



The use of plasma activated water in mouse models of disease

Roman Gardlik¹, Maria Suchonova¹, Lubomira Tothova¹, Barbora Konecna¹,
Katarina Kmetova¹, Slavomir Pasztor², Zdenko Machala²

¹Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia

²Division of Environmental Physics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia

E-mail: romangardlik@gmail.com

The beneficial effects of plasma activated water (PAW) have been previously shown mainly in decontamination of food industry products [1]. We prepared PAW by treating saline in non-thermal air plasma of transient spark discharge with water electro-spray (self-pulsing discharge with ~20 ns pulses of 1 kHz repetitive frequency and power ~2W; water flow rate 1 mL/min), as described in detail in [2]. The aim of our study was to investigate the effects of PAW on the growth of specific bacterial communities *in vitro* and *in vivo* in mouse models of urinary tract infection and colitis.

First, we tested antibacterial effects of PAW on the growth of uropathogenic strain of *Escherichia coli* (UPEC). 100 µL of liquid UPEC culture 10⁸ CFU/ml were treated with 1 mL of PAW and cultivated for 1, 2, 3, 4 or 5 minutes. Hydrogen peroxide (0,5%) was used as positive control. All tested times of cultivation showed significant inhibition of bacterial growth. In addition, we monitored the growth curve of UPEC liquid culture in the presence of PAW or control water. A clear inhibitory effect was shown when 10⁸ or less bacteria were incubated with PAW. These were preliminary *in vitro* studies for the subsequent *in vivo* experiment on mice.

Urinary tract infection (UTI) was induced by administration of the given UPEC strain into urinary bladder of mice. 24 h after the infection the bladder was washed three times with PAW or control water and the number of live bacteria was measured in the bladder and kidney homogenates 24 h after last wash. No bactericidal effect was observed in the bladder compared to control water. However, a trend of decreased number of live bacteria in the kidney of the infected mice was shown with PAW compared with control water. This indicates that PAW might have a bactericidal effect *in vivo*. This, however, needs to be investigated in more detail.

The effect of PAW was also tested in colitis (inflammation of the large intestine) in mice. Colitis is partially mediated by the presence of certain bacterial strains in the colon. We tested whether washing of the colon using PAW might provide a beneficial effect on the course of the disease. Colitis was induced by 7-day intake of 2% dextran sulphate sodium in tap water. PAW or control water was administered to the colon by colonoscopy instrument on day 4 and 6 of the experiment. Colon length as a marker of disease activity was slightly improved in the PAW group compared to control. However, none of the other inflammatory markers were significantly different in PAW group compared to control. Therapeutic effect of PAW in colitis was not proved in a reliable way.

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- [2] Z. Machala, B. Tarabová, *et al.*, *J. Phys. D: Appl. Phys.* 52, 034002 (2019)