

## PHARMACOLOGY

THE SHORT TERM EXPOSITION OF AgNO<sub>3</sub> ON 3T3 MOUSE  
FIBROBLASTS CELL LINETOMASZ DREWA<sup>1,2\*</sup>, KRYSZYNA SZMYTKOWSKA<sup>3</sup> and MICHAŁ CHABERSKI<sup>1</sup>

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**Abstract:** There is an increasing interest in using of silver coated (silver nitrate, silver alloy, silver oxide) catheters for hospital patients. Clinical trials with silver-coated urinary catheters have shown conflicting results. The most often performed catheterization is for a short period of time. The above observations have encouraged the authors to investigate the influence of silver nitrate (AgNO<sub>3</sub>) on 3T3 fibroblasts viability *in vitro* during a short time experiment (3 and 12 h). 3T3 fibroblast culture was established. The influence of AgNO<sub>3</sub> on the viability of murine 3T3 fibroblasts with the use of trypan blue staining was evaluated. The regression curves and lethal concentrations for 90, 50 and 10 % viability were calculated. The lethal concentrations of AgNO<sub>3</sub> after 3 h exposition were as follows LC<sub>10</sub>=0.98, LC<sub>50</sub>=6.44 and LC<sub>90</sub>=21.38. The lethal concentrations of AgNO<sub>3</sub> after 12 h exposition were as follows LC<sub>10</sub>=1.05, LC<sub>50</sub>=6.91 and LC<sub>90</sub>=22.96. The LC values were similar for 3 and 12 h exposure as well. In conclusion, the silver nitrate has the similar toxic effect on 3T3 fibroblasts during the short and long exposition. Attention should be paid when catheter has a close contact to injured urothelium even for a short period of time.

**Keywords:** silver ions, mouse fibroblasts *in vitro*, silver coated catheters, urinary tract infections

Urinary tract infections (UTI's) account for 40% of all nosocomial infections, and the most of them are associated with the use of urinary catheters. UTI's not only contribute to excess morbidity and mortality, but they also significantly add to the cost of hospitalization. There is an increasing interest in using of silver coated catheters (silver alloy, silver nitrate) for hospital patients. Clinical trials with silver-coated urinary catheters have shown conflicting results. Recent trials with silver-hydrogel urinary catheters have shown a non-significant reduction in nosocomial UTI's on the other side these catheters appeared to offer cost savings (1-3). The use of dressings containing 0.5% (31 × 10<sup>-4</sup> M) AgNO<sub>3</sub> or ointments containing silver compounds has been shown to prevent mainly infection, at the same time increasing the risk of inappropriate wound healing (4, 5). Several studies have demonstrated a cytotoxic effect from catheter materials, indicating that this may be of importance in urethral inflammation. However, the exact mechanisms behind this phenomenon are not known (6). The most often per-

formed catheterization is for a short period of time. Indwelling catheters are left for 3 days, usually after small surgery procedures or during diagnostic work-up.

The above observations have encouraged the authors to research the influence of silver nitrate (AgNO<sub>3</sub>) on 3T3 fibroblasts viability *in vitro* during a short time experiment (3 and 12 h).

## EXPERIMENTAL

## Murine 3T3 fibroblast culture

The medium used in 3T3 fibroblast culture consisted of Dulbecco's Modified Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (Sigma, Germany). The 5 × 10<sup>5</sup> cells were cultured in flasks with 25 cm<sup>2</sup> growth area (Greiner, Germany) at 37°C in 5% CO<sub>2</sub>. The 3T3 fibroblasts passages were done using a 0.1% trypsin and 0.02% EDDA solution (Sigma, Germany) when cells covered 70-80% of the flask area.

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### The evaluation of the influence of $\text{AgNO}_3$ on the viability of murine 3T3 fibroblasts with the use of trypan blue staining

The assessment of the influence of  $\text{AgNO}_3$  on the viability of murine 3T3 fibroblasts was performed with the use of trypan blue (Sigma, Germany) staining. 3T3 fibroblasts ( $10^4$  cells/well) were transferred to 12-well plates.  $\text{AgNO}_3$  solution was then added to the cultures in the following concentrations: 0.6; 1.2; 3.0; 4.5; 9.0; 12.0; 15.0; 30.0 and  $60.0 \times 10^{-4}$  M. 3T3 cells were incubated with PBS in volumes equal to  $\text{AgNO}_3$  solution volume in the control groups. The viability of 3T3 cells was evaluated after a short period of 3 h and then after 12 h of incubation with  $\text{AgNO}_3$ . In order to assess the viability, the fibroblasts were removed from the walls by trypsinization. Spun-down cells were suspended in 1 mL of culture medium. Next, a 50  $\mu\text{L}$  sample of the cell suspension was mixed with 50  $\mu\text{L}$  of dye (Trypan Blue, Sigma, Germany). Stained cells were placed in a Neubauer chamber and counted under a light micro-

scope magnification of  $100\times$ . The influence of each  $\text{AgNO}_3$  concentration was evaluated based on 10 separate measurements. The results were presented as mean values with standard deviation. Cell viability in a culture was estimated as the ratio of living cells to all cells in culture and presented as a percentage. The lethal concentrations of  $\text{AgNO}_3$  for 90.50 and 10% viability were calculated from previously calculated regression curves.

## RESULTS

The viability of 3T3 cells in the control group was very high, over 90%. The viability was assessed as 100% in the control group. 3 and 12-h exposure to low concentrations of silver nitrate ( $0.6$  to  $4.5 \times 10^{-4}$  M) has caused a drop to 40% in cell viability. In silver nitrate concentrations between 12 and  $60 \times 10^{-4}$  M low cell viability (below 20%) was observed during the 3 and 12 h experiment as well (Fig.1.A.B). The regression curve for the 12 h experiment was better adjusted than for 3 h (Fig.1.A.B). The lethal concentrations of  $\text{AgNO}_3$  after 3 h exposures were as follows  $\text{LC}_{10} = 0.98$ ,  $\text{LC}_{50} = 6.44$  and  $\text{LC}_{90} = 21.38$ . The lethal concentrations of  $\text{AgNO}_3$  after 12 h exposures were as follows:  $\text{LC}_{10} = 1.05$ ,  $\text{LC}_{50} = 6.91$  and  $\text{LC}_{90} = 22.96$ . The LC values were similar for 3 and 12 h exposure of  $\text{AgNO}_3$ .

## DISCUSSION

Urinary tract infections (UTI's) are the most common nosocomial infections experienced by patients and are responsible for significant morbidity and excess hospital costs. The introduction of a silver alloy, hydrogel-coated urinary catheter was associated with a significant decline in nosocomial UTI's and cost savings (1). Conclusions from another prospective, crossover study stated that silver-impregnated Foley catheters were not effective in preventing nosocomial UTI's (7). The Bardex I.C. catheter is a hydrogel latex Foley catheter with a monolayer of silver metal applied to the inner and outer surfaces of the catheter. The trial (five hospitals included) with a blind prospective study, had shown that exchanging the standard latex Foley catheter for the Bardex I.C. resulted in a trend toward an insignificant reduction in urinary tract infections with the use of the hydrogel/silver-coated catheter (3). However, the efficacy of antimicrobial catheters in hospitalized patients is still poorly defined. The meta analysis of twelve qualifying trials has shown that antimicrobial urinary catheters can prevent bacteriuria in hospitalized patients during short-term catheteriza-

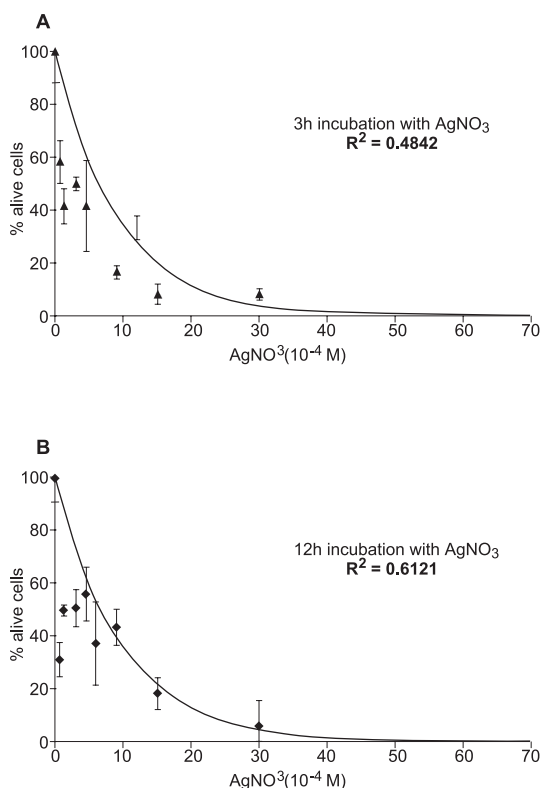


Figure 1. The viability of 3T3 line fibroblasts after 3 (A) and 12 h (B) of incubation with  $\text{AgNO}_3$ , evaluated with trypan blue staining. The percentage of viable cells compared to the control and standard deviation are plotted on the graph. Trend line was adjusted to visualize  $\text{AgNO}_3$  toxic effect.

tion, depending on antimicrobial coating and several other variables (8).

The data on the toxic effect of urological catheters and stents on urothelial layer is extremely sparse (9,10). The *in vitro* cytotoxicity studies often use immortalized cell lines and primary cultures as well. The use of primary cultures for such tests have certain difficulties. An important limiting factor is often the low success rate of culture establishment and heterogeneity of cells harvested from individuals (11-14). In our research, assessing the influence of AgNO<sub>3</sub> on 3T3 cell line was performed. It was previously proved that cell viability measured in the trypan blue assay correlates to the results of more detailed tests of cell culture viability (15). Human keratinocytes have undergone apoptosis when exposed to concentrations of silver, which were lethal for bacteria. Based on the burned patient treatment the nitrates should be used with caution especially under conditions where the epithelial layer has been destroyed (16,17). Urinary catheters are often introduced after small surgery and endoscopy procedures when epithelial layer is injured.

The aim of the study was to examine if the silver ions had a toxic effect during the short cell culture exposition. This experiment condition can reflect the possible influence of silver coated catheter on the cells during short term catheter indwelling. It was previously shown using a cell culture technique with a mouse fibroblast cell line (BALBc/3T3), and an animal model with implantation of catheter material into the urethra that especially latex materials do not have both cytotoxic effects and cause considerable inflammation within the urethral mucosa. It was speculated that by coating the catheters with silver, the cytotoxicity could be significantly reduced as compared with pure latex and hydrogel coated latex catheters (6). These data are opposite to the newest studies which have shown latex and silicone toxic influence on the urothelial cells *in vitro* (18). In an attempt to explain the inflammatory reaction within the urethra secondary to an indwelling catheter new studies should be undertaken. The next performed meta-analysis to estimate the effectiveness of silver-coated urinary catheters had clarified discrepant results among trials of silver-coated urinary catheters by revealing that silver alloy catheters are significantly more effective in preventing urinary tract infections than are silver oxide catheters (19). The study performed on Sprague-Dawley rats which were simultaneously implanted with two double-cuffed, silver-coated silicone rubber and standard silicone rubber catheters showed that there are sites where silver-coated catheters tend to induce less inflammation and infec-

tion and healed better than those with uncoated catheters. However, these data were statistically insignificant (20). The study performed on mouse fibroblasts culture using catheter extracts had shown that silver-coating had no toxic effect, whereas silver nitrate and silver sulfate coating did have a toxic effect (21). Our results strongly support these observations. The modern catheters are also coated with silver nitrate. It was shown that silver nitrate (10% by weight) and ofloxacin (5% by weight) blended caprolactone-L-lactide copolymer coated self-reinforced poly-L-lactic acid (SR-PLLA) urosprings left in the male rabbit urethra may provide possibilities of preventing bacterial adhesion to bioabsorbable stents and cause less tissue adverse reactions (22,23). In this study we have proved that silver nitrate had a similar toxic effect on mammalian cells in a short and long lasting *in vitro* experiment. There are many conflicting data concerning silver coated catheters. It was questionable if widespread use of silver coated catheters should be advised. Attention should be paid when silver coated catheter has a closed contact to injured urothelial layer.

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