Prevention of tumor cell reimplantation during transurethral resection: the in-vitro antiadhesive and cytotoxic properties of an irrigant containing polyethylene glycol 400

Raphael Z. Sangeda[a], Joachim Vandepitte[a], Ann Huygens[a], Ben Van Cleynenbreugel[b], Hendrik Van Poppel[b] and Peter A.M. de Witte[a]

A major challenge to urologists with respect to bladder cancer is the high rate of tumor recurrence after transurethral resection (TUR). Implantation of resected tumor cells on traumatized bladder urothelium is believed to be the main cause of tumor recurrence. The aim of this study was to find a safe irrigant fluid and modality that prevents reimplantation of malignant cells during TUR. Therefore, the cytotoxicity and antiadherence effects of polyethylene glycol 400 (PEG400) and PEG4000 were investigated and compared with currently used irrigant fluids, water and 1.5% glycine (G-IF), on the RT112 urothelial cell carcinoma cell line. PEG400 (20%), G-IF, water and to a lesser extent 10% PEG400 and PEG4000 showed dramatic cytotoxic effects, besides exhibiting interesting antiadherence characteristics. The presence of serum proteins did not interfere with the activity of PEG400. In a clonogenic assay, both water and 20% PEG400 showed a better cytotoxic profile than G-IF, and it was found that these two fluids were able to induce a 5-log kill. This study shows that a solution of 20% PEG400 in water is a promising irrigant with antiadhesive and cytotoxic properties, which could be used to prevent tumor cell reimplantation during TUR. The irrigant remains active in the presence of serum proteins, is transparent, inexpensive and possesses an excellent safety profile. *Anti-Cancer Drugs* 21:645–650 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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[a]Laboratorium Farmaceutische Biologie, Faculteit Farmaceutische Wetenschappen and [b]Dienst Urologie, Faculteit Geneeskunde, Katholieke Universiteit Leuven, Leuven, Belgium

Correspondence to Peter A.M. de Witte, Laboratory for Pharmaceutical Biology, Faculty of Pharmaceutical Sciences, Katholieke Universiteit Leuven, Campus Gasthuisberg, O&N 2, PB 824, Herestraat 49, Leuven 3000, Belgium
Tel: +32 16 323430; fax: +32 16 323460;
e-mail: Peter.deWitte@pharm.kuleuven.be

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Introduction

Bladder cancer was, in 2002, the ninth most common cancer worldwide for both sexes combined, and the fourth most common malignancy among men in the Western world, with an estimated 357 000 new cases per year and 145 000 deaths [1]. Bladder cancer is found to be increasingly nonmuscle invasive in 75–85% of all cases. According to the tumor-node system, these nonmuscle invasive tumors are staged as carcinoma *in situ*, nonmuscle invasive papillary tumor (Ta) and lamina propria invasive tumor (T1). Nonmuscle invasive bladder cancer has a high rate of recurrence (70%) after treatment and 25–30% of them eventually progress to muscle invasive disease [2,3].

Recurrence of nonmuscle invasive urothelial cell carcinoma of the bladder after transurethral resection (TUR) is a major clinical problem in urological oncology. Several mechanisms have been described that may lead to bladder cancer recurrence. First of all, this may be because of newly emerging tumors [4] or recurring tumors that were missed in the first resection, especially in the case of incipient tumors that were invisible endoscopically at the time of resection [3,5,6]. However, in addition, reimplantation of resected cancer cells in normal or resected areas of the epithelium during or after TUR is reported to be an important factor in the mechanism underlying the frequent recurrence of bladder tumors [7]. A general strategy to reduce recurrences after TUR is by immediately instilling chemotherapeutics such as mitomycin-c after TUR. Although this technique is considered to be generally safe, several adverse effects upon mitomycin-c instillation have been reported [8,9]. Besides, it results in a significant decrease in recurrences in the short term, but the effect is only marginal in the long term [10].

With a view to develop a safe irrigant for TUR that could reduce the chance of tumor recurrence, we recently screened for compounds that are cytotoxic for malignant urothelial cells and/or that can coat the bladder wall, thereby preventing tumor cell implantation. Interesting results were obtained with polyethylene glycol derivates (PEGs) (Fig. 1). These compounds are clinically used agents with profound chemopreventive properties in experimental colon carcinogenesis, an activity that has been associated with PEG-induced suppression of epidermal growth factor-receptor (EGF-R) protein of the mucosal cells [11]. Generally, PEGs are considered inert and possess low toxicity. On account of their low toxicity,

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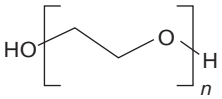
Fig. 1



Structure of polyethylene glycol (PEG), *n*=the number of ethylene oxide units. In PEG400 and PEG4000, *n* is 8–10 and 68–85, respectively.

viscosity and solubilizing effect, PEGs are used widely in human and veterinary pharmaceutical products intended for parenteral, oral, rectal and topical use [12,13].

In this study the cytotoxicity and antiadherence effects of PEG400 and PEG4000 were investigated and compared with the effects of water and 1.5% glycine constituted with 1% ethanol in water (G-IF), which are currently used as irrigants. Besides, the effect of serum proteins on the action of the irrigants was also determined.

Materials and methods

Chemicals and solutions

The irrigants tested were G-IF (containing 1.5% glycine in 1% ethanol) (Baxter, Lessines, Belgium) and water obtained from the Synergy Water System 185 (Millipore, Massachusetts, USA). PEG400 and PEG4000 were purchased from Fluka Chemie GmbH (Buchs, Switzerland).

Cell lines and culture conditions

The human RT112 cell line, which is a primary grade 2 TCC cell line (DSMZ, Braunschweig, Germany), was grown as a monolayer in minimum essential medium with Earle's salts containing 2 mmol/l L-glutamine under 5% CO_2 at 37°C. The medium was supplemented with 10% fetal calf serum (FCS), 1% antibiotic/antimycotic solution, 1% amino acids and tylosine (60 µg/ml).

MTT-based antiproliferative assay

The antiproliferative effect of the irrigants and PEG solutions on RT112 cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT) [14].

In brief, RT112 cells (200 µl, 2.5×10^4 cells/ml) were seeded in 96-well microplates and incubated for 24 h. After removal of the medium, the irrigant or PEG solution (± 10% FCS) was added (200 µl) and the cells were incubated (5–120 min, 37°C). After rinsing twice with phosphate buffered saline (Gibco BRL, Paisley, UK), the solution was then replaced with the medium (+ 10% FCS, 48 h, 37°C). Later, the medium was replaced with MTT solution (1 mg/ml, 200 µl, 4 h). Next, it was replaced by dimethyl sulfoxide and shaken to dissolve any formed formazan crystals. The plate was read on a microplate reader (FL 600 Microplate Fluorescence Reader, Bio-Tek Instruments, Winooski, Vermont, USA) at 550 nm. Absorbance taken from the cells grown in control conditions was considered as 100% (control). On the basis of the control, the antiproliferation effect was calculated and plotted using the GraphPad Prism 5.01 software (San Diego, California, USA). All experiments were performed in triplicate.

Clonogenic assay

RT112 cells were seeded onto six-well tissue culture plates (5×10^6 cells/well, 48h, 37°C). The medium was then replaced with the irrigant or PEG solution (+ 10% FCS) followed by incubation (2 h, 37°C). The fluid was collected and centrifuged to recover any floating cells. The cells remaining on the plates were trypsinized, combined with the floating cells and centrifuged (320*g*, 5 min). The pellet was resuspended in fresh medium. The cells were quantified (Coulter Electronic, Luton, UK) to determine an appropriate number of cells sufficient to yield 50–100 colonies and plated onto 100 mm Petri dishes in 10 ml of minimum essential medium. The Petri dishes were incubated for 9 days and then stained (2 min) with 1% methylene blue in methanol before the colonies were counted visually. As some test conditions can cause rapid necrosis of cells, a fraction of the cells originally present in the treated cells was not quantified by Coulter counting, and was therefore not processed in the clonogenic assay. This fraction (R1) was calculated from the ratio of the number of cells harvested immediately after treatment with a cytotoxic agent to the number of untreated controls cells. The final survival fraction was calculated according to formula survival fraction = $R1 \times R2$ in which R2 is the ratio of the cloning efficiency of treated cells to the cloning efficiency of control cells. All experiments were performed in triplicate.

Cell adherence assay

To study the antiadherence effects, the cells were seeded in 96-well plates (20 000 cells in 200 µl/well) containing the irrigant or PEG solution (± 10% FCS), or in the case of control conditions, containing the medium with 10% FCS [15] with a modification that the wells were not coated with an extracellular matrix earlier. The plates were centrifuged (71*g*, 5 min) to allow the cells to attach after which the cells were further incubated (15 min, 37°C). Then the plates were centrifuged upside down (9*g*, 5 min). The relative amount of attached cells was immediately assayed by using the MTT method.

Statistical analysis

The Graphpad Prism 5.01 software was used to calculate *P* values by using a one-way analysis of variance, if not mentioned otherwise. A *P* value of less than 0.05 was considered significant.

Results

Antiproliferative effect of PEG400 and PEG4000

The antiproliferative effect of PEG400 solutions with or without FCS was determined (Fig. 2a and b). From the nonlinear regression fits, IC_{50} values were calculated (Table 1). These values represent the concentration of PEG that is required to reduce cell proliferation by 50%. A maximal cytotoxic effect is present after 60 min and that the IC_{50} values obtained do not differ whether serum proteins are added or not. Conversely, after shorter treatment periods, the cytotoxic effects induced by PEG400 depended on the presence or absence of FCS.

PEG4000 (1–40%) showed a limited cytotoxicity; only after 120 min, the IC_{50} value was $22.3 \pm 1.0\%$ [mean $\% \pm$ standard deviation ($n = 3$)], whereas even the highest concentration of PEG4000 used (40%) did not induce 50% reduction in cell proliferation after shorter exposure times (15, 30, 60 min). At concentrations above 25%, the viscosity of PEG4000 is unacceptably high to be suitable as an irrigant during TUR of the bladder.

Comparison of the antiproliferative effect of PEG400, G-IF and water

As PEG400 showed a far better cytotoxicity profile as compared with PEG4000, we further compared the cytotoxic effects of 10 and 20% PEG400 with the effects of G-IF and water (Fig. 3; Table 2). It can be seen that (i) in general no differences existed between the cytotoxic effects of PEG400 in water versus PEG400 prepared in medium, (ii) 20% PEG400 and G-IF possessed more cytotoxic activity than 10% PEG and water and (iii) 10% PEG400 showed the lowest activity compared with the other tested conditions.

Clonogenic assay

To measure more accurately the cell killing effect, a clonogenic assay was performed for some conditions (20% PEG400 in water, G-IF and water, supplemented with 10% FCS, 2 h) that resulted in low cell proliferations, as observed in the earlier experiment. The results show that 20% PEG400 and water apparently killed all the cells present resulting in a greater than 5-log kill (Fig. 4). Somewhat surprisingly, G-IF was at least 10-fold less cytotoxic as compared with 20% PEG400 or water.

Adherence assay

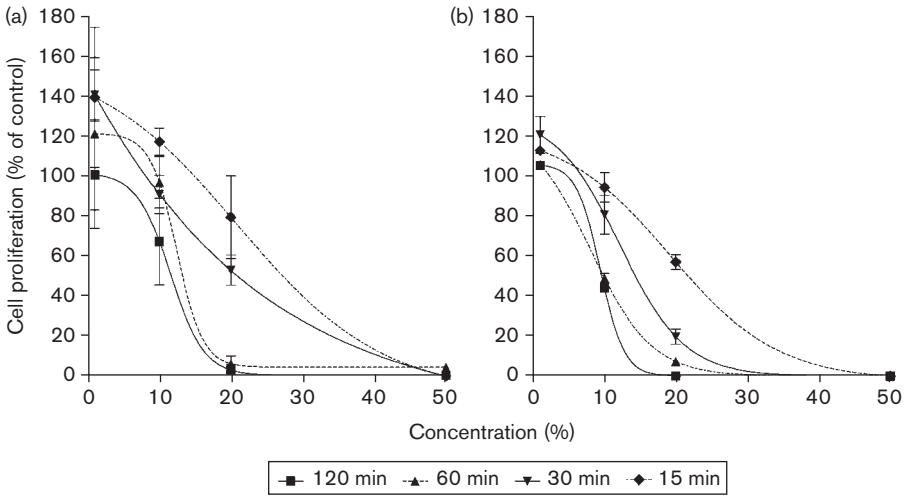
A cell adherence assay was conducted to determine whether irrigants or the PEG solutions affected cell adherence. Cytotoxic effects (Fig. 3) were minimized by limiting the exposure time to 15 min. In the presence of 10% FCS, water and G-IF prevented the adherence of more than 75% of RT112 cells (Fig. 5; Table 3). In the absence of FCS, this fraction further amounted to more

Table 1 Toxicity of PEG400 on RT112 cells with or without FCS

	IC_{50} value (%)		
	Without FCS	With FCS	*P* value[a]
15 min	33.8 ± 3.6	23.6 ± 1.1	*
30 min	24.3 ± 2.1	12.4 ± 1.0	**
60 min	11.8 ± 1.3	9.6 ± 1.0	NS
120 min	11.2 ± 1.2	9.6 ± 1.0	NS

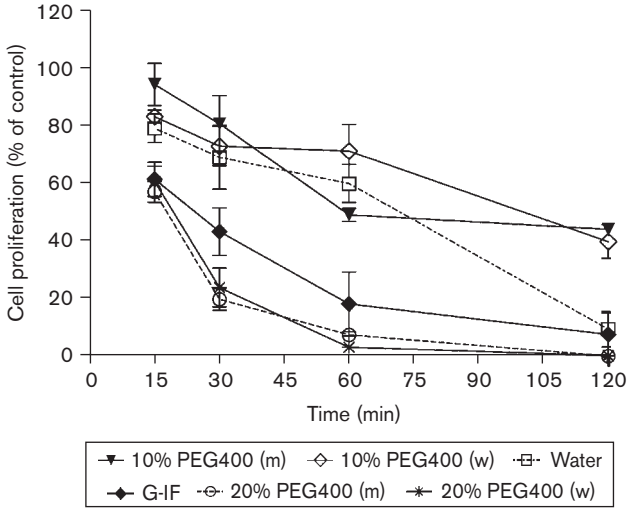
The data are expressed as IC_{50} values and represent mean % ± standard deviation ($n = 3$).
FCS, fetal calf serum; IC_{50}, concentration of PEG that is required to reduce cell proliferation by 50%; NS, not significant, PEG400, polyethylene glycol 400.
[a]*P* value calculated using the unpaired *t*-test.
*$P < 0.05$.
**$P < 0.01$.

Fig. 2



Cytotoxicity profile of polyethylene glycol 400 (1, 10, 20, 50%) without fetal calf serum (FCS) (a) and with FCS (b) after an incubation of the RT112 cells for 15, 30, 60 and 120 min, respectively. The results show the mean % ± standard deviation ($n = 3$).

Fig. 3



Cytotoxicity profile of polyethylene glycol 400 (PEG400) (10 and 20%), 1.5% glycine (G-IF) and water, all containing 10% fetal calf serum, after an incubation of the RT112 cells for 15, 30, 60 and 120 min, respectively. The results show the mean % ± standard deviation ($n=3$). m and w, PEG400 solutions made in medium or water, respectively.

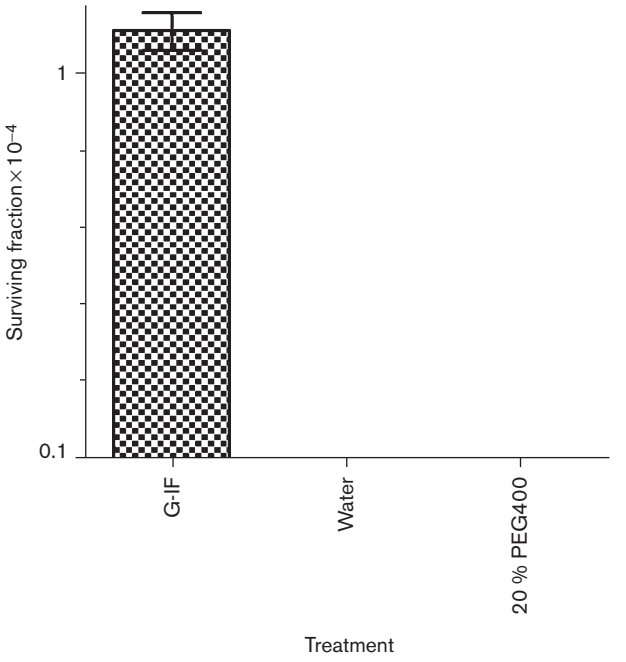
Table 2 One-way ANOVA with the Tukey post test to compare the antiproliferative effect on RT112 cells of cytotoxic fluids after 15, 30, 60 and 120 min treatment

	P value			
Cytotoxic fluid	15 min	30 min	60 min	120 min
10% PEG400 (m) vs. 10% PEG400 (w)	NS	NS	**	NS
10% PEG400 (m) vs. water	NS	NS	NS	***
10% PEG400 (m) vs. G-IF	***	***	***	***
10% PEG400 (m) vs. 20% PEG400 (m)	***	***	***	***
10% PEG400 (m) vs. 20% PEG400 (w)	***	***	***	***
10% PEG400 (w) vs. water	NS	NS	NS	***
10% PEG400 (w) vs. G-IF	**	*	***	***
10% PEG400 (w) vs. 20% PEG400 (m)	***	***	***	***
10% PEG400 (w) vs. 20% PEG400 (w)	**	***	***	***
Water vs. G-IF	*	*	***	NS
Water vs. 20% PEG400 (m)	**	***	***	NS
Water vs. 20% PEG400 (w)	*	***	***	NS
G-IF vs. 20% PEG400 (m)	NS	NS	NS	NS
G-IF vs. 20% PEG400 (w)	NS	NS	NS	NS
20% PEG400 (m) vs. 20% PEG400 (w)	NS	NS	NS	NS

ANOVA, analysis of variance; G-IF, 1.5% glycine; NS, not significant; PEG400, polyethylene glycol 400; w and m, PEG solution made in water and medium, respectively.
*$P<0.05$.
**$P<0.01$.
***$P<0.001$.

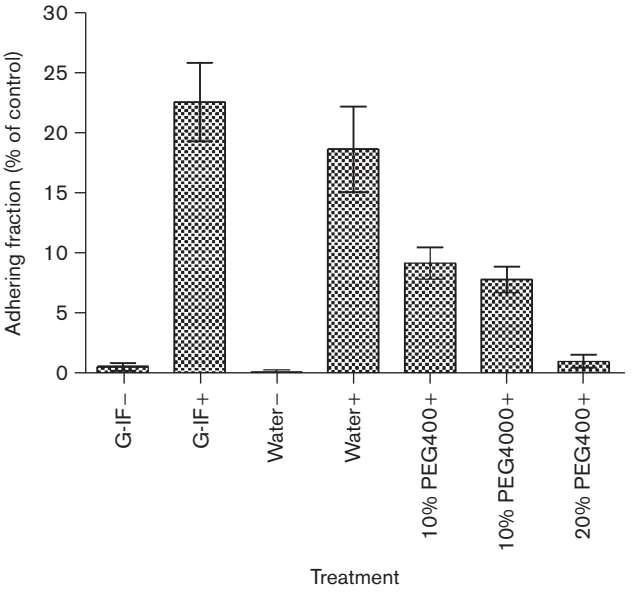
than 98%. PEG400 and PEG4000 used at 10% showed similar effects, reducing cellular adherence to less than 10%. As compared with the 10% condition, 20% PEG400 induced a more substantial effect.

Fig. 4



Clonogenic survival after treating RT112 cells with 1.5% glycine (G-IF), water and 20% polyethylene glycol 400 (PEG400) supplemented with 10% fetal calf serum for 2 h at 37°C. The results show the mean % ± standard deviation ($n=3$).

Fig. 5



Cell adherence after treatment of RT112 cells with water, 1.5% glycine (G-IF) without (−) or with (+) 10% fetal calf serum for 15 min. The results show the mean % ± standard deviation ($n=3$).

Discussion

During TUR of nonmuscle invasive bladder tumors, irrigants like G-IF and water give the surgeon a clear view of the operation field by removing blood and pieces of the resected tissue. The fluid also stretches the bladder allowing smooth endoscopic access. These irrigants,

Table 3 Student *t*-test analysis to compare the antiadhesion effect of cytotoxic fluids without (−) or with (+) 10% FCS

Cytotoxic fluid	*P* value
G-IF (+) vs. G-IF (−)	***
Water (+) vs. water (−)	***
10% PEG400 (+) vs. 10% PEG4000 (+)	NS
10% PEG400 (+) vs. 20% PEG400 (+)	**

FCS, fetal calf serum; G-IF, 1.5% glycine; NS, not significant; PEG400, polyethylene glycol 400.
**$P<0.01$.
***$P<0.001$.

however, also convey easily viable tumor cells that become suspended during resection throughout the bladder cavity [16], and hence local reimplantation of tumor cells is believed to be a major cause of recurrence of bladder cancer after TUR [7]. At present, there is an urgent need for effective agents used as an adjunct during or immediately after resection to prevent this regrafting of tumor cells. Ideally, these agents should be instantly cytotoxic to the resected tumor cells or coat the bladder to prevent tumor cell adherence. Besides, they should be harmless to the bladder wall, and as large amounts of irrigant fluid can become directly absorbed into the vascular system [17,18], they should be devoid of systemic toxicity. Therefore, it is not an option to include antitumoral drugs present on the pharmaceutical market in irrigant fluids.

In this study, we found that 20% PEG400, G-IF, water and to a lesser extent 10% PEG400 showed cytotoxic effects on RT112 cells. Conversely, PEG4000 showed limited potential as a cytotoxic agent. In general, a 2-h exposure was necessary to induce a dramatic effect, a procedure that can easily be translated into the clinic, that is, by instilling an irrigant in the bladder for an additional 2 h after TUR. Using a clonogenic assay we further showed that an exposure of RT112 cells for 2 h to water or 20% PEG400 induced a more than 5-log cell kill, indicating more than 99.999% reduction in the cell population.

The cytotoxic effects of water on human urothelial and colon cancer cell lines are well documented and are believed to be of clinical relevance during TUR of the bladder or abdominal lavage after surgery to lyse the remaining isolated cancer cells [19,20]. It has been shown that colon cancer cells exposed to a short hypotonic shock of up to 5 min undergo transitory cell swelling, followed by specific binding of extracellularly released ATP to P2 receptors and activation of apoptotic pathways [20], whereas short hypotonic stress induced apoptosis. Exposure times of longer than 15 min were necessary to lyse the cells by necrosis, likely because of cell membrane rupture that is secondary to massive cell swelling [20]. Although we did not investigate mechanistic details of the responses of RT112 cells to water exposure, it is a matter of fact that a similar necrosis underlies the results observed in this study.

As G-IF contains 1.5% glycine (concentration iso-osmotic with saline: 2.2%) and 1% ethanol (iso-osmotic concentration: 1.4%), the solution is slightly hyperosmotic. It is known that hyperosmotic stress can result in cell death [21], and hence the cytotoxicity induced by G-IF can be attributed to hypertonic conditions. In contrast, the irrigant also contains 1% ethanol, which is known to be cytotoxic at that concentration [22], and it is likely that the ethanol present in the irrigant also accounts partly for the effects observed.

As the concentration of PEG400 iso-osmotic with saline is 8.5%, the 10 and 20% PEG400 solutions are hyperosmotic as well. It was found that the cytotoxic effect of the PEG400 solutions made in water or cell medium containing isotonic concentrations of salts was comparable, however, and it likely mirrors specific cellular effects induced by PEG400 that are independent of its nonspecific osmotic activity. Of interest, it was shown that PEG800, but not osmotically equivalent concentrations of sorbitol or sodium chloride, induced apoptosis in colon cancer cell lines in a dose-dependent manner [23]. Further evidence for a possible specific interaction of PEG400 with cells comes from the observation that PEGs can be used as chemopreventive agents against colorectal cancer. This activity has been associated with a reduction of the EGF-R in epithelial cells through lysosomal degradation, effecting an antiproliferative activity involving the Snail/β-catenin pathway [8,23]. In these studies, the human colorectal cancer cell line HT-29 was incubated with 5% PEG3350 for 24 h, conditions that resemble somewhat the ones used in this study, that is, exposure of RT112 cells to 10–20% PEG400 for a maximum of 2 h. Whether similar intracellular effects underlie the present observations partially or fully is not known, and further studies elucidating mechanistic details regarding the cytotoxic and antiproliferative effects of PEG400 are necessary.

Both the irrigants and the PEG solutions dramatically reduced the fraction of cells that adhered to the surface of cell plates. In case of the irrigants (G-IF, water) it is unlikely that this is the result of a real coating of the plastic surface or cells, thereby limiting the interaction between cellular membrane proteins and the plastic matrix. Although the conditions used resulted in limited cytotoxicity, it is believed that rapid alterations in cell morphology induced by the hypotonic conditions (water) or 1% ethanol conditions (G-IF) brought about the antiadherence effects. Conversely, the activity of PEG400 and PEG4000 is probably related to their physicochemical properties, as amphiphilic polymers are prone to coat cells and/or plastic surfaces in a noncovalent manner. Significantly, PEG4000 induced a decrease of more than 90% of cell adherence, using a concentration and incubation time that does not induce cytotoxic effects. Conversely, and in line with the data showing that PEG800 induces apoptosis [24], PEG400 (8.5–20%) also produced

morphological changes of the cells within the first 30 min of incubation (results not shown), and it is therefore possible that these are related with the antiadherence effects as well.

Importantly, Solomon *et al.* [25] observed that a mean of 10.9% albumin serum-equivalent can be recovered in the waste irrigation fluid of patients undergoing TUR. As by binding to albumin or lipoproteins, compounds can become inactivated, it was critical to investigate the activity of PEG400 in the presence of serum constituents. Unexpectedly, PEG400 was slightly more cytotoxic to RT112 cells in the presence of FCS than in its absence after short incubation periods. This not only implies a lack of binding of PEG to serum constituents, but also seems to point to a specific interaction between cellular effects of PEG400 and at least a constituent of serum. This is an interesting observation that will be further investigated in detail in the near future, especially against the background of the influence of PEGs on the expression of the EGF-R in cells, as mentioned earlier.

Altogether, this study shows that 20% PEG400 is a promising cytotoxic fluid that warrants further in-vivo investigations in an appropriate animal model. The agent has antiadherence effect and remains a potent cytotoxic fluid in the presence of serum proteins. Besides, it is transparent, inexpensive and possesses an excellent safety profile.

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Conflict of interest: none declared.

References

1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *Cancer J Clin* 2005; **55**:74–108.
2 Sanford MA, Zaslau S. Updated cancer staging system. *Hosp Physician* 1999; **35**:14–16.
3 Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. *Lancet* 2009; **374**:239–249.
4 Jakse G, Algaba F, Malmström PU, Oosterlinck WA. Second-look TUR in T1 transitional carcinoma: why? *Eur Urol* 2004; **45**:539–546.
5 Klan R, Loy V, Huland H. Residual tumor discovered in routine second transurethral resection in patients with stage T1 transitional cell carcinoma of the bladder. *J Urol* 1991; **146**:316–318.
6 Spruck CH III, Ohneseit PF, Gonalez-Zulueta M, Esrig D, Miyao N, Tsai YC, *et al.* Two molecular pathways to transitional cell carcinoma of the bladder. *Cancer Res* 1994; **54**:784–788.
7 Boyd PJ, Burnand KG. Site of bladder-tumour recurrence. *Lancet* 1974; **2**:1290–1292.
8 Doherty AP, Trendell-Smith N, Stirling R, Rogers H, Bellringer J. Perivesical fat necrosis after adjuvant intravesical chemotherapy. *BJU Int* 1999; **83**:420–423.
9 Racioppi M, Porreca A, Foschi N, Delicato G, Destito A, D'Addessi A. A potential risk of early endoversicalchemotherapy with mitomycin c. *Urol Int* 2005; **75**:373–375.
10 Hendricksen K, Witje JA. Current strategies for first and second line intravesical therapy for nonmuscle invasive therapy. *Curr Opin Urol* 2007; **17**:352–357.
11 Wali RK, Kunte DP, Koetsier JL, Bissonnette M, Roy HK. Polyethylene glycol-mediated colorectal cancer chemoprevention: roles of epidermal growth factor receptor and Snail. *Mol Cancer Ther* 2008; **7**:3103–3111.
12 Hermansky SJ, Neptun DA, Loughran KA, Leung HW. Effects of polyethylene glycol 400 (PEG 400) following 13 weeks of gavage treatment in Fischer-344 rats. *Food Chem Toxicol* 1995; **33**:139–149.
13 Fruijtier-Pölloth C. Safety assessment on polyethylene glycols (PEGs) and their derivatives as used in cosmetic products. *Toxicology* 2005; **214**:1–38.
14 Huygens A, Kamuhabwa AR, Van Laethem A, Van Cleynenbreugel B, Van Poppel H, Agostinis P, *et al.* Enhancing the photodynamic effect of hypericin in tumour spheroids by fractionated light delivery in combination with hyperoxygenation. *Int J Oncol* 2005; **26**:1691–1697.
15 Bohle A, Jurczok A, Ardelt P, Wulf T, Ulmer AJ, Jocham D, Brandau S. Inhibition of bladder carcinoma cell adhesion by oligopeptide combinations in vitro and in vivo. *J Urol* 2002; **167**:357–363.
16 Muller F, Kraft R, Zingg EJ. Exfoliative cytology after transurethral resection of superficial bladder tumors. *Br J Urol* 1985; **57**:530.
17 Collins JW, Macdermott S, Bradbrook RA, Keeley FX, Timoney AG. Is using ethanol–glycine irrigating fluid monitoring and 'good surgical practice' enough to prevent harmful absorption during transurethral resection of the prostate? *BJU Int* 2006; **97**:1247–1251.
18 Hahn RG. Fluid absorption in endoscopic surgery. *Br J Anaes* 2006; **96**:8–20.
19 Solomon LZ, Birch BR, Cooper AJ. Water as a tumoricidal agent in bladder cancer. *Eur Urol* 1998a; **34**:500–504.
20 Selzner N, Selzner M, Graf R, Ungethuem U, Fitz JG, Clavien PA. Water induces autocrine stimulation of tumor cell killing through ATP release and P2 receptor binding. *Cell Death Differ* 2004; **11** (**Suppl 2**):S172–S180.
21 Burg MB, Ferraris JD, Dmitrieva NI. Cellular response to hyperosmotic stresses. *Physiol Rev* 2007; **87**:1441–1474.
22 Dolganiuc A, Szabo G. In vitro and in vivo models of acute alcohol exposure. *World J Gastroenterol* 2009; **15**:1168–1177.
23 Roy HK, Kunte DP, Koetsier JL, Hart J, Kim YL, Liu Y, *et al.* Chemoprevention of colon carcinogenesis by polyethylene glycol: suppression of epithelial proliferation via modulation of SNAIL/beta-catenin signaling. *Mol Cancer Ther* 2006; **5**:2060–2069.
24 Roy HK, DiBiase JK, Black J, Karolski WJ, Ratashak A, Ansari S. Polyethylene glycol induces apoptosis in HT-29 cells: potential for chemoprevention of colon cancer. *FEBS Lett* 2001; **496**:143–146.
25 Solomon LZ, Jennings AM, Foley SJ, Birch BR, Cooper AJ. Bladder cancer recurrence by implantation of exfoliated cells: is gamma-linolenic acid an effective tumoricidal agent? *Br J Urol* 1998b; **82**:122–126.