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SHORT COMMUNICATION

Hsp-90 Inhibitor Geldanamycin Attenuates Liver Oxidative Stress and Toxicity in Thiram-Induced Tibial Dyschondroplasia

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A B S T R A C T

An experiment was conducted to study the effects of hsp90 inhibitor geldanamycin (17-DMAG) on liver functioning and its antioxidant ability in thiram-induced tibial dyschondroplasia. One hundred and twenty commercial chicken broilers were allocated into three groups: 1) control, 2) thiram-induced and 3) 17-DMAG treated. Serum samples were collected on day 11 and 14 post-hatch to determine the liver ALT, AST and ALP activity. The liver samples were collected at the end of trial to determine the activity of SOD (superoxide dismutase), GSH-Px (glutathione peroxidase) and MDA (malondialdehyde) contents. The results depicted that thiram increased the level of serum ALT, AST and liver MDA contents while decreased the serum ALP and liver antioxidant enzymes (SOD, GSH-Px); however, by administering 17-DMAG, these values were observed close to normal range as compared to control group. In conclusion, the oxidative imbalance and damage to liver caused by thiram can be restored by using 17-DMAG.

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INTRODUCTION

Tibial dyschondroplasia (TD), an avian disease of growth plates at the proximal end of long bones, is characterized by the presence of a-vascularized, un-mineralized, non-viable cartilage that fails to resorb and replace by bone. Although etiology is unknown; however, abnormal chondrocytic differentiation, changes in the activities of matrix metalloproteinase and changes in genes encoding VEGF signaling pathways have been attributed to TD (Dan *et al.,* 2009; Velada *et al.,* 2011;Genin *et al.,* 2012). In commercial processing plants, TD has emerged as one of the most prevalent overt locomotive problems which account 30% among such cases in broiler flocks making it an animal welfare issue (Pelicia*et al.*, 2012).

Thiram (tetramethyl thiuram disulfide), a dithiocarbamate organic compound is used primarily and widely as fungicide and pest and rodent repellants in agricultural fields. It occurs as a contaminant in products being used for litter material and poultry feed ultimately leading to leg problems in poultry industry (Rath *et al.,* 2007). Being mainly metabolized in liver, this compound has also been reported to cause damaging and toxic effects to this organ (Gupta and Amma, 1993).

The 17-DMAG (17-dimethylaminoethylamino-17-demethoxy geldanamycin), a semi-synthetic derivative of geldanamycin is a water soluble stable compound which inhibits heat shock proteins 90 (hsp-90) by serving as antiangiogenic factor in tumor cells. Its therapeutic effect has recently been studied in the treatment of hepatocellular carcinoma (Leng *et al.,* 2012). The role of 17-DMAG has been evaluated in TD in recent times where, contrary to its antiangiogenic effect in cancer cells, it caused the invasion of blood vessels in TD lesion area and abrogated the lameness by interfering the VEGF signaling pathway (Herzog *et al.,* 2011; Genin *et al.,* 2012). The aim of this study was to investigate the effects of 17-DMAG on liver functioning and its antioxidant capability caused by thiram in tibial dyschondroplasia.

MATERIALS AND METHODS

The experiment was planned following the guidelines and approval of the Institution Animal Care and Use Committee of Huazhong Agricultural University Wuhan, China.

A total of 120-day-old male broiler chicks were raised under recommended temperature and standard hygienic conditions. Dyschondroplasia was induced by

dietary thiram at 50 mg/kg in feed in groups A (thiram group) B (treatment group) from day 3 post-hatch. The 17-DMAG (Selleckchem, Cat No S1142) in 600 µg was administered into wing vein of group B at day 7, 10 and 12 post-hatch. All control chicks (group C) were given basal diet. The experimental protocol and dose of 17-DMAG was selected based on the studies of (Herzog *et al.*, 2011; Genin *et al.*, 2012) to prevent and treat the TD development in chicken broilers.

During the experiment, 10 blood samples from each group were collected by cardiac puncture on day 11 & 14. The blood serum was separated and stored at -70^{o}C to determine the ALT, AST level and ALP activity. Immediately after death by cervical dislocation on day 14, the liver samples from each group were stored at -70^{o}C for later analysis of SOD, GSH-Px activity and MDA contents.

Liver SOD and GSH-Px activity were expressed in U per milligram of protein (U/mg protein) and liver MDA contents were interpreted in nanomoles per gram wet weight of tissue (nmoles/g). The values of serum ALT, AST and ALP activities were expressed as unit per litre (U/l). All commercial reagent kits were purchased from Jiancheng Biochem Company (Nanjing, China) following the protocol appropriate to each reagent kit.

Statistical analysis: All data are presented as means±SD. Comparison between mean values among the groups was carried out using one way ANOVA followed by Tukey's honest test for continuous variables. Differences were considered statistically significant if P<0.05.

RESULTSAND DISCUSSION

The effect of thiram and 17-DMAG on liver ALT and AST is shown in Table 1. Compared to control group, thiram caused damaging effects on liver significantly (P<0.05); however, this deleterious effect was counter acted by 17-DMAG with no significant difference (P<0.05) in group B as compared to control group. The serum ALP activity was hampered by thiram in group A (P<0.05) but by employing hsp-90 inhibitor in group B, it exhibited a normal picture as to control group (Table 1). Table 2 shows that, compared with control, dietary thiram decreased the SOD and GSH-Px and increased the MDA contents of liver in group A (P<0.05); however, these antioxidant enzymes in 17-DMAG treated group were close to control group with a significant decrease (P<0.05) in MDA levels.

With a major cause of lameness in meat type poultry, tibial dyschondroplasia has caused enormous economic losses. Since its discovery as a spontaneously occurring disease in 1965 by Leach and Nesheim, a variety of protocols have now been adopted to induce TD, all leading to same phenotype suggesting a common pathway. Thiram, as an agricultural fungicide and pesticide has drawn much attention due to its toxicity in commercial poultry feed and farming. As a TD-inducing agent, it has been employed to produce TD to investigate the pathogenesis of this abnormality in avian species.

Earlier, some reports suggested that thiram due to its main metabolism in liver, had caused hepatic toxicity by increasing the activity of serum AST and decreasing cytochrome P-450 in male rats and in thiram-induced TD in chicken broilers (Gupta and Amma, 1993; Li *et al.*, 2007). The higher serum concentrations of AST and ALT enzymes indicate the release of aminotransferase from hepatocytes into blood stream due to damaged liver. We investigated the levels of AST and ALT being considered as biomarkers to assess the liver functions. Our experiment depicted a significant growing rise of these two enzymes in thiram induced chicks (group A) as compared to control on 11 and 14 days post-hatch indicating hepatic toxicity. However, after treatment with 17-DMAG to group B, the severity of TD started subsiding along with the lower levels of serum AST and ALT with a possible decrease in liver damage (Table 1).

Table 1: Effect of thiram on ALT, AST and ALP activities in thiram-induced (50 mg/kg) broiler chicks (A) and 17-DMAG (600µg) treated group (B)

Days post-hatch	C (n=10)	A (n=10)	B (n=10)
ALT (U/l)			
11	33.41±1.2b	74.21±2.3a	62.71±1.4a
14	41.72±1.9b	93.72±2.9a	48.43±1.3b
AST (U/l)			
11	56.67±1.1b	135.74±2.3a	98.32±2.1a
14	62.32±1.6b	151.21±2.8a	71.43±1.6b
ALP (U/l)			
11	138.11±1.09a	98.31±0.6c	125.41±1.7b
14	151.43±1.4a	71±0.4c	141.32±1.3b

Values (mean±SD) were compared by one way ANOVA.Different letters represent level of significance (P<0.05) in rows.

Table 2: Effect of thiram on SOD, GSH-Px activities and MDA contents in thiram-induced (50mg/kg) broiler chicks (A) and 17-DMAG (600µg) treated group (B) on 14 days post-hatch

Parameter	C (n=10)	A (n=10)	B (n=10)
SOD (U/mg)	131.06±1.4a	91.3±1.1b	121.78±1.1a
GSH-Px (U/mg)	21.82±0.6a	10.36±0.4b	19.08±0.7a
MDA (nmoles/g)	27.42±1b	41.4±1.1a	29.3±0.9b

Values (mean±SD) were compared by one way ANOVA.Different letters represent level of significance (P<0.05) in rows.

The alkaline phosphatase (ALP) characterizes hypertrophic chondrocytes and is a marker of calcification thus relating to skeletal remodeling. Lack of calcification and failing of chondrocytes to undergo complete differentiation in tibial growth plate are one of the characteristic lesions of TD (Genin *et al.*, 2012). In our studies, the serum ALP levels were found significantly decreased in thiram-induced birds than control group depicting the lack of endochondral bone formation; however, on treating the chicks with hsp-90 inhibitor geldanamycin led to the normal tibial growth plate thus increasing the activity of this enzyme (Table 1). Genin *et al.*, (2012) also reported such type of ALP activity extracted from affected tibial growth plate. This enzyme was found absent in TD lesion and reappeared in that area as the lesion was abrogated.

Reactive oxygen species (ROS), produced constantly in animals serve as a common feature of "vicious cirlce" of oxidative stress. The antioxidant enzymes including glutathione peroxidase and superoxide dismutase remove these ROS. Thiram has been suggested as a potent oxidative agent; Marikovsky (2002) has reported this compound to inhibit SOD activity markedly and rapid depletion of GSH in human leading to an oxidative imbalance. Such type of stress results in the lipid peroxidation of cell membrane and releases MDA as an end product. The changes in GSH-Px and SOD activities and MDA contents can be evaluated to determine the

extent of oxidative stress in body. In this trial, a significant decrease in SOD and GSH-Px activities and increase in MDA contents were found in the liver of thiram fed group depicting the liver function damage. However, on treating the chicks with 17-DMAG, the oxidative imbalance was attenuated approximately to normal as compared to control group (Table 2). The presence of chaperon especially hsp90 in stressed-area has been evidenced in various studies. The inhibition of hsp90 by employing geldanamycin has been reported previously to reduce the oxidative stress in body and TD lesion (Herzog *et al.,* 2011; Genin *et al.,* 2012).

Conclusion: The oxidative imbalance and damage to liver caused by thiram can be restored by using 17-DMAG as exhibited by liver functioning enzymes and its antioxidant activities with parallel treatment for tibial dyschondroplasia.

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Authors' contribution: M Shahzad and JK Li participated in the design of study. M Shahzad, J Liu, Z Wang and F Nabi performed the experiments and M Shahzad, J Gao and D Zhang analyzed the data. M Shahzad wrote the manuscript. All authors read and approved the final manuscript.

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