Regulatory Toxicology and Pharmacology 73 (2015) 201–208

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Microbiological toxicity of tilmicosin on human colonic microflora in chemostats

Haihong Hao [b, 1], Junping Yao [b, 1], Qinghua Wu [c, d], Yajing Wei [b], Menghong Dai [b], Zahid Iqbal [b], Xu Wang [a], Yulian Wang [a], Lingli Huang [a], Dongmei Chen [a], Yanfei Tao [a], Zhenli Liu [a], Zonghui Yuan [a, b, *]

[a] National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[b] MOA Laboratory for Risk Assessment of Quality and Safety of Livestock and Poultry Products, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[c] College of Life Science, Yangtze University, Jingzhou, Hubei, China
[d] Faculty of Informatics and Management, Center for Basic and Applied Research, University of Hradec Kralove, Hradec Kralove, Czech Republic

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form
9 July 2015
Accepted 10 July 2015
Available online 16 July 2015

Keywords:
Tilmicosin
Human intestinal flora
Chemostat
Microbiological toxicity
Resistance
Virulence

ABSTRACT

To evaluate the microbiological safety of tilmicosin on human intestinal microflora, four chemostat models of healthy human colonic ecosystems were exposed to tilmicosin (0, 0.436, 4.36, and 43.6 μg/mL) for 7 days. Prior to and during drug exposure, three microbiological endpoints were monitored daily including short-chain fatty acids, bacterial counts and macrolide susceptibility. Colonization resistance of each community was determined by 3 successive daily challenges of *Salmonella typhimurium*. Genes associated with virulence and macrolide resistance in *Enterococcus faecalis* were determined by PCR. Transcriptional expression of the virulence gene (*gelE*) in *E. faecalis* was determined by real-time RT-PCR. Our results showed that different concentrations of tilmicosin did not disrupt the colonization resistance in each chemostat. During exposure to 4.36 and 43.6 μg/mL tilmicosin, the *Bacteroides fragilis* population was significantly decreased while the proportion of resistant Enterococci increased. After long-term exposure to the highest concentration (43.6 μg/mL) of tilmicosin, the *gelE* gene was significantly up-regulated in the high-level macrolide resistant strains that also contained the *ermB* resistance gene. This study was the first of its kind to evaluate the microbiological toxicity of tilmicosin using a chemostat model. These findings also provide new insight into the co-occurrence of macrolide resistance and virulence in *E. faecalis* under tilmicosin selective pressure.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Antimicrobial residues in meat products may have unintended harmful effects on the human intestinal flora. These potential consequences include changes in bacterial numbers and biochemistry, changes in the virulence and antibiotic resistance of resident bacteria as well as changes in the ability of the resident flora to prevent colonization by potential enteropathogens (MacNeil, 2005).

Tilmicosin, a new type of macrolide antibiotic, has been developed for treatment of respiratory diseases of economically important animals. As one of the most important veterinary drugs, the safety of tilmicosin in animal products is of global concern. The WHO has set the toxicological acceptable daily intake (ADI) as 0–40 μg/kg bw (WHO, 1998). However, systematic studies of the effects on the gut microflora (microbiological safety) have just started. Using a human gut flora associated (HFA) rodent model, the microbiological ADI of tilmicosin had been recommended at 4 μg/kg bw by the European Agency for the Evaluation of Medicinal Products (EMEA) and 40 μg/kg bw by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (EMEA, 2004; JECFA, 2010; Rumney and Rowland, 1995; WHO, 1998). However, these two microbiological ADIs were derived solely on the shifts in the enterobacterial population (Rumney and Rowland, 1995). The

* Corresponding author. National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan, Hubei 430070, China.
E-mail address: yuan5802@mail.hzau.edu.cn (Z. Yuan).
[1] The authors equally contribute to this work.

http://dx.doi.org/10.1016/j.yrtph.2015.07.008
0273-2300/© 2015 Elsevier Inc. All rights reserved.

effects of tilmicosin on other microbiological endpoints, like metabolic activity, development of resistance, colonization resistance and changes in virulence in the human intestinal floras are largely unknown.

To imitate the human intestinal microbial environment, a chemostat model has been developed and used for evaluating the impact of antibiotics on human colonic microflora (Carman et al., 2005; Carman and Woodburn, 2001). This chemostat model was recommended by United States Food and Drug Administration (FDA) for evaluating the microbiological safety of antimicrobial drugs and deriving a microbiological ADI (mADI) (FDA, 2005).

When evaluating the microbiological safety of macrolide drugs, a change in the incidence of resistant *Enterococci faecalis* populations is one of the most important endpoints. *E. faecalis*, a common species in human gut, accounts for nearly 10% of all nosocomial infections of the bloodstream, wounds, the urinary tract and the heart. Multiple virulence genes encoding aggregation substance (*asa1*), cytolysin (*cylA*), gelatinase (*gelE*) and cytolysin (*cylA*), have all been implicated as important factors for virulence of *E. faecalis* (Klibi et al., 2007). Two major mechanisms causing macrolide resistance in *Enterococci* are target modifications of the ribosomal RNA methylase encoded by *erm* genes and the membrane-bound efflux pumps encoded by the *mef* (*A*/*E*) and/or *msr* genes (Schwaiger and Bauer, 2008). Among these resistant genes, *ermB* gene plays a predominant role in the development of high-level resistance to macrolides, lincosamides and streptogramin B (MLS_B phenotype) in *Enterococcus* spp. (Harimaya et al., 2007).

In an effort to provide a complete microbiological safety spectrum, our study was designed to investigate the effect of tilmicosin on human colonic microflora by using a chemostat model. Under exposure to different levels of tilmicosin in chemostats, we measured changes in human colonic microflora including short-chain fatty acids (SCFA) concentrations, the populations of dominant bacteria, resistance development in facultative bacteria and colonization resistance (CR) against exogenous *Salmonella typhimurium* and Enterococci.

2. Materials and methods

2.1. Selection of four concentrations of tilmicosin

Previous studies have shown that gut microorganisms are exposed to approximately 40% of a tilmicosin dose administered orally and we factored this into our dose calculations for our chemostat experiments (EMEA, 2004). The colonic content of a 60 kg person is approximately 220 g. A low dose of tilmicosin (0.436 μg/mL) would be equivalent to the present ADI of tilmicosin (4 μg/kg bw) recommended by the EMEA (EMEA, 2004). We calculated an intermediate level at the ADI (40 μg/kg bw) of tilmicosin which would be 4.36 μg/mL a level recommended by JECFA (JECFA, 2010). The highest concentration of tilmicosin we used (43.6 μg/mL) was therefore equivalent to 400 μg/kg bw and was 100-fold greater than the lowest dose and we expected to have adverse effects on human intestinal microflora model. One group was set as drug control without the addition of tilmicosin.

2.2. Establishment of the chemostat model

Four separate chemostat models of the human large bowel ecosystem have been established by following previously published paper (Hao et al., 2013). A prepared fecal suspension of 50 mL was inoculated into each culture vessel and incubated for 7 h. Culture medium was then pumped through the vessels (35 mL/h) until they attained a steady state on day 7 and this was continued for another 7 days (days 8–13). On day 14, tilmicosin was added to three of the four chemostats to achieve final concentrations of 0.436, 4.36, 43.6 μg/mL. Tilmicosin was given for 7 days (day 14–20). The other chemostat containing no tilmicosin was taken as the negative control. Three samples (2 mL each) were collected daily from each culture vessel from days 7 through 20. These samples were then analyzed for the impact of tilmicosin on SCFAs, bacterial counts and resistance development. A new culture was started for each of the replicate test series.

2.3. Monitoring the change of SCFAs in the chemostats

Dynamic change in three primary SCFAs (acetic acid, propionic acid and butyric acid) was determined by replicating an established gas chromatographic method (Hao et al., 2013). The assay was performed in triplicate to obtain the mean concentration of SCFAs in each sample.

2.4. Monitoring the change of predominant bacteria counts in chemostats

We monitored the predominant bacterial species (*Escherichia coli, Enterococcus*, Bifidobacteria and *Bacteroides fragilis*) by culturing on selective agars according to the protocol given in microbiology laboratory guidebooks published by Ministry of Health in China (2003). Eosin methylene blue agar medium (EMB), bile esculin azide agar (BEA), BBL Medium and bacteroides bile esculin agar (BBE) were used for selective culture of *E. coli*, *Enterococcus* spp., *Bifidobacerium* spp. and *B. fragilis*, respectively. Triplicates were used to obtain the mean value of the bacteria number. Species identification was confirmed by classic biochemical tests and ABI 3130 system (Applied Biosystems, USA).

2.5. Monitoring the resistance development in E. coli and Enterococci

Resistant *E. coli* and *Enterococci* spp. were enumerated using EMB and BEA agar plates containing 4 × MIC of tilmicosin. The resistance rate was calculated using the mean number of resistant colonies divided by the total number of each strain identified selective plates lacking the drug.

Representative *E. coli* and *Enterococci* strains isolated from fresh fecal samples were subjected to minimum inhibitory concentration (MIC) determination under anaerobic condition (85% N_2, 10% H_2 and 5% CO_2) by the microbroth dilution test according to the methods defined in CLSI document M100-S19E (CLSI, 2010).

2.6. Assessment of colonization barrier of the chemostats

Following the method provide in previous paper, each chemostat was inoculated with 1 mL of 10^8 CFU/mL *S. typhimurium* (SI3) on the morning of days 20–22 and then allowed to run for another 7 days without further challenge (Carman et al., 2004). The chemostats were sampled starting on day 20 before the first inoculation and continued until day 29. Samples were collected in triplicate from each chemostat and the *S. typhimurium* population was quantified by plating on Bismuth Sulfite (BS) agar supplemented with 4 μg/mL ciprofloxacin. The dynamic change in the colonized *S. typhimurium* was used as a direct indicator for assessing the potential disruption of the colonization barrier.

2.7. Determination of virulence genes in E. faecalis

E. faecalis was isolated using BEA plates and further identified by Gram staining, the Manero method of Biochemical identification

Download English Version:

https://daneshyari.com/en/article/5856315

Download Persian Version:

https://daneshyari.com/article/5856315

Daneshyari.com