Wrażliwość uropatogennych szczepów *Escherichia coli* na fosfomycynę oraz jej interakcje z wybranymi ekstraktami roślinnymi stosowanymi w zakażeniach układu moczowego

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SUMMARY

Urinary tract infections (UTI) may be regarded as an important clinical problem. They belong to the most frequent infections both in hospitalized patients and outpatients. The highest percentage of UTI is caused by uropathogenic *Escherichia coli* strains (UPEC). These bacteria possess several virulence factors together with those responsible for colonization of lower urinary tract.

Fosfomycin is one of the antibiotics recommended for treatment of acute cystitis in women. This antibiotic is usually used as single 3 g dose. Its therapeutic concentration in urine, lasting for 1-2 days allows for elimination of the majority of uropathogens from urinary tract.

The aim of this paper was to assess the activity of fosfomycin *in vitro* against uropathogenic *E. coli* strains isolated from urine samples of outpatients with acute cystitis as well as interaction *in vitro* of fosfomycin with ciprofloxacin and the selected plant extracts used for treatment of UTI. The bacterial strains included in this study were from the collection of 74 clinical strains of Department of Pharmaceutical Microbiology, Medical University in Lublin. Besides, the reference strain *Escherichia coli* ATCC 25922 was used.

During studies, MIC (Minimal Inhibitory Concentration) for fosfomycin was estimated using the available methods: methods of serial drug dilution in solid or liquid medium as well as gradient-diffusion method. Also MBC (Minimal Bactericidal Concentration) for fosfomycin was determined. The growth rates of a chosen clinical strain in the presence of various fosfomycin concentrations was analyzed by the time-kill assay. Moreover, MIC for ciprofloxacin (the second`s generation fluoroquinolone) and the studied plants extracts was estimated together with their interactions with fosfomycin. Besides, the antioxidative activity of the extracts was determined in correlation with total polyphenol concentration.

On the basis of the obtained data it was assumed to be possible to assess efectiveness *in vitro* of fosfomycin against UPEC used in this study and the prevalence of fosfomycin-resistant strains as well as to propose new compositions of plant extracts showing additive or synergistic effects when used in combination with fosfomycin and good antioxidative properties.

First, activity of fosfomycin was estimated against 74 clinical strains of *E. coli* and the reference strain *E. coli* ATCC 25922 on the basis of MIC values determined by the reccomended metod of serial drug dilution in solid medium (Mueller-Hinton agar) supplemented with glucose-6-phosphate (25 mg/L). It was found that all of the strains studied were sensitive to fosfomycin (MIC $\leq$ 32/mg/L). Next, MIC values of fosfomycin were determined for a chosen 9 clinical strains of *E. coli* and the reference strain *E. coli* ATCC 25922 using three methods: the metod of serial drug dilution in solid medium (Mueller-Hinton agar) supplemented with glucose-6-phosphate, the gradient-diffusion method with commerical paper strips containing fosfomycin and glucose-6-phosphate as well as the method of serial drug dilution in liquid medium (Mueller-Hinton broth) supplemented with glucose-6-phosphate. The obtained MIC values were similar, irrespective of the metod used. Then, MBC (1-4 mg/L) and MBC/MIC (1-4) of fosfomycin for a chosen 9 clinical strains of *E. coli* and the reference strain *E. coli* ATCC 25922 were estimated; MBC/MIC $\leq$ 4 showed bactericidal effect of fosfomycin against the strains studied.

Subsequently, the time-kill assay was performed to assess the growth rates of a chosen clinical strain (MIC = 0,5 mg/L) in liquid medium (Mueller-Hinton broth) supplemented with glucose-6-phosphate in the presence of various fosfomycin concentrations (0,5-64 mg/L). The decrease of the bacterial population density after 6 h incubation was found (Δlog = 1,05-5,02), increasing with the increase of fosfomycin concentration in the range 0,5-32 mg/L, lasting at concentration of 64 mg/L. The phenomenon of re-growth of bacterial population (Δlog = 1,03-3,03) was observed at fosfomycin concentration in the range 0,5-32 mg/L after 24 h incubation; the higher fosfomycin concentration resulted in lower intensity of bacterial population re-growth. This phenomenon was not found at 64 mg/L fosfomycin.

Next, activity of ciprofloxacin was determined for a chosen 9 clinical strains of *E. coli* and the reference strain *E. coli* ATCC 25922 using the method of serial drug dilution in liquid medium (Mueller-Hinton broth). It was found that all of the strains were sensitive to ciprofloxacin with MIC = 0,04-012 mg/L.

Then, interactions *in vitro* between fosfomycin and ciprofloxacin against the reference strain *E. coli* ATCC 25922 were assessed using FIC index (Fractional Inhibitory Concentration

Index). The obtained results were analyzed in comparison with the thresholds of ∑FIC for various type of interactions such as addition, synergy, neutralism or antagonism. It was found that additon (∑FIC = 0,626) or synergism (∑FIC = 0,375) occurrred between fosfomycin and ciprofloxacin.

During next experiments, acitivity *in vitro* of 12 water plant extracts against a chosen 9 clinical strains of *E. coli* and the reference strain *E. coli* ATCC 25922 was determined as well as their interactions with ciprofloxacin and fosfomycin. First, MIC for the studied plant extracts was determined against a chosen 9 clinical strains of *E. coli* and the reference strain *E. coli* ATCC 25922 using the method of serial drug dilution in liquid medium (Mueller-Hinton broth). On the basis of the obtained results, the plant extracts were divided into 5 groups, depending on their antibacterial activity: (I) extract from alpine cranberry leaf (MIC_{50} = MIC_{90} = 8 mg/L), (II) extracts from bearberry leaf and european goldenrot (MIC_{50} = 8 mg/mL, MIC_{90} = 16 mg/L), (III) extracts from birch leaf, Java tea, majoram, rupturewort and knotgrass herb (MIC_{50} = MIC_{90} = 16 mg/L), (IV) extracts from nettle leaf, horsetail herb and restharrow root (MIC_{50} = 16 mg/L, MIC_{90} = 32 mg/L), (V) lovage root (MIC_{50} =MIC_{90} = 32 mg/L). The highest activity was found for extract from alpine cranberry leaf, while the lowest – for extract from lovage root. The obtained MBC values for the studiem extracts were similar to those of MIC, resulting in MBC/MIC = 1-2 which indicated the bactericidal effect of the extracts. MIC and MBC for the extracts against the reference strain *E. coli* ATCC 29522 were comparable to those for clinical strains.

Next, interactions *in vitro* between fosfomycin and plant extracts against the reference strain *E. coli* ATCC 25922 were assessed using FIC index. It was found that the combination of fosfomycin with the extracts from nettle leaf, bearberry leaf, european goldenrod and restharrow root resulted in additive effect (∑FIC = 0,625-0,75). The interaction such as neutralism was observed for combination of fosfomycin with all other extracts (ΣFIC = 1,25-3) as well as for combination of ciprofloxacin with all extracts studied (ΣFIC = 2-4).

Finally, antioxidative activity of the plant extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. This activity was differential. Q, that is percent of reduction of reactive oxygen species at a defined extract concentration (0,5 mg/mL) was within the range <5%-89,3%. This parameter was correlated with total polyphenol content (<0.1-119,1 mg gallic acid/100 g extract). The most active were extracts from bearberry leaf and alpine cranberry leaf, while the east active – extract from birch leaf (<5%).

Summarizing, data presented in this dissertation: (I) confirm high activity *in vitro* of fosfomycin against UPEC isolated from actute cystitis, (II) suggest a possibility of combination of fosfomycin with ciprofloxacin because of favourable interactions *in vitro* such as addition or synergism, (III) suggest a possibility of combination between fosfomycin and some plant extract, present in herbal medicinal products used for UTI treatment because of *in vitro* favourable interactions such as addition. The obtained results may be the valuable information about a possibility of combination between fosfomycin and herbal medicinal products for treatment of uncomplicated UTI.