

Effect Of Bottled Drinking Water On Cell Viability Of Human Urothelium

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Abstract

Diet is often linked to the irritable bladder as drinking of alcohol and coffee often aggravate the symptoms of Painful Bladder Syndrome/Interstitial Cystitis (PBS/IC) patients. The aim of this study was to assess the impact of different types of bottled water available in the market on the urothelium.

Methods: UROtsa cell line was used as in vitro models to assess the cytotoxicity from different brands of bottled waters, distilled, spring, mineral and ultra purified (Otsul water). The pH of water was measured and trypan blue dye exclusion test was used to measure cell viability.

Results: Overnight exposure to distilled water and most bottled water with reduced volume of growth media is harmful to cells. In contrast, exposure of ultra pure water under similar conditions caused minimal adverse effects.

Conclusion: The quality and source of water consumption may be important in PBS/IC. Ultra purified water should be considered as an adjuvant therapy for patients with irritable bladder symptoms.

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INTRODUCTION

Painful Bladder Syndrome/Interstitial Cystitis (PBS/IC) can be described as a chronic inflammatory condition of the bladder wall, characterized by urinary frequency and urgency, and severe suprapubic and/or pelvic pain, in the absence of bacterial cystitis¹. The cause of irritable bladder symptoms and of Painful Bladder Syndrome/Interstitial Cystitis (PBS/IC) is unknown. Current treatments are aimed at relieving the symptoms of PBS/IC². It has been reported that diet often contributes to the irritable symptoms of PBS/IC such as drinking alcohol and caffeine-containing beverages^{3, 4}.

Water constitutes a major portion of diet consumed by the PBS/IC patient population and they make a constant concerted effort to drink water that is good for their health. Unfortunately water devoid of any impurities does not exist in nature. Drinking water supply from surface or underground sources such as lakes or aquifers contains traces of substances derived from various origins. Water

from the natural sources is made fit for drinking by various treatment methods that aim to remove pollutants such as microorganisms, toxic minerals, metals, organic chemicals and radioactive substances.

Consumption of water that is not completely free from these undesirable pollutants may be linked to different diseases or symptoms of chronic diseases such as PBS/IC. The chance of this happening drastically increases in the PBS/IC patients because they drink large quantity of water. The increased amounts of water consumed increase the frequency of their voiding. Patients seem to desire that outcome, as the process of voiding seems to provide them with a degree of comfort from constant pain for a brief period.

We hypothesize that, extraneous constituents in water consumed by patients existing either as a pollutant or as additive from the purification process may have a direct effect on the human urothelium. We choose UROtsa cell line for our study as it is not a cancer derived cell line that lacks tumorigenicity but is immortal at the same time⁵. The cell line retains the features of normal human urothelium and it affords a good in vitro method to assess the impact of drinking water on urothelium. We determined viability of

cultured human bladder urothelium cells after incubating them with three brands of bottled waters. The effect of premium brands of bottled water on human urothelium has previously been not tested. Our study will help us determine if differences in constituents present in water can directly affect bladder urothelium.

METHODS

Cell-line: URO-tsa cells were grown in Dulbecco's Minimum Essential Medium (DMEM, high glucose), supplemented with 5% FCS, and antibiotics. 100,000 cells were plated in a two-well chamber slide and grown till 80% confluence. The plated cells were used as experimental in vitro models to assess the cytotoxicity from bottled waters.

Water: Four premium brands of bottled water were purchased from the local pharmacy and they were blindly labeled as Brand X, Y, Z. Brands X, Y and Z represent spring, mineral and aquifier water respectively while Brand of ultra pure water was prepared specially for patients suffering from bladder irritation (Otsu® water; www.otsuwater.com, Delithe Natural Products, Pittsburgh, PA).

Cell-culture and data analysis: Water from sealed bottles was removed aseptically and mixed with cell growth media to make final concentration of water to be 75% in mixture. 24 hours after adding the mixture of water and growth media, the cells were stained with Trypan blue dye. The dye selectively stains the dead cells blue, while it is unable to penetrate viable cell membrane. The values were analyzed using single factor ANOVA and statistical significant difference between different groups was analyzed with Dunnet multiple comparison test. Level of significance was checked at p<0.05.

RESULTS

The results of our study are presented in Tables 1 and Figure 1.

Figure 1

Figure 1: Representative photographs of UROtsa cells 24h after incubation with either distilled water or bottled waters. Panel A and B are untreated cells and cells treated with 75% of distilled water respectively. Panel C, D, E and F are Brand Z, Ultra pure water, brand X and brand Y respectively. The percentage of blue cells in panel A and D appear similar and panel B have the highest percentage of blue cells compared to unstained cells.

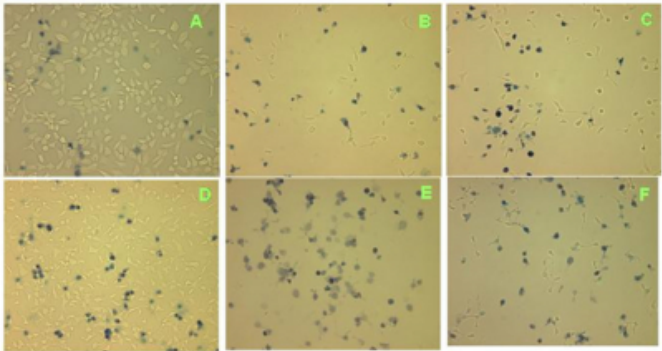


Figure 2

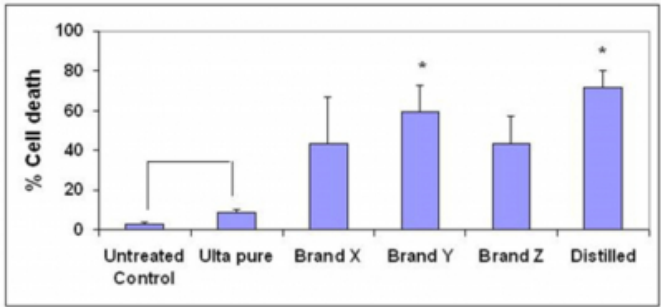
Table 1: Tabulation of percentage cell death from co-culturing of water and cell growth media. Distilled water showed the highest percentage of cell death followed by 75% of brand Y water. Brand X and Z caused less than 50% cell death but brand of ultra pure water behaved similar to the control untreated cells. All values are expressed as mean± SEM.

	Untreated Control	Ultra Pure	Brand X	Brand Y	Brand Z	Distilled
% Cell death (mean±SEM)	2.88±1.01	8.70±1.85	43.32±23.6	59.44±13.2	43.42±13.5	71.81±7.87

The cell chamber used as negative control in this study were only incubated with 100% cell growth media without any addition of bottled water (Panel A, Fig.1). The positive control for this study was distilled water making up to 75% fraction of the cell growth medium mixture (Panel B, Fig.1). Studies on bladder cancer cell line studies have shown that cytotoxicity of distilled water is comparable to chemotherapeutic agents 6. Trypan blue staining indicates dead cells after treatment with water as seen in (Panel B-F, Fig.1). Statistically, compared to premium brands of bottled water X, Y and Z, the ultra pure water showed significantly less cell death at 24h time point as summarized in Fig.2 (p<0.01).

Figure 3

Figure 2: Bar graph showing the mean cell death after treating with different types of water. Distilled water showed the highest percentage of cell death followed by 75% of brand Y water ($p<0.05$) indicated by *. The viability of cells in control well and those incubated with ultra pure water were not significantly different. Brand X and Z caused less than 50% cell death but brand of ultra pure water behaved similar to the control untreated cells.



We selected the time period of 24h to compare the differences in the effects of different brands of water on cell viability, as there was nearly complete cell death by the 48h time point. The effect of the cell viability is independent of the pH of the different water. The pH of the distilled and brands of bottled water X, Y, Z were 6.8, 6.1, 7.4, respectively.

Figure 4

Table 2: The results of pH measurement on different waters

Type of water	pH of Water
Ultra Pure Otsu	6.1
Brand Z	7.4
Brand X	7.2
Brand Y	6.8

DISCUSSION

The overactive or irritated bladder with sensation of urgency and urinary frequency affect over 17 million Americans. Although there are many causes such as aging and neurological diseases, many sufferers report bladder discomfort from irritants in their diet and liquid intake ⁷. The Painful Bladder Syndrome/Interstitial cystitis (PBS/IC) is estimated to affect 700,000 to 1,800,000 people in North America. While options for treating bladder irritation and

PBS/IC exist (i.e. oral and intravesical agents, neurostimulation, vaginal massage, surgery), but no uniformly effective regimen is currently available ². Removing dietary irritation such as caffeine, acid and carbonated beverages have been recommended for patients with both the overactive and painful bladder conditions ^{3, 4, 8}. Infact, management of patients with other urological diseases shows significant improvement with a sound nutritional support program⁸. A previous study has shown that changes in diet of spinal cord injured patients can affect the urothelium of their bladders. Cranberry juice has been shown to improve the lower urinary tract symptoms by preventing adhesion of uropathogenic bacteria to the urothelium by reducing biofilm production ⁹.

A popular recommendation for a variety of irritable bladder symptoms is to drink 8 glasses of water per day. But is regular tap or bottle waters the best choice for patients with irritable bladder? The hypothesis, which drove this study was that, ultra pure water, with its total lack of impurities, may relieve certain symptoms of PBS/IC and irritable bladder. The present study was undertaken to assess the effect of drinking water on bladder health specifically urothelium. The cells of UROtsa cell line cultured overnight in wells were used in the study. To avoid the risk of osmotic shock to cells, water was mixed with cell growth medium to assess the effect of water on cultured urothelium cells. A recently published study reported that monolayer cultures of human bladder cancer cells incubated with distilled water showed significant osmotic cytolysis ⁶. The cell killing effect of distilled water determined by microculture tetrazolium assay was comparable to similar exposure to mitomycin. Our results seem to confirm the results obtained with distilled water on cell survival ⁶. However, the cell line chosen for our study was not a cancer cell line but they were healthy normal cells ⁵.

The non-cancerous nature of cells used in our study increases the predictive value of these results for the IC/PBS population, as these patients are unlikely to have cancerous cells in their urothelium. Contents within the urine can have a direct effect on the bladder lining and on the inflammatory and immunoregulatory cells that resides in the bladder wall ¹⁰. In this study, we evaluated cell killing, which is an end stage response of cellular toxicity, and therefore long time period of 24h incubation was chosen. Future studies in this direction may evaluate other short term endpoints for cytotoxicity such as release and activity of intracellular enzymes such as lactic acid dehydrogenase LDH or acid

phosphatase activity¹¹. The levels and activity of these enzymes increases in cells experiencing insults or stressful environment.

The membrane integrity method used in our study to examine cytotoxicity using trypan blue is more suited for such exposure times. Sensitive bladder of IC/PBS patients is often exposed to trace undesirable constituents in urine derived from their drinking water during the storage phase of voiding. The storage phase is spread over a wide time frame from less than an hour to 4-5 hours. Therefore, to simulate the exposure of urothelium at low concentrations of contaminant for prolonged duration, we exposed the cells to higher concentration of 75% water used our study by following the principle of accelerated stability studies¹². Although the mechanism behind these observations obtained in our study is unclear, the absence of impurities in water appears to have a distinct impact on cell survival of human urothelium cells.

The correlation of in vitro - in vivo toxicity makes a number of assumptions and simplifications. Toxicity in vivo may arise either from the direct effect of any undesirable ingredient existing in water or from a toxic metabolite generated from that ingredient in the body. Toxicity in vivo is ultimately a process that occurs at the cellular level and thus in vitro methods are ideally suited for its study. The minimum in vitro drug concentration which induces changes in cell morphology, or up to 50% cell mortality is assumed to correspond to the drug dose in vivo which gives rise to initial or mild toxic signs, while the minimum in vitro drug concentration which elicits over 90% cell mortality is assumed to correspond to the in vivo dose which gives rise to marked clinical signs.

In our study, distilled water and Brand Y that is sourced from an aquafier showed more than 50% mortality in cultured human urothelium cells. These results encourages us advice IC/PBS patients from consuming water obtained from aquafiers for better managements of their symptoms. These results also support the recommendation that removing of any chemical, mineral or toxins may be an important part of helping symptoms of the irritable bladder¹³. Can something as easy as water completely free of harmful ingredients help? People all over the world used to turn on their faucets when they craved a drink of clear, cool water. Today, concerned about the safety of water supplies, they're turning to the bottle. This may also be true for people

suffering with irritable bladder issues.

CONCLUSIONS

Variation in water composition can directly affect bladder urothelium function and survival. However, water pH in the range of 6 to 7.5 did not correlate with urothelial effect. The quality and source of water consumption may be important in PBS/IC, an important woman's health issue. Our study suggests that ultra purified water; such as Otsu® water used in this study may be considered as an option or adjuvant therapy for patients with irritable bladder symptoms.

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