

Oxidative damage of the anticoccidial drug toltrazuril in mice liver and intestine

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Abstract: Coccidiosis in poultry is caused by protozoan parasites of the genus *Eimeria*, which is responsible for worldwide economic losses. Animals were divided into two groups of 10 mice per group. The first group was considered as the control group which gaved distilled water twice a week. Toltrazuril 2.5% solution was given orally twice at a 1-week interval at a dose rate of 20 mg/kg body weight. Toltrazuril induced a significant increase in the level of intestinal and blood serum malondealdehyde. Also, toltrazuril induced a significant increase in the level of nitric oxide in the liver and blood of mice after one week post-treatment. Moreover, mice treated with toltrazuril for one week showed a significant increase in the blood level of glutathione. The current work investigated the antioxidant properties of toltrazuril in the mice liver, intestine and blood serum.

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1. Introduction

Toltrazuril is active against all intracellular stages of coccidia, including schizonts, micro and macrogamonts. It interferes with the division of the protozoal nucleus, the activity of the mitochondria and damages the wall forming bodies in the microgametes. Toltrazuril produces severe vacuolisation of the protozoal endoplasmic reticulum in all intracellular development stages (Mehlhorn 2008).

Toltrazuril chemically, 1-methyl-3-[3-methyl-4-[4(trifluoromethylthio)phenoxy]phenyl]-1,3,5-triazinane-(trifluoromethylthio)phenoxy]phenyl]-1,3,5-triazinane-spectrum anticoccidial and antiprotozoal agent (Figure 1).

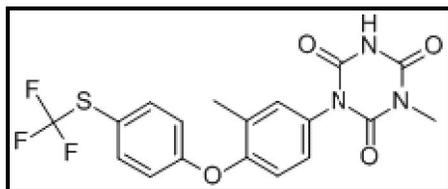


Figure 1. Chemical structure of Toltrazuril

Coccidiosis may cost the US chicken industry about \$127 million annually and likewise similar losses may occur worldwide (Chapman, 2009). Thus coccidiosis is probably the most expensive and wide spread infectious disease in commercial poultry systems.

Control of coccidiosis mostly depends upon the chemoprophylaxis by using anticoccidial drugs, however, managerial skills are also important to get maximum anticoccidial effect of these drugs (Tewari and Maharana, 2011). Chemical anticoccidial feed

additives has played a vital role in the growth of the poultry industry and has also facilitated better availability of affordable and good quality poultry meat and other products to the consumers during last 50 years. But due to frequent and irrational use of these anticoccidial drugs, varying degree of resistance has been developed against almost all the available anticoccidial drugs in various parts of the world (Abbas et al., 2011). Moreover, drug residues in poultry products may be potentially toxic to human beings.

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Valko et al., 2005). Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA (Evans and Cooke 2004). Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

Little information has been published regarding the antioxidant activity of toltrazuril. The current study aimed to investigate the antioxidant properties of toltrazuril in the mice liver, intestine and blood serum.

The protective role of glutathione, as an antioxidant and detoxifying agent, has been demonstrated in various clinical studies (Simopoulos, 2004). It is a ubiquitous

compound that is synthesized rapidly in the liver, kidney and other tissues, including the gastrointestinal tract. In animal cells, glutathione acts

as a substrate for glutathione peroxidase, which reduces lipid peroxides that are formed from polyunsaturated fatty acids in the diet and as a substrate for glutathione-S-transferase, which conjugates electrophilic compounds. Many evidences showed that glutathione obtained from the diet is directly absorbed by the gastrointestinal tract and thus dietary glutathione can readily increase the antioxidant status in humans (Jones et al., 1989).

2. Material and Methods

Animals and treatment

Swiss albino mice fed a standard diet and water *ad libitum*. The experiments were performed only with male mice at an age of 9-11 weeks and were approved by state authorities for animal protection.

Animals were divided into two groups of 10 mice per group. The first group was considered as the control group which gavage distilled water twice a week. Toltrazuril 2.5% (Bycox) solution (Marcyl Pharmaceuticals Industries, El-Abour City, Egypt) was given orally twice at a 1-week interval at a dose rate of 20 mg/kg body weight according to Pilarczyk et al. (1999).

Sample collection

After seven days, animals were anesthetized by ether. Blood was taken from heart of all mice into non-heparinized tubes to separate the serum for biochemical studies then stored at -80°C until use. Liver and intestine were separated and cut into small pieces.

Biochemical analysis

Liver and intestine were immediately homogenized to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose (Tsakiris et al. 2004). The homogenate was centrifuged at 500 g for 10 min at 4°C . The supernatant (10%) was used for the various biochemical determinations.

Glutathione (GSH) was determined chemically in jejunum homogenate using Ellman's reagent (Ellman 1959). The method is based on the reduction of Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid) with GSH to produce a yellow compound. The chromogen is directly proportional to GSH concentration, and its absorbance was measured at 405 nm.

Lipid peroxidation in plasma and jejunum homogenate were determined according to the method of Ohkawa et al. (1979) by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67%, followed by heating in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as malondialdehyde (MDA) equivalents formed.

The assay of nitric oxide (NO) in plasma and jejunum homogenate was done according to the method of Berkels et al. (2004). In acid medium and in the presence of nitrite the formed nitrous acid diazotises sulphanilamide, which is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish-purple color which was measured at 540 nm.

Statistical analysis

One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's test using a statistical package program (SPSS version 17.0). All p values are two-tailed and $P < 0.05$ was considered as significant for all statistical analysis in this study.

3. Results

Results in table 1. Showed that there are no significant changes in the level of MDA in the liver after treatment of mice with toltrazuril for one week while in the intestine and in blood serum the level is significantly changes to be 0.537 nmol/g and 105.77 nmol/ml, respectively.

Table 1. Toltrazuril induced changes in liver, intestine and blood serum malondealdehyde.

| Organ | Control mice | Toltrazuril treated mice |
|--------------------------------|--------------|--------------------------|
| Liver MDA (nmol/g tissue) | 0.298±0.01 | 0.368±0.02 |
| Intestinal MDA (nmol/g tissue) | 0.430±0.02 | 0.537±0.01* |
| Serum MDA (nmol/mL) | 83.33±2.34 | 105.77±2.74* |

Values are means \pm SD. *: Significant against non-treated control group at $p < 0.05$

Toltrazuril induced a significant increase in the level of NO in the liver of mice after one week post-treatment and the NO concentration reached 0.856 $\mu\text{mol/g}$ (Table 2). Also, the blood NO level was 131.72 $\mu\text{mol/L}$ (Table 2). In the intestine the level of NO was non-significantly changed.

Table 2. Toltrazuril induced changes in liver, intestine and blood serum nitric oxide.

| Organ | Control mice | Toltrazuril treated mice |
|--|------------------|--------------------------|
| Liver NO ($\mu\text{mol/g tissue}$) | 0.597 \pm 0.02 | 0.856 \pm 0.06* |
| Intestinal NO ($\mu\text{mol/g tissue}$) | 0.652 \pm 0.01 | 0.766 \pm 0.02 |
| Serum NO ($\mu\text{mol/L}$) | 84.41 \pm 2.81 | 131.72 \pm 5.86* |

Values are means \pm SD. *: Significant against non-treated control group at $p < 0.05$

Mice treated with toltrazuril for one week showed a significant increase in the blood level of GSH (Table 3) while the GSH level in both of liver and intestine did not change significantly (Table 3).

Table 3. Toltrazuril induced changes in liver, intestine and blood serum glutathione.

| Organ | Control mice | Toltrazuril treated mice |
|------------------------------|-------------------|--------------------------|
| Liver GSH (mg/g tissue) | 0.340 \pm 0.01 | 0.326 \pm 0.01 |
| Intestinal GSH (mg/g tissue) | 0.248 \pm 0.01 | 0.219 \pm 0.02 |
| Serum GSH (mg/L) | 111.10 \pm 1.31 | 92.66 \pm 1.97* |

Values are means \pm SD. *: Significant against non-treated control group at $p < 0.05$

4. Discussions

Attention has been focused on the protective effect of naturally occurring antioxidants in biological systems against parasites (Al-Quraishy et al. 2011; Dkhil et al. 2011).

Toltrazuril is active against all intracellular stages of coccidia, including schizonts, micro and macrogamonts (Mehlhorn 2008). It interferes with the division of the protozoal nucleus, the activity of the mitochondria and damages the wall forming bodies in the microgametes. Toltrazuril produces severe vacuolisation of the protozoal endoplasmic reticulum in all intracellular development stages.

In recent years, evidence has accumulated for a role of reactive oxygen metabolites as a mediator of tissue injury in several animal models (Fukui et al., 2010; Sato et al., 2011). Although, the exact mechanisms of free-radical generation are not yet completely understood, it is postulated that the antioxidant GSH depletion by the intestinal parasites may be a trigger for the production of reactive oxygen species (ROS) (Cam et al., 2008). Generation of ROS in the cytoplasm of cells may increase the mitochondrial hydrogen peroxide production and lipid peroxidation of cell and mitochondrial membranes, resulting in loss of membrane integrity and finally cell necrosis or apoptosis (Valko et al., 2007).

Although toltrazuril is an effective against coccidian but it has some adverse effects on the organs of animals. It is recommended to use natural products against coccidian than the use of chemical compounds.

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