Aquatic Toxicities of Major Antimicrobial and Anthelmintic Veterinary Pharmaceuticals and their Potential Ecological Risks

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Abstract

The acute toxicities of two major anti-pathogenic veterinary medicines, i.e., ciprofloxacin and enrofloxacin, and six benzimidazole anthelmintics, i.e., albendazole, thiabendazole, flubendazole, febantel, fenbendazole, and oxfendazole, were evaluated with a marine bacterium, *Vibrio fischeri*, and invertebrate *Daphnia magna*. These veterinary medical products have been widely used for farm animals, but their impact on aquatic fauna has seldom been investigated.

In general, daphnids responded as much as 3 orders of magnitude more sensitively to the tested pharmaceuticals than the microbes. For *Daphnia*, the most toxic product among the tested anthelmintics was fenbendazole, followed by flubendazole > albendazole ≈ febantel > thiabendazole > oxfendazole. Daphnids' EC50 values obtained from 48 to 96 hrs of fenbendazole exposure ranged from 2.7 to 6.3 ug/L. The mixture toxicity of the test pharmaceuticals was generally additive in nature and was well predicted by a concentration addition model. Using the predicted no effect concentrations (PNECs) of the benzimidazole derivatives estimated from this study, and predicted environmental concentrations (PECs) of these pharmaceuticals, the risk quotients of each anthelmintics were calculated. Most of the test anthelmintic compounds resulted in risk quotients greater than 1. Especially, risk quotient for fenbendazole was 2,791, which strongly indicates this compound might cause severe ecological consequences, should no future action be taken. This study is the first report on the aquatic toxicities and potential ecological risk of major anthelmintic and antimicrobial veterinary products in Korea. The result of this study provides information necessary for conducting more detailed ecological risk assessment of pharmaceutical products in ambient water and guiding proper management decision.

Introduction

Veterinary pharmaceutical products are widely used worldwide to treat diseases and improve productivity in livestock farming. Veterinary products applied for medicinal purposes or in feed additives may find their way into the aquatic environment through various pathways (Figure 1). There are growing concerns on these pharmaceutical residues in ambient water for their potential impact on aquatic ecosystem. However, only limited information is available on the ecological risks associated with the use of these pharmaceutical products.

Veterinary pharmaceutical market has grown considerably: its market size is circa 412 billion Korean Won as of year 2003. Antibiotics, especially antimicrobials and anthelmintics, currently occupy the biggest market share. In the present study, we conducted a series of aquatic toxicity assays with major antimicrobial and anthelmintic veterinary medicines, and estimated potential ecological risks due to these pharmaceutical residues. The result of this

study will help better understand the ecological consequences of veterinary medicines in the environment, and establishing proper management decision.

Materials and Method

1. Veterinary pharmaceutical products
Antimicrobials such as enrofloxacin and ciprofloxacin, were obtained from Fluka (St. Louis, MO USA) and Baker Korea, respectively. Four benzimidazole anthelmintics including albendazole, thiabendazole, flubenazole, fenbendazole were purchased from Sigma-Aldrich (St. Louis, MO USA). In addition, two anthelmintics, oxfendazole and febantel, were obtained from Kanto (Tokyo, Japan) and Baker Korea, respectively.

2. Toxicity assays
Microtox Assay: For the bacterial assay, the Microtox Model 500 toxicity analyzer was used. The lyophilized *Vibrio fischeri* bacteria were obtained from SDI (Newark, DE, USA). "81.9% Basic test protocol" was utilized after a minor modification (Azur Environmental Corp, 1998). The bacterial luminescence, the endpoint of this assay, was measured for each pharmaceutical product and their binary mixtures after 5 and 15 min of exposure at 15 °C. Water quality parameters such as pH and dissolved oxygen of the test solutions were measured.
Daphnid toxicity test: Daphnids were cultured and maintained in-house. All aspects of culturing and testing were performed following the U.S. EPA guideline (2002) with minor modifications when necessary. Immobility of daphnids was measured everyday throughout the test period for individual pharmaceutical products and their binary mixtures. Test temperature was maintained at 21 +/- 1 °C throughout the test periods. Water quality parameters such as pH, temperature, dissolved oxygen, and specific conductivity of the test solutions and control were measured, and recorded daily during the exposure. The dissolved oxygen, and specific conductivity were determined following American Public Health Association, American Water Works Association, and Water Pollution Control Federation standard methods (1992). Reference toxicity tests were conducted on regular basis to assure comparable sensitivities of the daphnids over time.

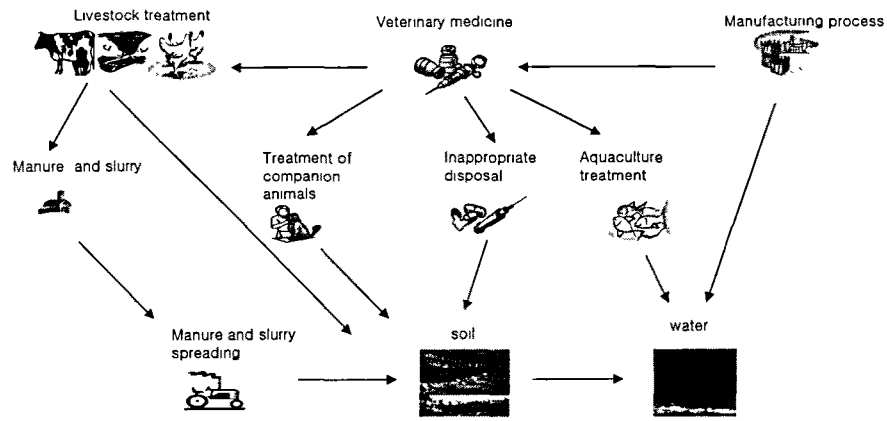

Fig. 1. Pathways into the environment for veterinary medicine.

Results and Discussion

Toxicity of individual veterinary antimicrobials and anthelmintics

Table 1 shows the summary of microbial toxicities of the test pharmaceuticals. The marine bacterium showed relatively low sensitivities to the two antimicrobials. The median effective concentration of ciprofloxacin and enrofloxacine obtained from the Microtox assay was 159 and 272 mg/L, respectively. The test bacterium, *V. fischeri*, does not seem to be as sensitive as some pathogens for which these antimicrobials are effective. With anthelmintics, *V. fischeri* exhibited greater sensitivities. The most toxic pharmaceutical to *V. fischeri* was albendazole that inhibited the bacterial metabolism by 50% at 770 ug/L.

Table 1. Microbial toxicity of major veterinary antimicrobials and anthelmintics

	EC_{50} (5 min exp)	EC_{50} (15 min exp)
Antimicrobials		
Ciprofloxacin (CF) (mg/L)	159.3 (130.75~194.1)	176.45 (144.7~215.15)
Enrofloxacin (EF) (mg/L)	272.25 (169.2~438.05)	306.35* -
Anthelmintics		
Albendazole (ABZ)	770.2 (714.4~830.2)	862.4 (763~974.6)
Thiabendazole (TBZ)	29640* -	194** -
Flubendazole (FLU)	400** -	852.8* -
Febantel (FBT)	2752* -	31.42** -
Fenbendazole (FBZ)	1570.6 (1351.2~1825.8)	798.4 (561.4~1134.8)
Oxfendazole (OFZ)	2210 (434.6~11250)	1985 (550~7166)

Values in parentheses are 95% confidence intervals.
* EC50 estimated from 2 points; ** highest effect; - Not available.

Table 2 summarizes acute toxicities of the pharmaceutical products to aquatic invertebrate *D magna*. Daphnids were in general more sensitive to anthelmintic chemicals, up to by the order of 4. The most toxic product among the test chemicals was anthelmintic fenbendazole, followed by flubendazole > albendazole ≈ febantel > thiabendazole > oxfendazole. Average EC50 values obtained from 48 to 96 hrs of fenbendazole exposure to *Daphnia* ranged from 2.7 to 6.3 ug/L.

Delayed toxicity of antimicrobials and anthelmintics

Organisms in affected area tend to escape to nearby un-impacted patch. In addition, most of the toxic discharges are not continuous, but intermittent. To simulate environmentally relevant exposure scenario, daphnids were removed to clean medium after exposure to each pharmaceuticals, and delayed expression of toxicity was observed for additional ten days. Significant mortalities were not observed in groups once exposed to lower concentrations up to ten days. (data not shown) However, it should be noted that sublethal endpoints, e.g., growth or reproduction, were not evaluated in this study.

Table 2. *Daphnia* toxicity of major veterinary antimicrobials and anthelmintics

	24 hr EC50	48 hr EC50	72 hr EC50	96 hr EC50
Antimicrobials				
Ciprofloxacin (mg/L)	223.9 (56.9~881.0)	146 (55.0~384.0)		
Enrofloxacin (mg/L)	26.75 (21.6~33.2)	15.7 (11.9~20.7)		
Anthelmintics				
Albendazole	72 (69.4~74.7)	63.7 (57.2~71.0)	57.4 (49.9~66.00	42 (36.0~49.0)
	>100	72 (69.4~74.5)	68.3 (63.9~72.9)	43.5 (37.9~50.0)
Thiabendazole	>2000	1002.6 (811.8~1238.2)	533.4 (407.0~697.9)	334.6 (267.0~419.3)
	2584.9 (1474.6~4531.3)	684.5 (570.7~820.9)	510.1 (397.1~655.2)	282.9 (222.1~360.2)
Flubendazole	>20	>20	20.1 (8.4~47.8)	5.2 (3.7~7.4)
	>20	33.3 (15.0~73.9)	10.1 (7.4~13.9)	2.8 (2.3~3.6)
Febantel	>500	>500	148.4 (60.5~363.9)	16.2 (11.1~23.8)
	>1000	216.5 (146.4~320.1)	63.2 (45.1~88.4)	24.3 (18.2~32.6)
Fenbendazole	30.4 (15.9~57.8)	5.6 (4.7~6.6)	3.3 (2.5~4.3)	2.7 (2.0~3.6)
	24.3 (19.9~29.7)	6.9 (5.9~8.0)	2.3 (1.9~2.9)	2.6 (2.1~3.4)
Oxfendazole	1309.1 (1112.0~1540.9)	1086.7 (931.0~1267.6)	883.9 (721.9~1082.2)	540.5 (431.0~677.7)
	>5000	1250 (1073.0~1455.0)	718.4 (610.0~845.7)	540 (428.0~680.5)

Units in ug/L unless otherwise noted; Values in parentheses are 95% confidence intervals.

Toxicity interactions of binary mixture

Microbial and invertebrate toxicity tests were carried out for a binary mixture of antimicrobials, and six anthelmintic binary mixtures to understand the mode of toxic interaction. Sum of toxic units for all the binary mixtures, calculated based on concentration addition model, generally indicates additive mode of interaction.

Potential ecological risk of benzimidazole anthelmintics

In order to predict potential risks of anthelmintics, one needs to have an exposure estimate and toxicity information. Since enough information to predict environmental concentration levels of each anthelmintics is not available, we used a predicted environmental concentration for benzimidazole derivatives in general and annual production data for each anthelmintics, to estimate predicted environmental concentrations (PECs) in surface water for each anthelmintic compound. Predicted no effect concentrations (PNECs) of each anthelmintic were estimated by dividing daphnid EC50 values with an assessment factor of 1,000 according to European Commission guideline (2003). Risk quotients, which are calculated by dividing PEC with PNEC, greater than unity would indicate potential environmental problem warranting further investigation and potentially management decision. Table 3 shows that most of the tested anthelmintic compounds have risk quotients greater than 1. Especially, risk quotient for fenbendazole was 2,791, which strongly indicates this compound might result in severe ecological consequences should no future action be taken.

Table 3. Potential Ecological Risks associated with Anthelmintics

Chemical	Annual production (Kg)	$PEC_{surfacewater}$ (㎍/L)	PNEC (㎍/L)	Risk Quotient
Albendazole	8,306	0.66	0.07	9.60
Thiabendazole	NA	NA	0.84	NA
Flubendazole	1,305	0.10	0.03	3.10
Febantel	3,475	0.27	0.22	1.27
Fenbendazole	220,821	17.44	0.01	2790.85
Oxfendazole	1,564	0.12	1.17	0.11

NA: Not available

Conclusion

Based on the acute microbial and *Daphnia* toxicities of two veterinary antimicrobials and six benzimidazole anthelmintics, some of the widely used anthelmintics have high potential of ecological risk. Especially risk quotient estimated for fenbendazole was 2,791, which strongly indicates this compound might cause severe ecological consequences in Korea, should no future action be taken. The result of this study provides information necessary for more detailed ecological risk assessment of pharmaceutical products in ambient water and guiding management decision.

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