Urol Res (1987) 17–20

Urological Research

Intravesical Chemotherapy: Combination with Tween 80 Increases Cytotoxicity in vitro

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Accepted: August 8, 1986

Summary. Tween 80 was shown to enhance significantly the cytotoxic activities of the four drugs (adriamycin, epodyl, mitomycin-c, thiotepa) most frequently administered intravesically to treat superficial bladder cancer. The colony forming ability of a human bladder cancer cell line, RT112, was measured following a 1 h exposure to each of the four drugs both alone and in combination with 0.1% and 0.3% Tween 80. Cell survival was not reduced by 0.1% Tween 80 alone. We conclude that the combination of Tween 80 with these drugs might increase the therapeutic index of intravesical chemotherapy.

Key words: Intravesical Chemotherapy, In vitro, Tween 80, Cytotoxicity.

Introduction

Intravesical chemotherapy is used in the management of superficial bladder cancer, either as an adjuvant to surgery or as definitive treatment for widespread disease difficult to control surgically. Complete or partial responses of varying duration are achieved in approximately two-thirds of patients, but the major cause of treatment failure is the initial presence or subsequent development of drug resistant disease.

Enhancement of the antitumour activities of chemotherapeutic drugs by Tween 80 (Polyoxyethylene sorbitan monooleate) was demonstrated in experimental [4, 5, 13, 14] and in vitro studies [11, 14, 15]. Tween 80 is a non-ionic surface-active detergent which may promote drug uptake by increasing cell membrane permeability [3, 12]. Promising clinical data were obtained also using Tween 80 in combination with adriamycin for intravesical treatment of carcinoma in situ of the bladder [8]. Complete responses, on the basis of negative cytology, were achieved in 3 out of 6 patients with adriamycin resistant tumours and in 2 out of 3 previously untreated patients [8].

In this study we further investigated the potential value of Tween 80 in the treatment of bladder cancer. The reproductive cell survival of a human bladder cancer cell line was measured following exposure to the drugs most frequently used for intravesical chemotherapy (adriamycin, epodyl, mitomycin-c, thiotepa) both alone and in combination with Tween 80.

Materials and Methods

The cell line used in these studies, RT112, was derived from a papillary transitional cell carcinoma of the human bladder and continues to produce tumours with a histopathology similar to that of the original biopsy on transplantation into nude mice [10]. The cells were maintained as monolayer cultures in plastic 25 cm^2 flasks (Nunc, Gibco, Paisley, Scotlanc), in RPMI 1640 medium (Gibco) supplemented with 5% heat inactivated foetal calf serum (Seralab: Sussex, England), and 2 mM L-glutamine (Flow, Irvine, Scotland), at 36.5 °C in a humidified atmosphere of 5% CO_2 in air. For the purposes of this study, a single batch of serum was used throughout. In order to minimise any changes occurring as a result of long-term culture, the cells were used over a restricted range of ten passages, from 35–44. Mycoplasma was not detected using nutrient agar culture or acetoorcein stained monolayers [10].

Colony Forming Assays

The influence of each drug alone and in combination with 0.1% and 0.3% Tween 80 on reproductive cell survival was measured by inhibition of colony formation. Exponentially growing cells were detached using a mixture of 0.05% trypsin (Difco 1:250; London, UK) in 0.016% versene (theylenediaminetetra-acetic acid, disodium salt; EDTA) (BDH Chemicals, Poole, UK). The cells were counted using a haemocytometer and viability was estimated using trypan blue dye exclusion. 1,000 single viable cells were plated in 5 cm plastic petri dishes (Nunc) containing 5 ml of medium and incubated for 48 h at 36.5 °C in a humidified atmosphere of 5% CO_2 in air. For the controls, the medium was replaced with fresh medium alone or supplemented with 0.1% or 0.3% Tween 80

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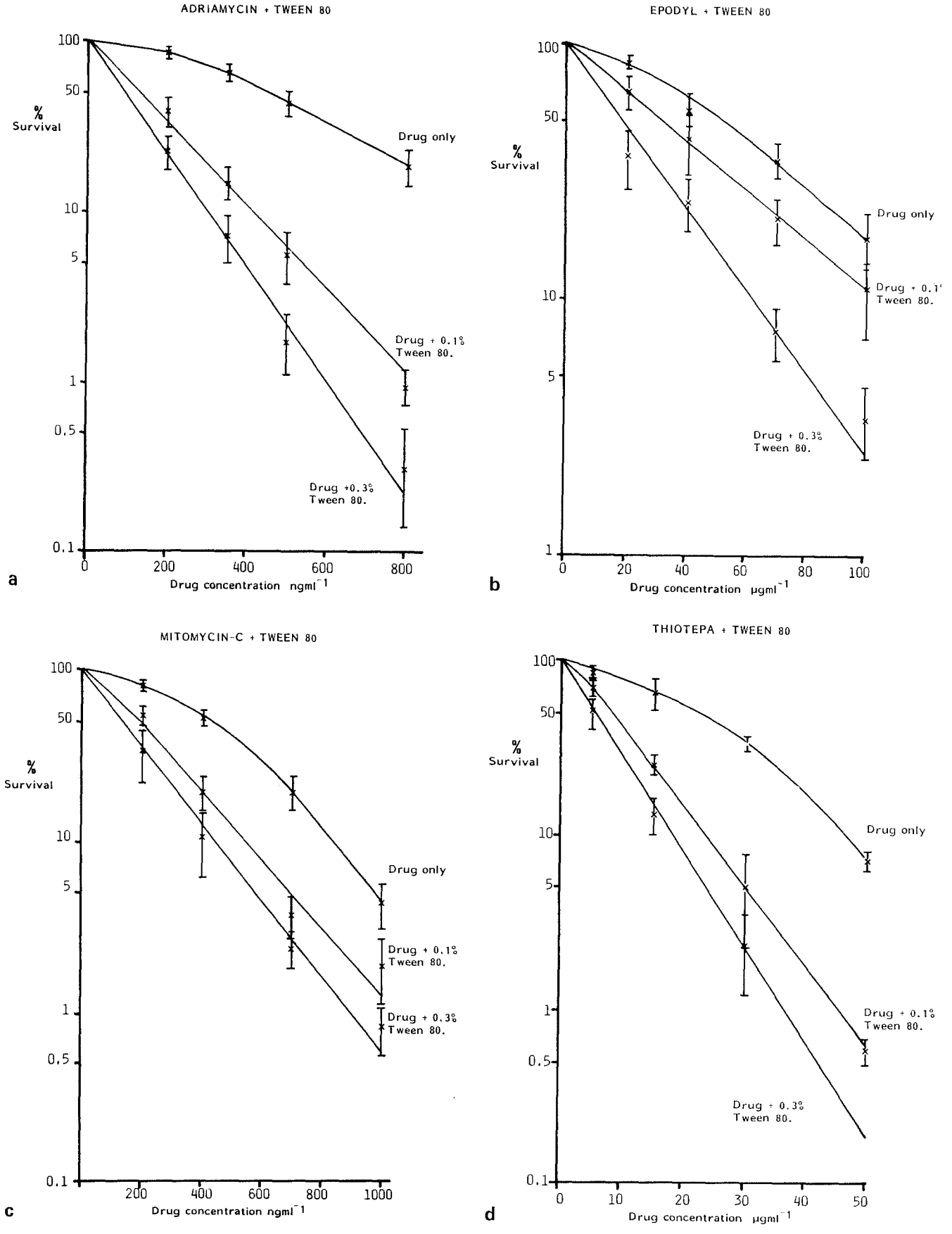

Fig. 1a–d. Percentage colony-forming ability of RT112 cells following a 1 h exposure to (a) adriamycin, (b) epodyl, (c) mitomycin-c and (d) thiotepa alone and in combination with 0.1% and 0.3% Tween 80. The bars indicate one standard error either side of the mean

(five replicate dishes each). For the drug treated cells (three replicate dishes for each drug concentration), the medium was replaced with fresh medium containing a range of drug concentrations either alone or supplemented with 0.1% or 0.3% Tween 80. Following a 1 h exposure period the medium was removed, the cells washed with three 5 ml aliquots of serum and glutamine-free medium, and 5 ml of complete medium was then added. After 14 days incubation, the cells were fixed in methanol (BDH) and stained with 10% Giemsa (BDH). Colonies consisting of more than 50 cells were scored as survivors using a binocular dissecting microscope. The mean number of colonies in the treated dishes was expressed as a percentage of that in the respective control dishes (medium alone or supplemented with 0.1% or 0.3% Tween 80). The data are derived from a minimum of three experiments for each drug.

Chemicals

Clinical preparations of adriamycin (doxorubicin HCl; Farmitalia Carlo Erba, Barnet, Herts, England), mitomycin-c (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan.), thiotepa (triethylenethiophosphoramide; Lederle Laboratories, Gosport, Hants, England) and epodyl (ethoglucid, Imperial Chemical Industries, Macclesfield, Cheshire, England) were made up immediately prior to use in calcium and magnesium-free phosphate buffered saline [7] and diluted to a final concentration in either medium alone or medium supplemented with 0.1% or 0.3% Tween 80.

Tween 80 (Serva; Heidelberg, Germany) was dissolved in RPMI 1640 medium at a concentration of 10%, and diluted to 0.1% or 0.3% in medium containing serum and glutamine.

Statistics

Examination of statistical significance was carried out by least squares regression of log survival against dose, although the curves on Fig. 1a–d have been drawn by eye to illustrate the shoulder at low dose.

Results

The dose-response of RT112 cells exposed for 1 h to adriamycin, epodyl, mitomycin-c and thiotepa alone and in combination with 0.1% and 0.3% Tween 80 are shown in Fig. 1a–d. The colony-forming ability of cells exposed for 1 h to 0.1% Tween 80 alone was not significantly different from that of untreated controls (96.7% ± 4.9, the latter figure being one standard error either side of the mean from 14 separate experiments), but was reduced by 0.3% Tween 80 to 62.1% ± 16.4 (the latter figure being one standard error either side of the mean from 14 separate experiments).

The combination of 0.1% or 0.3% Tween 80 significantly enhanced the cytotoxicities of adriamycin, mitomycin-c and thiotepa ($P < 0.01$). The enhancement of epodyl cytotoxicity by 0.1% Tween 80 was not quite significant ($P < 0.06$), whereas that of 0.3% Tween 80 was significant ($P < 0.04$).

The combination of each drug with 0.3% Tween 80 reduced colony-forming ability to a greater extent than did the combination with 0.1% Tween 80. The intercepts, reflecting the shoulder region, were significantly different with adriamycin ($P < 0.02$), epodyl ($P < 0.03$), mitomycin-c ($P < 0.01$) and thiotepa ($P < 0.03$), whereas the slopes of the dose-response curves differed only with adriamycin ($P < 0.03$) and with thiotepa ($P < 0.04$).

Discussion

We have demonstrated that Tween 80 significantly enhances the in vitro activities of the chemotherapeutic drugs used intravesically to treat superficial bladder cancer. These studies using human bladder cancer cells confirm and extend earlier observations on experimental animal tumours in vitro and in vivo [3–5, 11, 12, 14, 15]. Furthermore, the results support the conclusion that the addition of Tween 80 may enhance the therapeutic index of adriamycin for the treatment of bladder cancer [8].

The mechanism by which Tween 80 increases intracellular drug levels and potentiates cytotoxicity is probably related to cell membrane permeability. This conclusion is based mainly on measurements of the uptake and efflux of structurally unrelated compounds, using animal tumour cells in vitro [1, 3, 11, 14]. These permeability changes do not result in increased intracellular levels of all compounds, since little effect was observed on the uptake of a glucose analogue in the presence of Tween 80 [3]. It was concluded that Tween 80 did not cause a complete loss of the permeability barrier, but may only increase the passive uptake of relatively hydrophobic compounds [3]. This conclusion is supported by our finding that Tween 80 had least effect on the cytotoxicity of epodyl the least hydrophobic of the four drugs.

Tween 80 has an extremely low acute toxicity in animals and no deleterious effects have been seen in man [13]. It reduced the systemic absorption of adriamycin [9] and carbamates [2] from rat bladders. Furthermore, combination with Tween 80 did not increase systemic toxicity following intravesical administration of adriamycin [8] or mitomycin-c [6] in patients. Our data indicate that the administration of chemotherapeutic drugs with Tween 80 might enhance the therapeutic value of chemotherapy for superficial bladder cancer, and provide support for further clinical tials.

Acknowledgments. We are grateful to Dr. S. Eksborg for his advice and to the friends and relatives of Dr. Alan Sharp for a bequest in his memory towards the cost of these studies.

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