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The mixture toxicity of environmental contaminants containing sulfonamides and other antibiotics in Escherichia coli: Differences in both the special target proteins of individual chemicals and their effective combined concentration

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HIGHLIGHTS

- The joint effects between sulfonamides and other antibiotics on E. coli were determined.
- The difference of the joint effects between sulfonamides and different antibiotics were discussed.
- The reasons why different joint effects occurred between sulfonamides and different antibiotics were explored.

ARTICLE INFO

ABSTRACT

Organisms in the environment are exposed to mixtures of multiple contaminants, leading to serious environmental harm. These mixtures pose an ecological risk and have attracted an increasing amount of attention; however there has been little in-depth research the toxicity of mixtures, such as antibiotics. To determine how different mixtures of antibiotics affect organisms, the individual and mixture toxicity of sulfonamides and several antibiotics were determined using Escherichia coli as a target organism in our study. The results show that additive effects occur between sulfonamides and quinolones or with a portion of β-lactams, synergistic effects appear between sulfonamides and their potentiators or cefotaxime sodium, and antagonistic effects arise between sulfonamides and tetracyclines or penicillin V potassium salt. In addition, the toxicity mechanism of binary mixtures is further discussed and the results reveal that the joint effect differences depend not only the target proteins of individual chemicals but also on their effective combined concentration based on the approach of Quantitative Structure Activity Relationships (QSARs) and molecular docking. This study introduces the concept of the "effective concentration" to provide insight into understanding the mechanism of binary mixtures, which will be beneficial for evaluating the ecological risk of antibiotics.

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1. Introduction

Antibiotics, which have been widely produced in very large volumes, have been used in the medical industry and animal husbandry in the past few decades because of their extraordinary antibacterial ability, are they drawing of increasing concern at home and abroad. It is estimated that each year approximately 210,000 tons of antibiotics are produced and consumed in China (Li et al., 2015). Because antibiotics are hard to metabolize and absorb by humans and animals, a considerable inestimable portion of antibiotics is released into the environment via urine or feces every year, leading to large quantities of antibiotic residue in water and soil sediments, which cause serious harm to microorganisms or other non-target creatures (Christensen et al., 2006; Kümmerer,

2009; Baran et al., 2011). Antibiotic residues also cause great ecological damage, such that antibiotics recognized as a class of potentially environment-threatening pollutants (Daughton and Ternes, 1999; Białk-Bielińska et al., 2013). Therefore, a toxicity study of antibiotics, especially of mixture toxicity, as environmental contaminants is urgently needed to evaluate their ecological risk.

In the field of environmental toxicology, there has been a great deal of research regarding the single toxicity of antibiotics on organisms over the last few decades, and most of the mechanisms of toxicity mostly have been determined. For example, Brown (Brown, 1962) identified that sulfonamides are effective inhibitors of the biosynthesis of folic acid compounds by studying cell-free extracts of Escherichia coli (E. coli), which functions by competing with p-aminobenzoic acid as a substrate in the enzyme system so that dihydropteroate synthase (DHPS) cannot be produced. Tetracyclines can prevent aminoacyl-tRNA (aa-tRNA) from binding to the A site of the 30s ribosome subunit by combining with itself, leading to protein inhibition (Day, 1966; Craven et al., 1969; Geigenmüller and Nierhaus, 1986). The toxicity mechanisms of individual chemicals, such as trimethoprim, quinolones and so on, have been studied and confirmed (Bushby and Hitchings, 1968; Backhaus et al., 2000; Oliphant and Green, 2002). Nevertheless, as is known, organisms in the environment are generally not exposed to single compounds, but rather to mixtures of a variety of chemicals; however, there is little literature related to mixture toxicity. M. González-Pleiter et al (González-Pleiter et al., 2013). examined the individual and combined toxicity of five antibiotics in two organisms representative of the aquatic environment, and the results showed that cyanobacterium, as a target organism, is more sensitive than the green alga, a non-target organism, to the toxic effect of the tested antibiotics. These results show that different tested organisms have very different mixture toxicity to the same mixtures. Therefore, what could happen if different antibiotic mixtures act on the same organism? Will these joint effects be very different? If so, why are they different? These questions cannot be answered based on the available data from previous research. This study addresses these problems.

QSAR models, namely, Quantitative Structure-Activity Relationship models, have been extensively employed to predict the toxicity of individual chemicals and mixtures (Lin et al., 2003; Castillo-Garit et al., 2008; Tian et al., 2013a). In a previous study, based on the approaches of QSARs and molecular docking, by which we can determine the value of $E_{binding}$ (a parameter representing the binding energy between a single chemical and its target protein), we revealed the differences between acute and chronic mechanisms containing sulfonamides and their potentiators in Photobacterium phosphoreum (Zou et al., 2012). In that article, the concept of the "actual concentration" was introduced to describe the concentration of individual chemical-binding-receptor proteins, and the results indicated that the dissimilarity of target proteins as well as the "actual concentration" led to the difference of joint effects in which acute toxicity is antagonistic but the chronic toxicity is synergistic. Additionally, a case study on the mixture toxicity of cyanogenic toxicants and aldehydes in Photobacterium phosphoreum employed similar methods. The results showed that for mixtures, their joint effects resulted from an intracellular chemical reaction and their underlying toxicological mechanism depended on not only their interaction with target proteins but also their transmembrane actions and their concentrations (Tian et al., 2013b). Therefore, these tools, including QSARs and molecular docking, can be used to investigate the toxicity mechanism of binary mixtures between sulfonamides and other antibiotics in E. coli.

In this paper, we investigate the half maximal effective concentration (EC_{50}) of single toxicants containing sulfonamides and several other antibiotics, as well as their mixtures, in a model organism, E. coli. These compounds are typical antibiotics that are widely used, and they are most frequently detected in the environment; therefore, our study has practical significance (Hu et al., 2010; Jiang et al., 2011; Li et al., 2015). As a consequence, the purposes of this study are as follows: (1) to evaluate the difference of joint effects between sulfonamides and other distinct antibiotics and (2) to analyze the possible reasons why different joint effects occur between sulfonamides and different antibiotics in the same organism by developing QSAR models.

2. Materials and methods

2.1. Chemicals and organism

The tested pharmaceuticals, including 12 sulfonamide antibiotics, 4 tetracyclines, 3 quinolones and 4 sulfonamide potentiators (Table 1), were all purchased from Sigma-Aldrich Company, except for 5 β-lactams, which were obtained from Aladdin Industrial Corporation. All of these chemicals were of analytical reagent grade or above (≥95%) and used as received without further purification. The tested bacterium, Escherichia coli K-12 MG1655, was purchased from Biovector Science Lab, Inc., and then revived and maintained on Luria-Bertani agar slants at 4 °C.

2.2. Toxicity experiment

The chemicals were appropriately dissolved with the help of DMSO at a concentration of no more than 0.1% and were then diluted with a 1% NaCl solution. A concentration-gradient of series antibiotic solutions and bacteria liquid were successively added into 96-well plates. Then, the mixture systems were cultured for 12 h at 37 °C. The initial and final values of the optical density (OD) were determined and based on the relationship between OD corresponding antibiotic concentrations decreases; EC_{50} was determined.

The EC_{50mix} and toxicity units (TU), which are parameters used to describe the mixture toxicity effect, were calculated by Eqs. (1) and (2) as follows:

$$EC_{50mix} = \frac{C_M}{\frac{C_A}{EC_{50A}} + \frac{C_B}{EC_{50B}}} \tag{1}$$

$$TU = \frac{C_A}{EC_{50A}} + \frac{C_B}{EC_{50B}} \tag{2}$$

where C_A and C_B were the concentrations of components A and B in the mixtures at median inhibition and were calculated according to the EC_{50mix}. EC_{50A} and EC_{50B} were the median effective concentrations of the single chemicals A and B, respectively. Simple addition was characterized by $0.8 < TU < 1.2$, while $TU > 1.2$ represented antagonism and $TU < 0.8$ indicated synergism (Broderius et al., 1995). More details regarding the difference between EC_{50mix} and TU can be found the research of Tian et al. (Tian et al., 2013a).

In addition, the toxic ratio for most of the binary mixtures was set to 1:1 based on the Climax Hypothesis that we proposed in our previous research (Lin et al., 2005), which indicated that the joint effect was closely associated with the toxic ratio of each component and that the most obvious effect would appear at the equitoxic ratio.

2.3. Molecular docking

The protein crystal structures of different antibiotics in E. coli