment, and was not seen on simultaneous exposure to drug and DMSO. In the animal studies described by Thuning et al. [17], the animals were given DMSO in their drinking water for 6 days before treatment with diverse chemotherapeutic drugs. 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, but this approach is unlikely to be acceptable to patients because of the characteristic smell produced by even small amounts of DMSO.

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 common intravesical chemotherapeutic agents studied. Drug enhancement by DMSO in other models may be produced by its effects on differentiation before administration of the anticancer agent.

Acknowledgement. The authors wish to thank the Peel Medical Research Trust for financing this study.

References

1. American Cancer Society (1980) Cancer statistics. Cancer 30:24
2. Borenfreund E, Steinglass M, Korngold G, Bendich A (1975) Effect of dimethyl sulfoxide and dimethyl formamide on the growth and morphology of tumor cells. Ann NY Acad Sci 243:164–171
3. Borzelleca JF, Harris TM, Bernstein S (1968) The effect of Dimethyl Sulfoxide on the permeability of the urinary bladder. Invest Urol 6:43–52
4. Eksborg S, Edsmyr F, Nasland I (1982) Intravesical instillation with adriamycin + Tween 80 in patients with superficial bladder tumours. Eur Urol 8:213–215
5. Groos E, Walker L, Masters JRW (1986) Intravesical chemotherapy: studies on the relationship between pH and cytotoxicity. Cancer 58:1199–1208
6. Hastings RJ, Franks LM (1981) Chromosome pattern, growth in agar, and tumorigenicity in nude mice of four human bladder carcinoma cell lines. Int J Cancer 27:15–21
7. Kim YS, Tsao D, Siddiqui B, Whitehead JS, Arnstein P, Bennett BS, Hicks J (1980) Effects of sodium butyrate and dimethylsulfoxide on biochemical properties of human colon cancer cells. Cancer 45:1185–1192
8. Lum BL (1983) Intravesical Chemotherapy of Superficial Bladder Cancer. Recent Results Cancer Res 85:3–36
9. Masters JRW, Hepburn PJ, Walter L, Highman WJ, Trejdosiewicz LK, Povey S, Parkar M, Hill BJ, Riddle P, Franks LM (1986) Tissue culture model of transitional cell carcinoma: characterisation of 22 human urothelial cell lines. Cancer Res 46:3630–3636
10. Mickey DD, Meadows LM, Vassiliades TA, Fried FA (1983) Dimethylsulfoxide (DMSO) induced growth inhibition and differentiation of human prostatic adenocarcinoma cells in vitro. Proceedings of the 78th Congress of the American Urological Association, p 41A
11. Parris CN, Arlett CF, Lehmann AR, Green MHL, Masters JRW (1988) Differential sensitivities to gamma radiation of human bladder and testicular tumour cell lines. Int J Radiat Biol 53:599–608
12. Parris CN, Masters JRW, Walker MC, Newman B, Riddle PR, English PJ (1987) Intravesical chemotherapy: combination with Tween 80 increases cytotoxicity in vitro. Urol Res 15:17–20
13. Riehm H, Biedler JL (1972) Potentiation of drug effect by Tween 80 in Chinese hamster ovary cells resistant to actinomycin-D and daunomycin. Cancer Res 32:1195–1200
14. Schoenfeld RH, Belville WD, Jacob WH, Buck AS, Dressner ML, Insalaco SJ, Ward GS (1983) The effect of dimethyl sulfoxide on the uptake of cisplatin from the urinary bladder of the dog: a pilot study. J Am Osteopath Assoc 82:570–573
15. Spruance SL, McKeough MB, Cardinal JR (1983) Dimethyl sulfoxide as a vehicle for topical antiviral chemotherapy. Ann NY Acad Sci 411:28–33
16. Stewart BH, Branson AC, Hewitt CB, Kiser WS, Straffon RA (1972) The treatment of patients with interstitial cystitis with special reference to intravesical DMSO. J Urol 107:377–380
17. Thuning CA, Fanshaw MS, Warren J (1983) Mechanisms of the synergistic effect of oral dimethyl sulfoxide on antineoplastic therapy. Ann NY Acad Sci 411:150–160
18. Tofilon PJ, Vines CM, Milas L (1985) Enhancement of in vitro chemotherapeutic activity by dimethyl sulfoxide. Clin Exp Metastasis 33:141–150
19. Torti FM, Lum BL (1984) The biology and treatment of superficial bladder cancer. J Clin Oncol 2:505–531
20. Utz DC, Hanash KA, Farrow GM (1970) The plight of the patient with carcinoma in situ of the bladder. J Urol 103: 160–164
21. Walker MC, Parris CN, Masters JRW (1987) Differential sensitivities of human testicular and bladder tumor cell lines to chemotherapeutic drugs. J Natl Cancer Inst 79:213–6
22. Warren J, Sacksteder MR, Jarosz H, Wasserman B, Andreiotti PE (1975) Potentiation of antineoplastic compounds by oral dimethyl sulfoxide in tumour-bearing rats. Ann NY Acad Sci 243:194–208

Lawrence Walker, MD
Department of Pathology
Institute of Urology
University College London
St. Paul's Hospital
24, Endell Street
London WC2H, 9AE
United Kingdom