INFLUENCE OF A NEW-QUINOLONE ANTIBACTERIAL AGENT, TEMAFLOXACIN, ON NORMAL HUMAN INTESTINAL MICROFLORA

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The effect of temafloxacin (TMFX), a newly synthesized quinolone antibacterial agent, given orally for 7 days to five healthy male volunteers, on intestinal microflora was investigated. Faecal specimens were cultured quantitatively for aerobic and anaerobic bacteria before, during and after administration.

Marked suppressions of Enterobacteriaceae and *Streptococcus*, including *Enterococcus*, were observed during TMFX administration. The anaerobic bacteria, *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Veillonella* and Gram-positive cocci were also reduced by TMFX administration in most subjects. Lecithinase-positive *Clostridium* was eliminated, though, lecithinase-negative *Clostridium* was not affected. Recovery of the levels and composition of the intestinal microflora was not delayed.

Key words: Intestinal flora; Quinolone; Temafloxacin

Introduction

Temafloxacin (TMFX) (Abbott Laboratories, USA) is a newly developed quinolone antibacterial agent, which has good activity against a broad-spectrum of microorganisms including anaerobic bacteria[4,6,8]. Therapy with broad spectrum antimicrobial agents induces changes in the intestinal flora[2] These changes in the faecal microflora may induce diarrhea, overgrowth of *Clostridium difficile*[1,7] and fungi, and impairment of colonization resistance in the digestive tract[9].

The present study was performed to investigate the influence of TMFX on intestinal microflora in healthy male volunteers under steady state conditions.

Materials and Methods

1. Subjects

Five healthy male volunteers, aged 30～43 years, were studied. None of the volunteers had taken any antimicrobial drugs or experienced gastrointestinal disorders for at least 1 month before the study. Written informed consent was obtained and the volunteers were admitted to the clinical study units during the period of TMFX use.

2. Treatments

A dose of 300 mg of TMFX was given orally to five subjects followed by subsequent doses of 300 mg at 12 h intervals for 7 days.

3. Microbiological studies

Faecal samples were collected before administration of TMFX, on the fourth day of treatment and then on one day (day 8), one week (day 14), 2 weeks (day 21) and 4 weeks (day 35) after cessation of treatment. The faecal samples were put into Gaspak pouches (BBL, Microbiology Systems, Cockeysville, Md. USA) and transported to the laboratory immediately after defecation into sterile plastic containers. One gram of stool was transferred to 9 ml of prereduced phosphate buffered saline with 0.1 per cent agar, serial 10-fold dilutions were made and 0.05 ml volumes were seeded on various media for detection of intestinal bacteria. The media for detection of intestinal bacteria and the incubation method have been described previously[10] Non-selective media for total

aerobes and total bacteria, and selective media for Enterobacteriaceae, *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Lactobacillus*, yeast, lecithinase positive *Clostridium*, *Bifidobacterium*, *Eubacterium*, *Bacteroides*, *Bacteroides fragilis* group, *Veillonella*, *C. difficile* and *Fusobacterium* were used. Selective media and non selective medium for total aerobes were inoculated with 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} dilution of the faecal samples. Non selective media for total bacteria were inoculated with 10^{-6}, 10^{-7} and 10^{-8} dilution. Plates were inoculated at 37℃ for 24～48 h for aerobic cultures or for 48～72 h in anaerobic jars for anaerobic cultures.

Different colonies on selective and non-selective media were counted and identified to genus level by Gram stain characteristics and cell morphology. Lecithinase-positive strains on Negler Neomycin agar[10] were identified with *Clostridium perfringens* differentiation strip (Nissui Seiyaku, Tokyo, Japan). Different colonies on selective media for Enterobacteriaceae, *Enterococcus* and *Bacteroides* were counted and identified to species level biochemically with Biotest No. 1 (Eiken Kagaku, Tokyo, Japan), API STREPT (Analytab Product, La Balme Les Grottes, France) and API 20A (Analytab product), respectively.

Results

Microbiological changes in the faeces of each volunteer during antibacterial agent use and after treatment are listed in Table 1-1, 1-2. In general, total aerobes were eliminated during treatment and one day after cessation of treatment. Total anaerobes were suppressed on the fourth day of treatment in 3 of 5 volunteers and returned to the pretreatment levels one day after treatment. The levels of Enterobacteriaceae and *Enterococcus* decreased markedly during administration of TMFX and had returned to pretreatment levels by 7 days after treatment. In subject C, 2.3 logs of *P. aeruginosa* was detectable 7 days after treatment. The numbers of *Bacteroides* decreased on the fourth day of treatment in all but subject A. In subject A, the viable count of *Bacteroides*, especially the *B. fragilis* group, increased on the fourth day of treatment. The administration of TMFX induced decreases in Enterobacteriaceae, *Streptococcus* and *Enterococcus*, and also caused a decrease in anaerobes including the *B. fragilis* group, *Eubacterium*, *Bifidobacterium* and Gram-positive cocci. However, the levels of total bacteria and total aerobes returned to the same level as before administration one day and 7 days after treatment, respectively. Other predominant anaerobic bacteria, *Eubacterium*, Gram-positive anaerobic cocci and *Bifidobacterium* were eliminated during antibacterial agent intakes in all subjects. In subject A, *Eubacterium* and *Bifidobacterium* decreased markedly, and in subject B, *Eubacterium* and Gram-positive anaerobic cocci decreased, and in subject C, Gram-positive anaerobic cocci decreased. *Veillonella* also decreased on the fourth day of treatment, but increased from 7 days to 28 days after treatment in all subjects. Lecithinase positive *Clostridium* was detected in faeces of 3 subjects before treatment and the levels of these organisms were decreased below the lowest detectable level during and after treatment. However, lecithinase negative *Clostridium* was not affected by TMFX administration. The major anaerobic bacteria had returned to pretreatment levels by one day after treatment.

TMFX eliminated *Escherichia coli* that was detected in faeces of most subjects before treatment and enumerated Enterobacteriaceae other than *E. coli* by 7 days after the last dose of TMFX. *E. coli* had returned to the pretreatment levels by 14 days after treatment (Table 2). During the TMFX administration period, the level of *Enterococcus* decreased markedly and had returned to normal by one week after cessation of treatment. There was no significant increase in *Enterococcus* (Table 3). The composition of *Bacteroides* species in the faeces of each subject varied during the period of this study.

B. ruminicola was detected dominantly in faeces of subjects B, D and E before treatment and decreased markedly during TMFX use (data not shown).

C. difficile was not recovered from the faeces of any of the volunteers.

Discussion

Intestinal flora can be influenced by antimicrobial agents, especially by drugs which are excreted extensively in the bile. The altered gastrointestinal microflora may permit potentialiy invasive pathogens to overgrow[5] or lead to a diminution in colonization resistance to exogenous microorganisms[9]. TMFX has

been reported to be excreted mainly in the urine[3,8]. However, the concentrations of TMFX in faeces might be sufficient to affect both aerobes and anaerobes in the intestine. None of volunteers complained of abdominal pain, nausea or diarrhoea.

In vitro, TMFX was more active against the *B. fragilis* group than other new quinolone agents such as ciprofloxacin, ofloxacin and enoxacin[6]. With *in vivo* human testing, TMFX decreased the level of *Bacteroides* more markedly than did sparfloxacin[10]. Although the antibacterial activity of TMFX against faecal aerobes was almost same as those of other quinolone drugs, the levels of faecal anaerobes, especially the *B. fragilis* group, decreased during TMFX use and recovered promptly after administration. Thus, TMFX administration might not evoke side reactions such as diarrhoea.

Taken together, these results show that TMFX is

Table 1-1. Counts* of microorganisms in faecal flora of subjects A, B and C receiving 300 mg of temafloxacin orally at 12 h intervals for 7 days

Bacteria	Subject A						Subject B						Subject C					
	before	during	after				before	during	after				before	during	after			
		day 4	day 8	day 14	day 21	day 35		day 4	day 8	day 14	day 21	day 35		day 4	day 8	day 14	day 21	day 35
Enterobacteriaceae	8.0	—	—	—	8.8	9.0	7.1	—	—	8.0	9.1	7.4	8.0	—	—	8.7	8.1	8.0
Streptococcus	8.0	—	3.6	7.1	—	4.4	2.3	—	—	7.4	8.1	7.9	—	—	—	6.6	4.9	6.6
Enterococcus	5.9	—	—	6.9	7.8	3.4	5.6	—	—	7.5	7.6	—	8.0	—	—	7.0	6.3	6.6
Staphylococcus	3.8	—	—	—	—	—	—	—	—	—	2.3	—	—	2.6	2.3	—	—	2.8
Pseudomonas aeruginosa	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.3	—	—
Candida albicans	3.2	—	—	3.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lactobacillus	—	—	—	5.0	2.6	2.9	7.6	—	—	6.2	3.7	3.9	6.0	4.1	3.8	6.1	5.8	6.8
Bacteroides	10.2	10.6	10.6	10.6	10.5	10.6	10.8	9.9	10.7	10.8	10.8	10.6	10.5	10.1	10.1	10.3	10.3	10.1
B. fragilis group	9.7	10.4	10.5	10.3	10.3	10.6	10.7	9.4	10.5	10.0	10.6	10.2	10.3	9.8	10.0	10.0	10.0	9.9
Fusobacterium	8.2	5.9	8.3	8.7	8.6	8.5	—	—	—	5.3	6.5	3.5	8.1	5.3	9.9	—	7.3	7.0
Eubacterium	9.5	—	—	9.6	9.8	9.7	9.8	—	9.5	9.3	10.0	9.5	10.3	9.8	8.9	9.1	9.2	9.3
Anaerobic Gram-positive cocci	9.2	7.1	9.4	8.9	8.6	9.1	9.6	—	9.6	9.6	10.0	9.4	7.9	—	8.6	9.3	8.3	8.1
Bifidobacterium	10.1	2.8	9.5	9.6	10.2	9.9	10.5	7.6	8.8	9.4	9.7	9.2	10.4	7.9	9.3	10.1	9.8	10.0
Veillonella	—	—	—	7.3	8.1	7.1	4.4	—	2.3	8.3	7.7	9.1	5.4	2.9	6.6	8.9	7.8	9.3
Megasphaera	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clostridium	6.6	7.3	6.3	9.4	7.6	7.9	9.3	8.6	9.6	9.4	9.3	8.6	8.2	9.9	7.0	8.3	8.8	9.1
C. difficile	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C. perfringens	—	—	—	—	—	—	—	—	—	—	—	—	5.3	—	—	—	—	—
Total bacteria	10.5	10.6	10.7	10.7	10.7	10.8	11.1	9.9	10.8	10.8	10.9	10.7	10.9	10.4	10.2	10.6	10.5	10.5

* Counts are expressed as $\log_{10}$ per gram wet faeces.
—: no organisms detected (counts of <2 $\log_{10}$ per gram).

Table 1-2. Counts* of microorganisms in faecal flora of subjects D and E receiving 300 mg of temafloxacin orally at 12 h intervals for 7 days

Bacteria	Subject D						Subject E					
	before	during	after				before	during	after			
		day 4	day 8	day 14	day 21	day 35		day 4	day 8	day 14	day 21	day 35
Enterobacteriaceae	8.8	—	—	7.0	6.8	7.4	7.3	—	—	5.0	7.3	5.5
Streptococcus	7.6	—	—	6.8	6.6	7.0	—	—	—	6.9	6.8	—
Enterococcus	6.7	—	—	7.4	4.8	3.1	8.3	—	—	6.8	6.2	7.5
Staphylococcus	3.2	—	3.2	—	—	4.0	2.3	—	2.6	2.9	3.3	—
Pseudomonas aeruginosa	—	—	—	—	—	—	—	—	—	—	—	—
Candida albicans	—	—	—	—	—	5.9	—	3.7	—	2.3	—	—
Lactobacillus	6.5	6.6	5.3	6.2	6.8	6.1	5.1	2.3	4.3	7.6	8.0	7.6
Bacteroides	10.4	9.9	10.7	10.5	10.7	10.6	10.2	10.3	10.2	10.3	10.6	10.7
B. fragilis group	10.2	9.3	10.3	10.2	10.1	10.2	10.2	9.7	9.1	9.2	9.1	9.7
Fusobacterium	—	2.9	3.7	2.6	—	—	8.8	9.2	9.2	8.1	9.2	7.8
Eubacterium	9.9	8.6	10.0	10.2	10.1	10.2	9.6	7.8	7.3	9.8	9.6	10.1
Anaerobic Gram-positive cocci	9.6	8.6	9.6	9.8	9.7	9.7	9.2	9.8	8.4	9.4	10.1	9.3
Bifidobacterium	10.0	7.3	9.6	10.3	10.3	10.3	9.6	9.3	9.7	9.9	10.1	10.1
Veillonella	6.5	—	5.3	7.3	7.1	7.4	3.8	—	5.6	9.3	9.3	5.5
Megasphaera	—	—	—	—	—	—	7.3	9.2	—	—	—	—
Clostridium	9.1	9.4	9.8	9.4	9.8	9.8	8.8	9.7	9.5	9.7	10.2	9.1
C. difficile	—	—	—	—	—	—	—	—	—	—	—	—
C. perfringens	7.4	—	—	—	—	—	3.8	—	—	5.6	—	—
Total bacteria	10.7	10.0	10.8	10.9	11.0	11.0	10.4	10.5	10.4	10.7	10.9	10.9

* Counts are expressed as $\log_{10}$ per gram wet faeces.
—: no organisms detected (counts of <2 $\log_{10}$ per gram).

Table 2. Levels* of Enterobacteriaceae in faeces of volunteers receiving 300 mg of temafloxacin orally at 12 h intervals for 7 days

Subject	Bacteria	Before	During	After			
			day 4	day 8	day 14	day 21	day 35
A	*E. coli*	7.8	—	—	—	8.7	9.0
	K. pneumoniae	—	—	—	7.1	—	—
	C. freundii	—	—	—	8.7	—	6.9
	H. alvei	7.0	—	—	7.5	—	—
	P. mirabilis	—	—	—	—	6.9	—
B	*E. coli*	7.1	—	—	—	8.6	—
	K. pneumoniae	4.8	—	—	5.9	7.4	5.1
	C. freundii	—	—	—	7.8	8.9	6.5
	S. odorifera	—	—	—	7.6	—	—
	M. morganii	—	—	—	—	7.7	—
	E. cloacae	—	—	—	—	—	5.7
	E. aerogenes	—	—	—	—	—	7.0
C	*E. coli*	—	—	—	—	7.7	7.3
	K. pneumoniae	6.6	—	—	7.0	6.3	6.8
	C. freundii	7.3	—	—	8.6	8.0	6.8
	E. cloacae	6.6	—	—	—	—	6.9
	E. agglomerans	6.3	—	—	—	—	—
	Salmonella sp.	—	—	—	6.3	—	—
	Gram-negative rod	—	—	—	—	—	6.3
D	*E. coli*	8.0	—	—	—	6.3	6.9
	C. freundii	—	—	—	6.2	6.3	—
	H. alvei	—	—	—	6.2	—	—
	E. cloacae	—	—	—	5.8	—	4.3
	Gram-negative rod	—	—	—	—	—	4.3
E	*E. coli*	6.6	—	—	4.3	6.9	4.8
	K. pneumoniae	—	—	—	2.6	6.6	—
	C. freundii	—	—	—	4.8	5.1	4.8
	E. cloacae	—	—	—	4.3	—	5.3
	E. agglomerans	4.3	—	—	—	—	—

* Counts of different colonies on Enterobacteriaceae selective medium (DHL) expressed as $\log_{10}$ per gram wet faeces.
—: no organisms detected (counts of <2 $\log_{10}$ per gram).

Table 3. Levels* of *Enterococcus* spp. in faeces of volunteers receiving 300 mg of temafloxacin orally at 12 h intervals for 7 days

Subject	Bacteria	Before	During	After			
			day 4	day 8	day 14	day 21	day 35
A	*E. faecium*	5.9	—	—	—	—	—
	E. faecalis	—	—	—	6.9	7.8	3.2
	E. gallinarum	—	—	—	7.1	—	—
	E. durans	—	—	—	—	—	—
B	*E. faecium*	5.6	—	—	—	7.6	—
	E. gallinarum	—	—	—	7.5	—	—
C	*E. faecium*	8.0	—	—	6.6	6.3	6.6
	E. faecalis	—	—	—	6.3	5.5	—
	E. durans	—	—	—	6.6	—	—
D	*E. faecalis*	6.7	—	—	7.1	4.8	3.1
	E. durans	—	—	—	7.0	—	—
E	*E. faecium*	8.3	—	—	—	—	7.5
	E. faecalis	—	—	—	6.9	5.7	—
	E. durans	—	—	—	5.0	—	—
	E. gallinarum	—	—	—	—	—	—

* Counts of different colonies on enterococci selective medium expressed as $\log_{10}$ per gram wet faeces.
—: no organisms detected (counts of <2 $\log_{10}$ per gram).

active against aerobic and anaerobic Gram-negative rods, *Enterococcus* and anaerobic Gram-positive cocci. Microbial levels were almost normal within one week after cessation of administration.

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新しいニューキノロン系抗菌剤，temafloxacinのヒト腸内細菌叢に及ぼす影響

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新しく合成されたキノロン系抗菌剤であるtemafloxacinを5人の健常成人男子に経口で7日間投与し，腸内細菌叢への影響を調べた。投与前，投与中および投与終了後の糞便サンプルを培養し，糞便中の好気性菌及び嫌気性菌の菌数を測定したところ，投与期間中，大腸菌群および腸球菌の著しい減少が観察された。バクテロイデス，ビフィドバクテリウムおよびユウバクテリウム等の腸内優勢嫌気性菌も，投与期間中多くの被験者で減少した。レシチナーゼ陽性クロストリジウムも減少したが，レシチナーゼ陰性クロストリジウムの菌数に変化はなかった。菌数および菌叢の構成は投与終了後すみやかに回復した。

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