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Influence of water and food consumption on inadvertent antibiotics intake among general population

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ABSTRACT

Antibiotic entry into the water environment has been of growing concern. However, few investigations have been performed to examine the potential for indirect human exposure to environmental antibiotic residues. We evaluated the contribution of drinking water and major food consumption to inadvertent intake of antibiotic residues among general human population in Korea. We estimated daily human intake of six antibiotics, i.e., sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfathiazole (STZ), trimethoprim (TMP), enrofloxacin (EFX), and roxithromycin (RTM), by measuring the concentrations of the antibiotics and their major metabolites in urine from general population in Korea ($n=541$). In addition, we measured antibiotics from source water of drinking water as well as in tap water samples, and surveyed water consumption rates among the study population. To assess the contribution of dietary factor, we also surveyed consumption pattern for several major foods which are suspected of antibiotics residue. SMZ, Sulfamethazine-N4-acetyl (SMZ-N4), TMP, EFX, ciprofloxacin (CFX), and RTM were detected up to 448, 6210, 11,900, 6970, 32,400, and 151 pg/ml in the urine samples, respectively. Estimates of daily intake of major antibiotics did not appear to be related with consumption of drinking water although antibiotics were frequently detected in source waters (10–67 ng/l). Consumption of several foods correlated significantly with urinary excretion of several antibiotics. Daily intake estimates of EFX and CFX were associated with consumption of beef, pork, and dairy products; those of SMZ and TMP associated with pork and dairy products; and that of TMP related with raw fish. Daily antibiotics intake estimates however did not exceed the acceptable daily intake levels.

1. Introduction

There are growing concerns regarding the entry of antibiotics into the environment (Witte, 1998). Continuous release of antibiotics into the environment poses unique environmental challenges. Reports have indicated that roughly 80–100 active pharmaceutical ingredients and their metabolites, many of which are antibiotics, have been detected in sewage, surface water, groundwater, and even in drinking water worldwide (Brun et al., 2006; Fent et al., 2006; Heberer, 2002; Hernando et al., 2006; Jones et al., 2002; Lishman et al., 2006; Metcalfe et al., 2003a, 2003b; Roberts and Thomas, 2006; Sarmah et al., 2006). These findings raised public health concerns regarding potential inadvertent human exposure to pharmaceuticals via contaminated water.

To date, few studies have evaluated the potential for indirect antibiotic exposure via contaminated drinking water or resident fish consumption (Christensen, 1998; Cunningham et al., 2009; Webb et al., 2003). Webb et al. (2003) reported that conservative estimates of exposure to pharmaceuticals through drinking water consumption in the United Kingdom were negligible as even the lifetime consumption amount of pharmaceuticals through contaminated drinking water would be less than the daily recommended therapeutic dose in most cases. However, no studies based on actual measurement of pharmaceuticals in drinking water or in human biological samples have been reported.

In the present study we estimated daily inadvertent antibiotic intake in the general population and aimed to understand the contribution of drinking water to such exposure. As certain meat and dairy products may contain antibiotic residues, we also surveyed the population for consumption patterns of major food items and compared them to the inadvertent antibiotic intake

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levels in the general human population. The results of this study may be useful in establishing appropriate management of antibiotics in the environment.

2. Materials and methods

2.1. Selection of target antibiotics

Six antibiotics, i.e., sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfathiazole (STZ), trimethoprim (TMP), enrofloxacin (EFX), and roxithromycin (RTM), were chosen as target compounds based on their production volume, levels of detection in the Korean environment, or status of being regulated as food residue (e.g., maximum residue limit (MRL); Table 1). The sulfonamide-based antibiotics (SMZ, SMX, and STZ) and TMP are widely used for therapeutic and prophylactic purposes in both humans and animals (Wang et al., 2006) and can be excreted as *N*4-acetylated metabolites in urine. EFX is one of few fluoroquinolone-based antibiotics that are used in veterinary medicine. RTM is a semi-synthetic macrolide antibiotic used in humans and has been identified as an antibiotic of potential ecological concern in Korea (Kim et al., 2006).

2.2. Urine sample collection and questionnaire

The study group consisted of age- and sex-stratified individuals ($n=541$, with 265 males and 276 females) living in the Han and Nakdong river basins in Korea (Fig. 1 and Table 2). The present study was approved by the Institutional Review Board of School of Public Health, Seoul National University, and written statements of informed consent were obtained from all participants. Urine samples were collected in 50-ml polypropylene tubes and stored at $-20\,^{\circ}C$ prior to analyses.

We designed an extensive questionnaire to gather information on the consumption patterns of water and several foods that might contain antibiotic residues. We also surveyed the details of the participants' medical histories and recent medications with special attention to therapeutic antibiotic treatments. General demographic information including sex, age, body weight, and height were also collected. People on antibiotic medication within 30 days of the survey were excluded from this study. To assess the contribution of water consumption, we investigated tap water consumption rates using 24-h recall questionnaire with a pictorial guide. Tap water was defined as local water either as it is or as boiled for beverages (United States Environmental Protection Agency, 2000). To assess the influence of dietary factors, a 48-h recall questionnaire was designed, and the consumption frequencies of beef, pork, poultry, sheep, goat, duck, turkey, seafood, and dairy products were asked. We provided participating subjects with a sample questionnaire 3 days before the survey to help them better prepare the actual questionnaire.

2.3. Study area and water sample collection

The Han and Nakdong river systems were selected as the study area primarily because of their importance as sources of drinking water for about 13 million people living in the Seoul Metropolitan and Gyeongsangdo areas. We sampled sources of drinking water including mainstreams and upstreams of both rivers (Fig. 1). Sampling was performed twice, i.e., in low and high water flow conditions because the flow conditions could affect antibiotic occurrence levels (Choi et al., 2008; Kolpin et al., 2004). We collected the first set of water samples ($n=38$) between 18 and 21 June 2007 (representing low water flow) and the second sampling ($n=39$) between 16 and 18 August 2007, shortly after a rainy period. We also collected a total of 29 tap water samples from the homes of study participants (15 from Han and 14 from Nakdong River basin). Sampling homes were selected based on the location of the home to maximize geographical diversity. Water was grab-sampled in amber glass bottles (3 l) that were pre-rinsed several times with deionized water. The glass bottles were rinsed again with sample water on site. Samples were shipped on ice and delivered to the laboratory within 8 h.

2.4. Preparation and analysis of urine samples

Urine samples were analyzed using liquid chromatography tandem mass spectrometry with column-switching techniques (LC/MS/MS; API 4000, Applied Biosystems, Framingham, MA, USA). We analyzed the major metabolites of some antibiotics (SMZ, SMX, STZ, and EFX) as well. The isotope-labeled internal standards (SMZ-phenyl-$^{13}C_6$, SMX-ring-$^{13}C_6$, TMP-ring-$^{13}C_3$, ciprofloxacin (CFX)-2, 3,-carboxyl-$^{13}C_3$, CFX-quinoline-^{15}N, and simeton) were obtained from Cambridge Isotope Laboratory (Andover, MA, USA). Sulfamethoxazole-N4-acetyl (SMX-N4) and sulfathiazole-N4-acetyl (STZ-N4) were kindly provided by Proviond Corporation (Seoul, Korea).

The frozen urine samples were thawed, vortexed, and centrifuged for 10 min at 6750 rpm at room temperature. The supernatant (10 ml) was adjusted to pH=3.0 with 0.5 N HCl (~100 μl). Solid-phase extraction was performed on a 6-ml Oasis hydrophilic–lipophilic balance (HLB) sorbent cartridge (200 mg; Waters, Milford, MA, USA) at a flow rate of 2 ml/min. The cartridges were preconditioned with 6 ml of MeOH, 0.5 N HCl and water; after the sample elution, each cartridge was washed with 6 ml of water and a water:MeOH solution (80:20, v/v). After removing the water via vacuum suction, the analytes were eluted with 5 ml MeOH, dried to 300 μl using a Speed-Vac (CVE-3100, Eyera, Japan), and diluted with 0.1% (v/v) formic acid (eluent A; 1:1). Prior to injection, the extracts were filtered through a nylon syringe filter (0.2 μm, Whatman, Kent, UK), and 10 μl of the filtrate was injected into the LC/MS/MS system.

The semi-micro HPLC system (Nanospace SI-2, Shiseido, Tokyo, Japan) consisted of two binary pumps, an autosampler, a column heater, a vacuum degasser, and a six-port switching valve. The on-line cleanup was performed with a pre-column ($2.0\times150\ mm^2$ inner diameter Cadenza HS-C_{18}, 5 μm particle size, Imtakt, Japan), and the separation of the antibiotics was performed on a reverse phase C_{18} column ($2.0\times75\ mm^2$ inner diameter Cadenza HS-C_{18}, 3 μm particle size, Imtakt). The column temperature was maintained at 40 °C for the pre-column and 25 °C for the analytical column. The flow rate was set to 200 μl/min. The mobile phase was 0.1% formic acid in water (v/v, eluent A) and acetonitrile with 0.1% formic acid (v/v, eluent B).

The system was operated according to the following procedure: In Step 1 (valve A; switchover 0–3.1 min) a urine sample was injected onto the pre-column, which was washed with the mobile phase at a flow rate of 200 μl/min to remove proteins and endogenous interfering compounds. In Step 2 (switchover 3.1–8.0 min) the valve was switched to position B and the enriched compounds were eluted from the pre-column to the analytical column to separate the analytes from the co-eluted endogenous compounds. In Step 3 (valve A; switchover 8.0–17.0 min) the mobile phase was returned to its initial condition.

The spectra of each antibiotic were detected by mass spectrometry (API 4000) with a mass spectrometer equipped with an electrospray ionization (ESI) mode. The ESI conditions for the analysis of the targeted antibiotics were optimized to

Table 1
Environmental load and occurrence level in Korea and fate of target antibiotics.

Antibiotics	Sulfonamides				Quinolone	Macrolide
	SMZ	SMX	STZ	TMP	EFX	RTM
Usage	V	H	H	H,V	V	H
Amount of production (kg/year)*	26,864	14,791	96,403	7572	24,971	44,778
Load of environment (kg)[a]	4836	4437	64,590	765	4245	27,992
Maximum concentration (ng/l)						
WWTP influent	6021	1029	577	246	201	241
WWTP effluent	1558	922	357	150	133	195
Surface water	123	270	123	587	333	36
Half-life in body (h)	4.75	12.5	1.3	4.6	5.9	12
Half-life in water (days)	38	38	38	60	180	180
Maximum residue level (mg/kg)	0.1 Muscle, fat				0.3	(Cow) Liver
	0.025 Milk				0.2	(Cow) Kidneys
					0.1	Muscle, fat
					0.0	Fish egg

Abbreviation: H—human, V—veterinary, WWTP—wastewater treatment plant.

[a] Based only on human use (*Source*: Park, 2006).