ANKEM Derg 2005;19(2):77-79.

IN-VITRO SUSCEPTIBILITY OF CLINICAL ISOLATES OF *ESCHERICHIA COLI* TO FOSFOMYCIN TROMETAMOL AND OTHER ANTIBIOTICS

İlhan AFŞAR, Berna GÖNÜL, Aslı Gamze ŞENER, Metin TÜRKER

Atatürk Training Hospital, Department of Microbiology, İZMİR

SUMMARY

Escherichia coli is the most commonly isolated microorganism in uncomplicated urinary tract infections. Among the large number of currently available antimicrobial agents, fosfomycin trometamol is a useful alternative for other antibiotics. We studied the in-vitro susceptibility of 236 clinical urinary isolates of E.coli to fosfomycin trometamol in comparison with some other antibiotics. The percentages of susceptible strains were found to be 99 % for imipenem and amikacin, 97 % for fosfomycin, 87 % for cefepime, 81 % for ceftriaxone, 67 % for amoxicillin/clavulanic acid, 63 % for ciprofloxacin, 62 % for sulphamethoxazole/trimethoprim and cephalothin, and 32 % for ampicillin. No significant differences were found in the efficacies of fosfomycin, imipenem and amikacin ($p>0.05$) and these three antibiotics were found significantly more active than others ($p<0.001$).

Keywords: *Escherichia coli, fosfomycin trometamol, urinary infections*

ÖZET

Escherichia coli'nin Klinik İzolatlarının Fosfomisin trometamol ve diğer Antibiyotiklere in-vitro Duyarlılığı

Komplikasyonsuz üriner sistem infeksiyonlarında en sık izole edilen mikroorganizma E.coli'dir. Fosfomisin trometamol bu infeksiyonlarda kullanılabilecek iyi bir alternatiftir. Çalışmamızda 236 klinik üriner E.coli izolatında, fosfomisin trometamol duyarlılığı bazı diğer antibiyotiklerle karşılaştırılmıştır. Duyarlı suş oranları imipenem ve amikasin için % 99, fosfomisin için % 97, sefepim için % 87, seftriakson için % 81, amoksisilin/klavulanik asit için % 67, siprofloksasin için % 63, sulfametoksazol/trimetoprim ve sefalotin için % 62, ampisilin için % 32 olarak bulunmuştur. Fosfomisinin etkinliği ile imipenem ve amikasinin etkinliği arasındaki fark anlamsız bulunmuş ($p>0.05$), fakat bu üç antibiyotik ile diğerleri arasında anlamlı etkinlik farkı saptanmıştır ($p<0.001$).

Anahtar sözcükler: *Escherichia coli, fosfomisin trometamol, üriner infeksiyonlar*

INTRODUCTION

Escherichia coli is the most commonly isolated microorganism in uncomplicated urinary tract infections (UTI)[14]. Other responsible microorganisms include other *Enterobacteriaceae* (*Klebsiella, Enterobacter, Proteus* spp...), *Staphylococcus saprophyticus, Streptococcus, Enterococcus, Pseudomonas* strains[4]. The optimal treatment duration required for lower UTI is still under discussion. However, several studies indicate that single-dose therapies may obtain cure rates similar to conventional treatments. The advantages of the single-dose may also include minimising the risk of side effects and the development of resistant bacterial strains[2]. An ideal agent for single-dose therapy of UTI are listed in table 1[11].

Correspondence address: İlhan Afşar. Atatürk Training Hospital, Microbiology Department, İZMİR
Tel.: (0232) 244 44 44/2439
e-mail:iafsar@yahoo.com
Received: 26.01.2005, accepted: 23.03.2005

Table 1: *Ideal properties for a single-dose agent.*

Antimicrobial	Activity (Low MIC) Cidal (Low MBC) Spectrum (Broad enough) No emergence of resistance
Pharmacokinetics	Urine concentration high Slow excretion No inactivation
In-vivo	Active Acceptable No change commensal flora

Fosfomycin trometamol (FT) is bactericidal against a wide range of Gram positive and Gram negative bacteria and is nontoxic[8].

The bactericidal effect of fosfomycin is due to blocking bacterial wall synthesis. Two main transport systems, the L-α-glycerophosphate system and the hexose phosphate uptake system, promote fosfomycin passage across the bacterial cell to reach its target site. The blocking of cell wall synthesis is accomplished by the inhibition of pyruvyl transferase, an enzyme necessary for the first step in the synthesis of bacterial cell walls[13]. Recommended dosage for women 18 years of age and older is one 3.0 g sachet (powder) of FT mixed with water. Only a single dose of FT is recommended for the treatment of acute cystitis. Studies in children have utilized a single 2.0 g dose of FT[13].

The bioavailability of oral FT in adults is approximately 40 %[1]. Fosfomycin attains maximum serum concentrations (Cmax) in about two hours. Pharmacokinetic profile of FT is in table 2[13].

Table 2: *Pharmacokinetic profile of fosfomycin trometamol.*

Tmax (hours)	2.5 + 0.8
Cmax (mg/L)	26.2 + 8.1
T1/2 (hours)	3.6 + 0.44
Urinary recovery over 48 hours (% of dose)	43.3 + 9. 4

Usage of 50 mg/kg dose

Fosfomycin has a large apparent volume of distribution (140 L) and is not higly bound to plasma proteins [10].

The purpose of the study was to determine the in-vitro susceptibility to FT and other antibiotics of clinical isolates of *E.coli*.

MATERIAL AND METHODS

Female patients between 18-65 years of age with semptoms (dysuria, pollakisuria without fever, urgency and suprapubic pain,i.e.) of uncomplicated UTI were included in the study. Patients were excluded from the study on the basis of: history of chronic urinary tract infection, renal insuficiency or renal abnormalities, pregnancy.

Total 236 patients were included. Midstream urine samples were first examined by dipslide and sediments were examined for leucocyturia.

All bacteria occuring in urine samples at $\geq 10^4$ cfu/ml were carried out for conventional culture and bacteriological identification. Bacteria occuring at $<10^4$ cfu/ml were excluded from study. *E.coli* species were identified by their biochemical reaction profile using BBL Crystal E/NF identification products (Becton-Dickinson, USA) for confirmation. Identified *E.coli* species were stored on microbeads and frozen at -70ºC until for susceptibility test. The in-vitro susceptibility of FT in comparison with ampicillin (AMP), cephalothin (KF), amoxicillin/clavulanic acid (AMC), sulphamethoxazole/trimethoprim (SXT), ceftriaxone (CRO), ciprofloxacin (CIP), imipenem (IMP), cefepime (FEP), amikacin (AK) and fosfomycin trometamol (FT) were determined by the disk diffusion method performed in accordance with National Committee for Clinical Laboratory Standards (NCCLS) procedures[7]. Muller Hington agar (Oxoid, England) was used as culture medium. Zone sizes were calculated according to the recommendations of NCCLS (Table 3). *E.coli* ATCC 25922 was used as control strain.

Table 3: *NCCLS zone diameter of used antimicrobial agents.*

Antimicrobial agent	NCCLS zone diameter			μg/disk
	R	I	S	
Ampicillin	≤13	14-16	≥17	10
Cephalothin	≤14	15-17	≥18	30
Amoxicillin/clav. acid	≤13	14-17	≥18	20/10
Sulpha./trimethoprim	≤10	11-15	≥16	1.25/23.75
Ceftriaxone	≤13	14-20	≥21	30
Ciprofloxacin	≤15	16-20	≥21	5
Imipenem	≤13	14-15	≥16	10
Cefepime	≤14	15-17	≥18	30
Amikacin	≤14	15-16	≥17	30
Fosfomycin trometamol	≤12	13-15	≥16	200/50

R: Resistant I: Intermediate S:Sensitive

RESULTS

FT was very effective against 236 clinical isolates of *E.coli*. Resistance to FT was observed in six clinical isolates. The results obtained for AMP, KF, AMC, SXT, CRO, CIP, IMP, FEP, AK in comparison to FT were given in table 4.

Table 4: *Results of disk diffusion method.*

Antimicrobial agent	Number of isolates		
	Sensitive	Intermediate	Resistant
Imipenem	234	-	2
Amikacin	233	-	3
Fosfomycin trometamol	230	-	6
Cefepime	205	-	31
Ceftriaxone	191	-	45
Amoxicillin/clav. acid	158	5	73
Ciprofloxacin	148	2	86
Sulpha./trimethoprim	147	2	87
Cephalothin	146	7	83
Ampicillin	75	-	161

DISCUSSION

Escherichia coli is the most frequently isolated pathogen in UTI[5,6,9,14]. Generally, AMP, SXT, AMC, CIP are used in patients with uncomplicated lower UTI. These antibiotics must be used at least 7 days and sometimes side effects may be seen.

Comparative clinical trials suggest that a single 3 g dose of FT is as clinically effective as 7-10 day treatment regimens of standard agents such as nitrofurantion, norfloxacin and SXT used to treat UTI. FT is well tolerated and has poor side effects. FT treatment populations have included young women (including pregnant patients), elderly women and children with acute uncomplicated lower UTI[12].

Our study has shown that FT is in-vitro effective against *E.coli* strains (97.5 % susceptibility rate). There are a lot of studies for FT treatment effectivities of uncomplicated UTI in women. FT has cure rates of 75-80 % [9], pathogens of monoinfections were eradicated in 87.1 % of 284 patiens treated with FT[6]. FT eradicated in 81.2 % of patients[8] and cure rate was 89 % in patients treated by FT[4]. Our study has shown that IMP and AK were the most effective antibiotics for *E.coli* and followed by FT, but the differences were insignificant in these three agents ($p>0.05$). But the differences between these three antibiotics and others were significant ($p<0.001$).

Basic criteria for effective lower UTI treatment (efficacy, tolerability and low cost) are met by FT with an advantage of single dose treatment[14].

In conclusion, having a good in vitro antibacterial spectrum to *E. coli* strains, and known favourable pharmacokinetic properties with high urine concentrations, FT is an appropriate antibiotic for the single dose treatment of uncomplicated UTI.

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