Journal of
Complementary Medicine & Alternative Healthcare

Juniper Publishers
key to the Researchers

Short Communication
Volume 1 Issue 4 -February 2017

J Complement Med Alt Healthcare

Examination of Immunological Effects of Homeopathic *Escherichia coli* extract (*E. coli extractum* 4x-8x) on Bladder Epithelial Cells

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Submission: January 31, 2017; **Published:** February 22, 2017

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Abstract

The homeopathic extract *E. coli extractum* (Sanukehl®Coli) of gram-negative *Escherichia coli* bacteria in the potency 6Xis applied in treating bacterial infections, especially of chronic cystitis. As a cause of chronic cystitis, intracellular infections are discussed. This study analyzes the *in vitro* effect of *E. coli extractum* 4X-8X in T 24 human bladder cancer cells (T24) on the release of proinflammatory TNF-α, IL-6, IL-8 and the anti-inflammatory cytokine IL-10.TNF-α and IL-8 are responsible for activation and migration of granulocytes into the tissue, a reaction of the innate immune system. Furthermore, the function of IL-6 goes even further. It stimulates, inter alia, differentiation factors of B-cells to antibody-producing plasma cells, and consequently the humoral immune system. IL-10 is one of the anti-inflammatory cytokines. The results of this study show, that the homeopathic preparation of *E. coli extractum* can modulate the release of cytokines of the innate and humoral immune system.

Keywords: Cystitis; Cytokines; *E. coli extractum;* 4X-8X; Human bladder epithelial cells; Lipopolysaccharide (LPS)

Introduction

The urinary tract is extremely susceptible to infection by different pathogens. Most E. coli strains are facultative pathogenic gram-negative bacteria in cystitis, which could also exist intracellularly [1,2]. In homeopathic therapy *E. coli extractum (DSMZ Nr. 14345)* is used to treat cystitis [3], due to its ability to activate the innate immune system of the bladder and, in particular, of the bladder epithelial cells (BECs). The activation of the innate immune system to initiate the healing process is mediated e.g. by the binding of uropathogenic bacteria to Toll-like receptor 4 (TLR4) [4]. Studies on TLR4 signaling in monocytes, macrophages, and dendritic cells have revealed that the binding of lipopolysaccharide (LPS) to the TLR4 receptor triggers a signaling cascade, resulting in the activation of nuclear factor-κB (NF-κB) which in term regulates the expression of several immunomodulatory cytokines, like TNF-α, IL-6, IL-8, and IL-10 [5-7]. TNF-α regulates the induction of apoptosis in epithelial cells, and is involved in the defense of intracellular pathogens which in turn leads to an exfoliation of diseased surface cells [8].

In this study, we analyzed the effects of the homeopathic *E. coli* extract in the potency 4X-8X and present here the data for potency 6X on the cytokine release in vitro, on both, non-inflamed, immunologically inactive, and on inflamed, immunologically active T24 human bladder cancer epithelial cells. In this cell culture system, the non-inflamed T24 cells are defined by the cells being cultivated without LPS. Inflamed cells, in contrast, are cultivated in the presence of LPS.

Cells were treated with different concentrations of the test substances for 24 hours, followed by an MTT assay (Sigma Aldrich, Steinheim, Germany). Only living cells can convert yellow MTT reagent to purple formazan by mitochondrial reductase enzymes. The absorbance of formazan was measured at 550nm. At 24h none of the tested concentrations were cytotoxic.

Discussion and Conclusion

In non-inflamed cells (absence of LPS), *E. coli extractum*6X increased the expression of IL-6 and IL-8 significantly. Also, a significant TNF-α release was also visible in T24 cells (Figure 1). A concentration dependency was detectable (data not shown). In inflammed cells (presence of LPS) *E. coli extractum* 6X slightly decreased IL-6, IL-8, and TNF-α expression in presence of LPS, significantly (Figure 2). IL-10, the anti-inflammatory cytokine [9], was undetectable in the supernatants of T24 bladder cells.

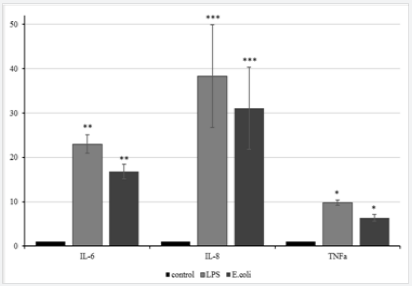

Figure 1: Effects of *E.coli* extractum 6X on the IL-6. IL-8 and TNF-α release in non-inflamed T24 cells. Cells were stimulated with *E.coli* extract for 24h. LPS (1µg/ml) was used as a positive control. IL-6, IL-8, and TNF-α expression in supernatants was measured in a microtiter plate reader (TriStar LB 941) at A460 nm. Values are relative to control; p-values are depicted with p<0.05: *, p<0.01:**, and p<0.001:***.

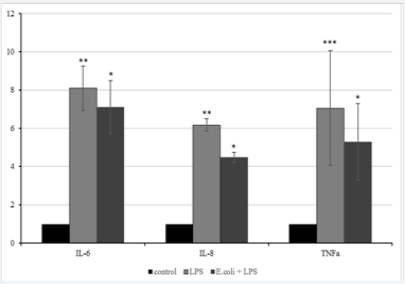

Figure 2: Effect of E.coli extractum 6X on the LPS-mediated IL-6. IL-8, and TNF-α release in inflamed T24 cells. Cells were pretreated with E.coli extract for 30min, and then additionally stimulated with LPS for 24h. Cytokine expression in supernatants was measured in a microtiter plate reader (TriStar LB 941) at A460 nm, respectively. Values are relative to control; p-values are depicted with p<0.05: *, p<0.01:** and p<0.001:***.

These results indicate, that homeopathic *E. coli extractum* 6X has an immunomodulating effect. In inflamed T24 cells, the release of proinflammatory cytokines is reduced while it is stimulated in non-inflamed, immunologically inactive, cells. This result supports the observation made in the therapeutic application, that *E. coli extractum* 6X reduces an existing inflammatory process. In contrast, in chronic infections caused, inter alia, by intracellular E. coli strains, where immunological defense is not active, the E. coli extract stimulates the immune system by cytokine production. This may be a part of the mechanism of action that supports the healing process, which should be investigated in further studies.

Acknowledgement

The author would like to acknowledge CONVIDIA clinical research GmbH, Von-Steuben-Strasse 10, 48143 Muenster, Germany, for their collaboration in the manuscript development, and VivaCell Biotechnology GmbH, Ferdinand-Porsche-Str. 5, 79211 Denzlingen, Germany, for conducting the experimental investigation.

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How to cite this article: Dieter S. Examination of Immunological Effects of Homeopathic *Escherichia coli* extract (*E. coli extractum* 4x-8x) on Bladder Epithelial Cells. J Complement Med Alt Healthcare. 2017; 1(4): 555566.