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Investigation of *in vivo* effect of florfenicol on metabolic-antioxidant enzymes' activities on Morkaraman normal and lactating sheep

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Abstract

Florfenicol is a broad-spectrum, primarily bacteriostatic, antibiotic with a range of activity including many gram-negative and gram-positive organisms. This study was carried out to determine the *in vivo* effect of florfenicol on the paraoxonase (PON), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities on Morkaraman normal and lactating sheep. For these studies, three normal and three lactating sheep groups (55–60 kg) were selected for each of intramuscular administration for 24 h of florfenicol (30 mg/kg). Three normal and three lactating sheep groups were included in the study for a control group, which were not subjected to drug administration. For florfenicol, the mean of the hemolysate paraoxonase, glutathione peroxidase, superoxide dismutase, catalase activities and milk paraoxonase, catalase, lactoperoxidase, superoxide dismutase activity was determined and compared to the control group. According to the research results, while PON1 and CAT enzymes were activated, SOD and GPX enzymes were inhibited by florfenicol in both normal and lactating Morkaraman sheep. While florfenicol did not change milk PON1 and SOD activities, it significantly inhibited milk CAT and LPO enzyme activities.

Keywords: Paraoxonase (PON); Catalase (CAT); Superoxide dismutase (SOD); Glutathione peroxidase (GPX); Lactoperoxidase (LPO); Florfenicol; Antibiotics

1. Introduction

Florfenicol (2,2-dichloro-*N*-((1R,2S)-3-fluoro-1-hydroxy-1-(4-(methylsulfonyl)phenyl) propan-2-yl)ethanamide) has been demonstrated to be active *in vitro* and *in vivo* against many gram-negative and gram-positive organisms [30]. In the treatment of bovine respiratory disease, florfenicol may be considered as bactericidal agent against some *Mannheimia* (*Pasteurella*) *hemolytica* and *Pasteurella multocida* when it is administered to achieve minimum inhibitory concentrations (MICs) [7]; the minimum bactericidal concentrations (MBCs) are very close to the MICs.

There are also oxygen and reactive nitrogen species as well as superoxide radical, hydrogen peroxide and hydroxyl radicals in the body. Radicalic and reactive

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intermediates are chemically very active and they can oxidize nucleic acids, proteins and lipids in the environment leading to the reduction and elimination of them in their biological functions can create negative consequences in the body [17]. Against free radicals produced by the organism itself and the toxic effects of normal oxygen metabolism endogenous antioxidant system consists of antioxidant enzymes, catalase (CAT), paraoxonase (PON), glutathione peroxidase (GPX) and superoxide dismutase (SOD). These antioxidative enzymes prevent resulting/possible oxidative damages by eliminating radicals and reactives. They also take task in detoxification of xenobiotics, some antineoplastic drugs and certain metabolic end-products and enzymatic defense systems [3,13,24,26,28,34].

The enzymatic antioxidant defenses include paraoxonase (PON), superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Subsequent studies have shown that PON enzyme (paraoxonase/arylesterase) (PON; aryldialkylphosphatase, EC 3.1.8.1) is an organophosphatase with broad substrate specificity, including aromatic carboxylic acid esters such as phenyl acetate [6,27]. The first functions of paraoxonases are hydrolysis of toxic organophosphates. PON can also be hydrolyzed to the carbonate esters, aromatic lactone, statin type drugs and pharmaceutical substances. In addition, PONs play a role in preventing the oxidation of LDL and the prevention of atherosclerosis, hypercholesterolemia, diabetes, and coronary vascular disease [14,19–21,23,29].

Catalase (CAT, H_2O_2: H_2O_2 oxidoreductase; EC 1.11.1.6) is one of the principle antioxidant enzymes. In the presence of molecular oxygen, the primary functions of the CAT are catalyzed of dismutation reaction of a peroxide such as hydrogen peroxide and ROOH is synthesized in some positions of metabolism. It also especially prevents from irreversible damage in membranes [11,22].

Superoxide dismutase (EC 1.15.1.1) is also an antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and H_2O_2 [5,17,32]. SOD families include cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD and extracellular Cu, Zn-SOD (ECSOD).

SOD plays a major role in the first line of antioxidant defense and high SOD activities are correlated with high immune competence [31].

GPX (EC 1.11.1.19) catalyses the reaction of hydroperoxides using GSH, protecting mammalian cells against oxidative damage [1].

Reactive oxygen species (ROS) such as the superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2), have been implicated in many of the events leading to the development of diseases such as cancer, allergy, atherosclerosis, and Alzheimer's disease [4,12,15]. Peroxidase acts as preventive antioxidants to detoxify damaging from blood and organic substrates [4].

As decreased activity of PON, GPX, LPO, SOD and CAT has been acknowledged as a risk factor for cancer, allergy, coronary vascular disease, organophosphate toxicity, damaging to the structure of the membrane and DNA the factors affecting antioxidative enzymes activities must be well addressed.

While sheep are producing milk, nutrient requirements of sheep are especially high and their blood flows increases during lactation. Although florfenicol is widely used in sheep, their effects on the antioxidant enzymes activities of lactating and non-lactating sheep are not known. It is hypothesized that sheep producing milk eliminates the effects of florfenicol with antioxidative enzymes more than sheep not producing milk during lactation. Although florfenicol are being used commonly in sheep, the exact effect of this drug on paraoxonase, glutathione peroxidase, superoxide dismutase, catalase has been unknown in therapies on lactating and non-lactating sheep. There is also a deep need of understanding of the impact of commonly used this drug on the activity of these antioxidative enzymes. Therefore, the aim of this study was to examine the effects of commonly used florfenicol on normal and lactating sheep PON, GPX, LPO, SOD and CAT activities *in vivo* on lactating and non-lactating sheep.

2. Experimental

2.1. Chemical and reagent

All chemicals used in this study were obtained from Sigma Chem. Co and Merck (Germany) and they were analytical grade.

2.2. Animals, experimental design and sample collection

In this study, 12 mature clinically healthy Morkaraman sheep ($n = 12$) were used. The sheep had an average weight of 55–60 kg. Sheep were housed during 15 days in stables of Ataturk University. This animal experiment was approved ethically with protocol in Ethical Committee of Ataturk University.

We allocated 12 sheep (36 weeks old) to four groups of 3 animals: (1) normal control sheep, (2) lactating control sheep, (3) intramuscular administration with florfenicol normal sheep, and (4) intramuscular administration with florfenicol lactating sheep.