Hazard of Sulfonamides and Detection Technology Research Progress

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Abstract. As a kind of widely used antibiotic with typical characteristics, sulfonamides have been greatly applied in clinical medicine for long time. It can’t be effectively treated by pollutant disposal system during pharmaceutical process and utilization and will be discharged into natural environment to be one of the antibiotics with great effect. This kind of substance is difficult to be biodegraded and will be easy to accumulate in the environment, generating huge eco-toxicological effect with significant mutagenicity and teratogenic effect. It is the severe threat for ecological balance, human health and drinking water safety. Its environmental behavior and detection technology attract extensive attention home and abroad.

1. Introduction

Sulfonamides (SAs) are a kind of chemically synthesized broad-spectrum antibiotic with basic structure of sulfanilamide. The sulfonamides usually used on the market are basically the compounds further derived from the basic parent nucleus of para-position aminobenzenesulfonamide. Usually, the hydrogen on sulfamine can be replaced by different heterocyclic rings (R1 group) to form different types of sulfonamides. Compared with sulfanilamide, its titer is significantly increased, antibacterial spectrum is widened and toxicity is reduced. Para-position free amino groups are the bacteriostatic active groups. If they are replaced, antibacterial effect will disappear; however, in order to increase assimilation, some modifications (R2 group) will be made on the amino groups to form prodrugs. After being assimilated by human body or animals, it will release free amino groups through zymolysis in the body to recover bacteriostatic activity. The parent nucleus of sulfonamides is more close to para aminobenzoic acid in structure and functions; however, it will compete with para aminobenzoic acid (PABA) in sensitive bacteria to synthesize enzyme with dihydrofolic acid to constrain synthesis of folic acid, and growth and proliferation of bacteria can be effectively restrained since folic acid is the necessary nutritional ingredient for growth and proliferation of bacteria\(^{[1]}\). In addition, dihydrofolic acid in food can be directly taken in by human and animals to form tetrahydrofolic acid. Therefore, sulfonamides will not affect synthesis of tetrahydrofolic acid in human body and animals.

Sulfonamides gradually attract attention due to its wide application, easy accumulation and difficult degradation in natural environment. Despite that researches on control technology of sulfonamides are increasing every year; the range and depth of the researches are far from enough. Therefore, this paper analyses environmental behavior of sulfonamides and proposes corresponding control measures according to research results of recent years through combination with current situation of sulfonamides residue in the environment.
2. Hazard of sulfonamides in biological body and environment

2.1. Hazard of sulfonamides in biological body

2.1.1 Hazard for urinary system. Sulfonamides are mainly metabolized through kidney in human body in prototype or in acetylated form; however, acetylated sulfonamide metabolin is not active but retaining toxicity of sulfonamides. In addition, the acetylated metabolin is difficult to be dissolved and will precipitate in urine, especially in aciduria, which will cause damage to kidney. The precipitated sediment can cause hematuresis, backache and even anuria etc [2-3].

2.1.2 Allergic reaction. After entering human body, sulfonamides may bring antibody to human body and cause allergic reaction after binding with protein in human body. The less serious case may be drug fever, arthropathy and various types of drug eruptions (such as urticaria and erythema etc); the serious case may be exfoliative dermatitis, even shock that is life threatening [3].

2.1.3 “Three pathogenicity” effect (teratogenetic, carcinogenic, mutagenic). It is discovered in tests that some sulfonamides may cause cancers, fetal anomaly and genovariation etc. For example, continuous administration of sulfonamides such as sulfadimidine etc can induce thyroid hyperplasia to rodents with tendency of oncogenicity [4].

2.1.4 Drug resistance. Drug resistance means that gene mutation occurs after long-term and repeated contact between bacteria and drugs; sensitivity of bacteria for drugs is reduced and even disappears, and therapeutic effect of the drugs is lost. Bacteria will generate drug resistance for all kinds of antibiotics and sulfonamides are no exception. The bacterial sensitive to sulfonamides will generate drug resistance after long-term contact with insufficient sulfonamides no matter in vivo or in vitro; in addition, the speed and strength of drug resistance are closely related to variety of bacteria, administration cycle, concentration of drugs and contact time etc. Once bacteria generate drug resistance, cross resistance will be generated, which means that once one type of sulfonamides loses its effect, the other sulfonamides will lose effect as well. Gene mutation is likely to happen to the bacteria restrained for long time in body of livestock accepting multiple administrations of sulfonamides. Therefore, drug resistance is generated and drug resistant strains will bloom in animals’ bodies. If human eats meat products with drug resistant strains for long time, the drug resistant bacteria will affect human. It will be difficult to carry out treatment then.

2.1.5 Alteration of intestinal flora. Many kinds of microorganisms live in intestinal tracts of healthy persons in approximately fixed proportion. They depend on each other and restrict each other. These microorganisms are called intestinal flora. If meat or egg food containing sulfonamides is taken in, it may exert undesirable effect on normal flora in intestinal tract can cause drug sensitivity to sulfonamides; it will restrain the bacteria beneficial for human body and cause disequilibrium of flora in intestinal tract; in addition, it will cause prolonged diarrhea or make human body be short of certain vitamins; what’s severer, it may lead to dehydration, electrolyte disturbance and even shock.

2.1.6 Reaction of hemopoietic system. Sulfonamides can destruct human hemopoietic system, turn hemachrome into metahemoglobin, cause hemolytic anemia, intravascular hemolysis and hemoglobinuria. Meanwhile, sulfonamides will inhibit generation of myeloplast and cause agranulocytosis, thrombocytopenia and even aplastic anemia. These symptoms are rare but they can be irretrievable once they occur.

2.2. Effect on ecological environment
Sulfonamides are mainly metabolized in animals’ bodies in prototype or in acetylated form and the metabolite will be discharged through excrement such as feces and urine etc. The metabolite is still
active in the environment and will cause pollution to water and soil etc, and exert harmful effect on
microorganisms and insects etc in the environment. Meanwhile, the sulfonamides existing in the
environment can induce gene mutation of bacteria to form drug resistance. Once human is affected, it
will be incurable. Although it is shown in detection result that except the rabbitfishes in Daya Bay
Aquiculture Area, the sulfonamides residue in fish muscle of other aquiculture areas conforms to the
limit standard of national pollution-free aquatic products, if the pollution of sulfonamides residue to
water etc is not effectively controlled, its future hazard for food safety and human health is obvious.

3. Sulfonamides Residue and Detection Method

3.1. Sulfonamides residue
It is shown in related research data home and abroad that the large dose of veterinary drugs has caused
annually increased varieties and concentrations of veterinary drugs in the environment, among which
sulfonamides rank first. Some researches show that 25%-75% veterinary drugs for animals are
eliminated from the body through feces in form of parent drugs, and are retained for long time in soil.
Some researchers even believed that the veterinary drugs discharged with animals’ feces and urine
reaches 90% of dosage. It is notable that large proportion of veterinary drugs enters the environment
through animal feces in form of parent drugs or metabolite.

3.1.1 Sulfonamides residue in animal manure. Sulfonamide veterinary drug is a kind of typical
veterinary drug as feed additives for disease prevention and cure. It is widely used in breeding industry
home and abroad. Sulfonamides will be gradually decreased along animals’ growth and metabolism;
however, a large proportion of sulfonamides will be eliminated from body into natural environment
with feces. He et al detected sulfadiazine and sulfamethazine in sediments of a marine fish
aquiculture area and oyster aquiculture area in Daya Bay of Guangdong Province. Chen et al indicated in their research result that the detection rate of sulfonamides residue was generally higher,
among which the detection rates of sulfadimidine, prizzone and sulfaniamid were over 50%. Hamscher et al discovered that content of sulfamerazine in liquid organic fertilizer was up to 4.0
mg/kg through detection of concentrations of multiple kinds of antibiotics in feces of pig farm.
Martinez et al detected large dose of sulfadiazine drugs in chicken manure. Utilization of livestock
manure in land is the currently main disposal method; however, the high residue of sulfonamide
antibiotics brings great ecological risk to soil and water etc.

3.1.2 Sulfonamides residue in soil. The agricultural application of livestock manure is the main way
that veterinary drug antibiotic enters soil. The sulfonamides residue in feces and urines of livestock
and poultry industry enters the soil through organic fertilizer or direct agricultural application; during
aquiculture, sulfonamides are directly used in fishponds; the excessive drugs will enter the soil along
the sludge in fishponds and cause sulfonamides residue. Some researches show that the sulfonamides
entering farmland soil every year are massive and difficult to be biodegraded by microorganisms in
the soil. Li et al carried out study on pollution characteristics of 6 types of sulfonamide antibiotics in vegetable soil in Guangzhou and Shenzhen etc and the result showed that the main
detected substances were sulfamethoxazole, sulfamethoxydiazine and sulfadimidine. Paul et al carried out detection study on sulfonamides in 4 different kinds of soil, including sandy clay loam,
loam, sandy loam soil and clay loam, and 3 drugs including prizzone, oxytetracycline and tylosin were
detected in these soils.

3.1.3 Sulfonamides veterinary drug residue in water. Currently, the phenomenon of antibiotic residue
in surface water and drinking water is universal in the countries all over the world. Wattses et al detected sulfonamides in the rivers in UK and the content was 1μg/L. Kim et al indicated in their research that antibiotic drugs such as sulfonamides etc were detected in surface water, drinking water
and sewage in Korea. Xu et al detected sulfamethoxazole in rivers in Shenzhen and the content was
880 ng/L. In addition, sulfonamides were detected in underground water. Two types of antibiotics of erythromycin and sulfamethoxazole were detected in sample of underground well water in Badenia-Wirtembergia of Germany. Sarmach et al. detected tetracycline, oxytetracycline, lincomycin, sulfamerazine and antibiotic metabolite in underground water.

3.2. Detection method for sulfonamides

3.2.1 Detection method for sulfonamides residue in animal food
(1) Spectrophotometry
The advantages of spectrophotometry is no need of expensive instrument and reagent, low requirement for sample pretreatment and easy operation etc. It can be popularized in quality assurance departments and manufacturing enterprises without sophisticated hardware equipment. However, accuracy of result of this method is not very high; in addition, its lowest detectable limit is high with low sensitivity. Therefore, it can't be used as affirmative method.

(2) Immunoassay
Immunoassay includes radio immunoassay, enzyme-linked immunospot assay and biosensing technique, among which radio immunoassay is the earliest application with advantages of stable detection result, fast detection, high specificity and easy operation etc; enzyme-linked immunospot assay also plays an important role due to its advantage in qualitative and quantitative analysis; another drug residue detection technology is biosensing technique. It is gradually becoming the mainstream of drug residue detection technology due to its speediness, accuracy, high sensitivity and real-time detection.

(3) Chromatographic technique
Chromatographic technique is widely used in separation and analysis of multi-component mixture, especially suitable for quantitative analysis of organic compounds. Therefore, this method is frequently used for detection of sulfonamides residue in food.

(4) Capillary electrophoresis (CE) method
Capillary electrophoresis (CE) is also called high performance capillary electrophoresis (HPCE). It is a kind of electrophoretic separation taking capillaries as separation channel and high electrostatic voltage field as driving force. Compared with a series of ordinary electrophoresis method, its advantages are high sensitivity, high resolution, speediness, small sample amount and low cost etc. However, its shortcomings are small sample size and no detection of residue with low concentration.

(5) Coupling technique
Coupling technique is the analytical method integrating two or more than two kinds of methods. It is widely used in drug residue detection systems due to its high sensitivity, high accuracy, convenient operation and economy etc. The coupling method of gas chromatography – tandem mass spectrum (GC-MS) and high performance liquid chromatography – tandem mass spectrum (HPLC-MS) is frequently used for detection of sulfonamides residue in animal food. It is used by authority laboratories in USA and UK for detection of residue of 7 types of sulfonamides including sulfamethazine etc in tissues of cow, pig and chicken. HPLC/MS method does not require derivation for samples. Therefore, it is more frequently used.

3.2.2 Detection method for sulfonamides residue in water environment. Sulfonamide antibiotic is a kind of compound with strong water solubility and poor volatilility. Therefore, complicated derivative reaction is required during test and analysis by gas chromatography - mass spectrometer. The reproducibility of analytical result is greatly influenced by the uncontrollability during reaction so it is not used frequently now. Ma et al. detected three types of antibiotics including sulfonamides, tetracyclines and quinolones in soil by solid phase extraction – high performance liquid chromatography – tandem mass spectrometry. Tang et al. detected microscale sulfonamide antibiotics in municipal sewage by high performance liquid chromatography – tandem mass
spectrometry. Liquid chromatography–UV (LC-UV) and liquid chromatography–fluorescence detection (LC-FLD) are also frequently used in analysis of antibiotics. Wu et al. [23] detected 7 types of sulfonamide antibiotics in water environment by ionic liquid single drop microextraction–high performance liquid chromatography. Nancy et al. [25] carried out extraction and concentration for sulfonamide antibiotics in sewage of pig farm through 3mg HLB solid phase microextraction, and carried out detection by HPLC-UV; however, the recovery rate was not high while detection limit was high. The solid phase microextraction is gradually applied in test for veterinary drug antibiotics in environmental samples due to its speediness, large volume and easy operation etc.

4. Prospect

This paper researches environmental behavior and its process and mechanism of sulfonamides, including residue amount, form and transformation of sulfonamides in the research environment, and ecotoxicology of animals, plants and microorganisms based on formation and release mechanism of combined drugs in the environment, and micro-biological degradation and purification system of drugs in the environment.

With people’s increasing attention to food safety problems, the requirements for precision and accuracy of detection method for sulfonamides residue, especially veterinary drugs are gradually increased. People start to continuously explore and research the new detection technology applied for sulfonamides residue, and apply it into practical production and living after rational design. Instrument method is gaining attention during measuring of sulfonamides residual quantity. From spectrophotometry and fluorescence method in early stage to the recent chromatographic analysis, mass spectrum analysis method, capillary electrophoresis and supercritical fluid chromatography, nearly all the analytical theories and techniques for detection of sulfonamides residue are studied and applied in SAS residue analysis. The most frequently used screening method is reversed-phase high performance liquid chromatography (HPLC). The latterly developed enzyme linked immunosorbent assay (ELISA) is also widely used as a screening method. Liquid chromatography is still regarded as the common method for sulfonamides detection due to its advantages of high sensitivity, low detection limit and accurate repeatability. Pretreatment of LC/MS is easy and time consumption for detection is less with high sensitivity and low detection limit, which meets detection requirements; however, it is not suitable for massive application due to high cost and expensive instrument, but it can be the future development tendency. With rapid development of modern science and technology, ELISA kit can be rapidly used in detection of drug residue etc and will greatly promote detection efficiency; in addition, it can carry out simultaneous detections with multiple samples.

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