Effects of 2,4-D (dichlorophenoxyacetic acid) on blood anti-oxidant / oxidant balance and on tissues in lambs

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SUMMARY

In this study, the effects of the common herbicide 2,4-D (dichlorophenoxyacetic acid) on blood antioxidant / oxidant status and on various organs were investigated in Sakiz crossbred lambs. For that, 6 lambs were poisoned accidentally with the herbicide whereas 6 other healthy animals served as negative controls and plasma MDA and GSH concentrations as well as serum ascorbate, retinol and β-carotene concentrations were determined by spectrophotometry two days later. In parallel, conventional histological analysis was performed on lungs, liver, kidney and heart from a lamb dead after 2,4-D exposure. Blood antioxidant/oxidant balance was altered due to a significant increase of MDA concentrations coupled to a significant decrease of GSH concentrations in intoxicated lambs compared to controls. Congestive lesions and some haemorrhages were evidenced in lungs and kidneys and cell degeneration associated with lymphocyte infiltrate were observed in liver, kidneys and in heart. These results highly suggest that the herbicide 2,4-D induces an oxidative stress leading to cell destructions and tissue injury.

Keywords: 2,4-D (dichlorophenoxyacetic acid), lambs, glutathione, malondialdehyde, oxidative stress, necrosis, histopathological analysis.

RÉSUMÉ

Effets du 2,4-D (acide dichlorophénoxyacétique) sur le statut sanguin antioxydant / oxydant et toxicité tissulaire chez l’agneau

L’objectif de cette étude a été d’étudier les effets d’un herbicide commun, le 2,4-D (acide dichlorophénoxyacétique) sur le statut sanguin antioxydant / oxydant et sur différents organes chez des agneaux Sakiz croisés. Six agneaux ont été accidentellement intoxiqués par le 2,4-D alors que 6 autres animaux sains ont servi de contrôles négatifs et les concentrations plasmatiques de MDA et de GSH de même que les concentrations sériques d’ascorbate, de rétinol et de β-carotènes ont été déterminées par spectrophotométrie 2 jours après. En parallèle, une analyse histologique conventionnelle a été réalisée sur les poumons, les reins, le foie et le cœur d’un agneau mort après exposition à l’herbicide. L’équilibre sanguin antioxydant / oxydant a été altéré suite à une augmentation significative de la concentration en MDA couplée à une diminution significative de la concentration de GSH chez les animaux intoxiqués par rapport aux contrôles. Des lésions congestives et quelques hémorragies ont été mises en évidence dans les poumons et les reins et des phénomènes de dégénérance cellulaire associés à des infiltrats lymphocytaires ont été observés dans le foie, les reins et le cœur. Ces résultats suggèrent fortement que l’herbicide 2,4-D induit un stress oxydatif à l’origine de phénomènes nécrotiques et des lésions tissulaires.

Mots clés : 2,4-D (acide dichlorophén oxyacétique), agneau, glutathion, malondialdehyde, stress oxydatif, analyse histopathologique.

Introduction

In the recent time, world population has increased rapidly, as the total agricultural area has decreased. This fact necessitates increasing yield amount per unit area. For this purpose, agricultural combat methods are applied to avoid loss in agricultural product output. One of the most common methods of agricultural combat is chemical combat with herbicides, cited in pesticide group. The 2,4-D (dichlorophenoxyacetic acid) is an herbicide widely used all over the world for about 50 years [7, 19, 24, 29]. Herbicides introduced as yield booster, when used unconsciously and uncontrolled, could cause many global problems such as acute and chronic poisoning in humans, wild and domestic animals, by exhibiting teratogenic, mutagenic and carcinogenic effects, and by inducing deterioration of biological balance and environmental and food pollution [7, 20, 23].

Oxidative stress could be defined as an imbalance between antioxidant defence mechanisms and free radical production, causing peroxidation of cellular lipid layers. Lipids are the most sensitive structures to the free radicals induced damages. Free radicals easily react with unsaturated fatty acids, causing lipid peroxidation and oxidation of polyunsaturated fatty acids forms recurring chain reactions, leading to irreversible membrane damages [3, 29]. The malondialdehyde (MDA) is an important product accumulated during lipid peroxidation. Oxidative stress induced by herbicides consists in the superoxide formation resulting from electron accumulation in NADPH dependent reactions. As superoxide dismutases (SOD) don’t exist in lungs, lung tissues are highly sensitive to superoxide accumulation induced by herbicides. Some compounds like diethylthiocarbamate, inhibit the superoxide dismutase (SOD) efficiency and increase the peroxidation speed. In addition, others, particularly herbicides like amino-
Materials and Methods

ANIMALS AND PROTOCOL DESIGN

A total of 12 Sakız crossbred lambs, (7 males and 5 females), approximately 5.5 months old, stemming from the Suhut County Agriculture Office, were included in the present study. Six lambs were poisoned due to the accidental consumption of plants contaminated with 2,4-D (dichlorophenoxyacetic acid) herbicide (group I) whereas the others have not consumed contaminated plants and constituted the control group (group II).

Two days after plant consumption, blood samples were collected from v. jugularis puncture into tubes with heparin in one hand, and without anticoagulant in the other hand, 2 hours after appearance of the first sign of 2,4-Dichlorophenoxyacetic acid intoxication. The control lambs were sampled on the same occasions. After clotting at 4°C for 60 minutes, tubes without additives were centrifuged at 1 000 x g for 15 minutes at 4°C and sera were carefully harvested and stored at -70 °C until analysis of ascorbic acid, retinol and ß-carotene. Noxyacetic acid intoxication. The control lambs were sampled from v. jugularis puncture into tubes with heparin and without anticoagulant in the other hand.

RESULTS

Clinical parameters recorded in lambs were hypersalivation, coordination failure, muscle contractions and tremor. The death was observed in a lamb 3 hours after the first signs of 2,4-Dichlorophenoxyacetic acid intoxication. Dispnea, tachycardia, salivation, ataxia, tonic and clonic spasms were observed before and during death.

As shown in Table I, the circulating oxidant-antioxidant balance was significantly altered in poisoning lambs: plasma MDA concentrations were significantly increased (P < 0.01) compared to controls whereas GSH concentrations were depressed (P < 0.05). The other biochemical parameters investigated in the current study were not significantly affected by the herbicide intoxication. The alterations of MDA and GSH concentrations were used within the same day for determining MDA and GSH concentrations.
GSH concentrations were detected in all poisoned lambs, and a significant negative correlation \((r = -0.79, P < 0.05)\) was evidenced between the 2 biochemical parameters.

Morphological and histological examinations were performed on the unique dead lamb. The main macroscopic findings of the dead lamb were oedema, hyperaemia and petechial haemorrhage in lungs. In lung, vessels were hyperaemic and sporadic free erythrocytes were found in interstitial tissue and alveoli. Oedema fluid was also detected in some alveoli. Moreover, some alveoli were atelectatic and some were emphysematic (figure 1B) and partial degenerations of the bronchiole epithelium associated with sporadic lumen degeneration were scarcely recorded. In liver, vacuole degeneration of hepatocytes and lymphocyte infiltration in the peri-portal region were observed (figure 1A). Highly hyperaemic vessels and degenerative signs (coagulation necrosis, pyknosis) of tubular epithelial cells were commonly noted in kidney (figure 1C). In addition, hyaline degeneration of muscle fibbers coupled to a moderate lymphocyte infiltration was evidenced in the heart (figure 1D).

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Intoxicated lambs</th>
<th>Healthy lambs</th>
<th>(P) value</th>
</tr>
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<tbody>
<tr>
<td>MDA ((\mu)mol/L)</td>
<td>1.87 ± 0.25a</td>
<td>1.16 ± 0.12b</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td>GSH (mg/L)</td>
<td>407.7 ± 2.5a</td>
<td>423.9 ± 4.6b</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Vitamin C (mg/L)</td>
<td>28.3 ± 3.8</td>
<td>28.7 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin A ((\mu)g/L)</td>
<td>368.0 ± 11.2</td>
<td>366.0 ± 7.4</td>
<td>NS</td>
</tr>
<tr>
<td>(\beta)-carotene ((\mu)g/L)</td>
<td>127.9 ± 3.1</td>
<td>127.4 ± 3.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

**TABLE I:** Circulating oxidant-anti-oxidant balance in Sakiz crossbred lambs intoxicated with 2,4-D (Dichlorophenoxyacetic Acid) \((n = 6)\) and in healthy controls \((n = 6)\) 2 days after consumption of plants contaminated with the herbicide. Results are expressed as mean ± standard deviation.

**NS:** Not significant. Different superscripts \(a, b\) in the same line indicate significant differences between the 2 groups.

**FIGURE 1:** Histological lesions induced by 2,4-D (Dichlorophenoxyacetic Acid) intoxication in a Sakız crossbred lamb. Haematoxylin – Eosin. **A.** Liver section, inflammatory cell clusters (arrows) and small fat droplets (arrowheads) in hepatocytes, X100. **B.** Lung section, oedema fluid (arrows) and free erythrocytes (arrowheads) in alveoli, X100. **C.** Kidney section, degenerated tubular epithelial cells (arrows), X200. **D.** Heart muscle section, inflammatory cell clusters (arrows), X200.
Discussion

Insecticides and herbicides are the most common agricultural drugs in world pesticide market. In Turkey, insecticides come first, whereas herbicides are the second important parts of agricultural drugs [20, 23]. Overdose and untimely applications of 2,4-D (Dichlorophenoxyacetic Acid) leave residue on agricultural product, thus causing harmful effects on humans and animals consuming these products [7, 20, 23]. Free radicals play crucial roles in the toxicity of pesticides and environmental chemicals. Pesticides could cause oxidative stress, free radical formation and changes of antioxidants. Lipid peroxidation was reported to be involved in the poisoning cases caused by pesticides [16]. DELLA et al. [9] observed in their study conducted with seven different pesticides that GSH was consumed in rat liver. In the same way, CANA-CANKATAN et al. [8] demonstrated significant decreases of GSH contents in liver and erythrocytes from rats treated with carbosulphan application (a pesticide used in agriculture) coupled to marked increases of cellular MDA contents and SETH et al. [25] observed that carbamate insecticides decreased blood GSH concentrations and increased serum MDA concentrations in rats. In agreement with that, significant increases of plasma MDA concentrations and significant decreases of plasma GSH concentrations were evidenced in 2,4-D (Dichlorophenoxyacetic Acid) intoxicated lambs. Moreover, these 2 parameters were significantly and negatively correlated ($r = -0.79$, $P < 0.05$). In the literature, it was reported that as a result of lipid peroxidation based on oxidative damage, peroxidation products increase, while GSH concentrations decrease [6-11, 15, 21, 22, 29]. Indeed, BUKOWSKA [6, 7] observed decreases of GSH concentrations in blood and in tissues, particularly in human erythrocytes coupled to increases of the MDA concentrations after intoxication by the 2,4-D (2,4-Dichlorophenoxyacetic Acid). Furthermore, it was previously demonstrated that the 2,4-D causes haemolysis [10, 11] and exhibited hepatotoxicity [21].

Phenyl-like herbicides such as diuron, fenuron, linuron, monolinuron, monuron, metazol and neburon form azoic components after N-amplification reactions and these carcinogenic azoic components cause lipid peroxidation in cell membranes whereas others such as bipyridyl-like herbicides directly cause lipid peroxidation [15]. The occurrence of an oxidative stress induced by several pesticides / herbicides can result from a direct action of compounds onto antioxidant systems. ALICIGUZEL et al. [4] reported that inactivation of some enzymes of human erythrocyte antioxidant enzymes may exacerbate the toxicity of 4-chlorophenoxyaceticacid (a phenoxy herbicide). By inhibiting superoxide dismutases and/or catalase such as diethyldithiocarbamate and aminotriazol respectively, the inactivation of superoxide and hydrogen peroxide becomes limited, leading to peroxidation acceleration. In addition, SUWALSKY et al. [28] reported that 2,4-D (2,4-Dichlorophenoxyacetic Acid) corrupts phospholipids by oxidative transformation, leading to alteration of cell membrane integrity and morphologic changes in erythrocytes. POGOSYAN et al. [22] demonstrated a physical cell interaction with the 2,4-D binding to some phospholipids and stated that this direct association with cell membrane may exacerbate lipid peroxidation.

The toxicity of the 2,4-D (dichlorophenoxyacetic acid) was restrictively studied on rats and even, on some specific organs. In this way, OZDAS et al. [20] reported dose-dependent degenerative alterations including atrophy in seminiferous tubules, irregular arrangement of spermatogenic cells as well as a marked testicular damage especially prominent in high dose group in testis tissues after 2,4-D intoxication in rats. SUTER [26] reported that lindane (an organo-chloride pesticide) intake causes centrilobular necrosis in liver as well as necrosis of tubular epithelial cells and interstitial nephritis in kidneys. VIDELA et al. [30] observed severe necrosis in liver coupled to infiltration with lymphocytes and granulocyte leukocytes in rats after lindane exposure and FIDAN et al. [13] observed megalyoclastosis, periacinar degeneration, congestion and perportal lymphocyte infiltration in liver from rats subjected to overdose lindane (20 and 40 mg/kg), and also reported medullar and cortical bleeding in kidney and degeneration of proximal tubules. In the current study, the histopathological findings found in lambs intoxicated with 2,4-D (mainly, bleeding in lungs and kidneys, cell degeneration and lymphocyte infiltration in liver, kidney and heart) are compatible with these previous reports [13, 26, 30].

As a conclusion, the modifications of the antioxidant-oxidant equilibrium characterized by increase of MDA concentrations and decrease of GSH concentrations in blood associated to congestive and necrotic lesions in lungs, liver, kidneys and heart demonstrate the occurrence of an oxidative stress during 2,4-D (dichlorophenoxyacetic acid) intoxication in lambs. Therefore, the 2,4-D herbicide, taking its residue amounts into consideration, should be used more carefully and consciously.

References


