ASPECTS OF USING N-ACETYLCYSTEINE IN AFLATOXICOSIS AND ITS EVALUATION REGARDING SOME LIPID PEROXIDATION PARAMETERS IN RABBITS

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Received for publication August 30, 2004.

Abstract

In the study, 42 rabbits, 6 to 8 weeks of age, were divided into 6 groups. Groups 2, 3, and 4 were given, by a catheter directly into the stomach, aflatoxin (AF) (78.70% of AF B₁, 10.80% of AF B₂, 6.80% of AF G₁, 3.70% of AF G₂) in a dose of 0.4 mg/kg/bw/d in dimethyl sulfoxide (DMSO), while groups 1, 5, and 6 were simultaneously given the same dose of DMSO with no AF. Twenty-four hours after AF was given, N-acetylcysteine (NAC) was administered intramuscularly at a dose of 250 mg/kg/bw/d to groups 3 and 5 and at a dose of 500 mg/kg/bw/d to groups 4 and 6 for 6 d. At the beginning of the trial (day 0) and on days 1, 4, and 7 following the administration of AF, blood was taken into heparinized tubes, and malondialdehyde level and superoxide dismutase and catalase activities were examined. It was found that AF caused oxidative damage and NAC was effective, at least partly, in the prevention of this damage. Consequently, it was obvious that NAC can be used as ameliorative agent in combination with other drugs in order to alleviate the adverse effects of AF action.

Key words: rabbits, N-acetylcysteine, aflatoxicosis, lipid peroxidation.

Aflatoxins are toxic metabolites synthesised by certain kinds of fungi, belonging to Aspergillus genus (15). Several studies were made about different toxic effect of the compounds (8, 9) and alleviating of these in many kinds of animals (10-12, 26-30). Especially, there are numerous studies showing that aflatoxins cause lipid peroxidation (10, 33, 34). Lipid peroxidation occurs when unstable and active compounds exceed their maximum level and the compensation by cells and cell defence systems are insufficient to destroy these compounds (17-19, 31). The cell membrane, which is highly sensitive and contains the high level of unsaturated fatty acids, undergoes peroxidation and protein/lipid ratio of cell membrane changes. Since these changes will disturb the diffusion of intra- and extracellular compounds through the cell, they trigger several adverse effects which can take place in the body (14, 17, 19). Aflatoxins bind glutathione and deplete its stores in the body by causing extreme damage to the liver, where it is primarily synthesised (2). Recently, the views about the main mechanism of the various adverse effects of aflatoxins (carcinogenic, mutagenic, teratogenic) gained momentum because of its ability to cause oxidative damage (2, 6, 21).

In this context, the most realistic approach will be to determine whether the treatment that can be performed in cases of poisoning is successful or not, basing on the mechanisms of the action of these compounds which lead to poisoning. In practice, there are lots of studies available about methods of the protection from aflatoxins (10-12, 26-30, 33). Yet, the studies conducted to find the most realistic curative choice are limited in such cases where the poisoning occurred (33, 37). So far there are no available data of using N-acetylcysteine (NAC), an intracellular glutathione precursor (5), for the treatment of domestic animals poisoned with aflatoxins. In this study, it will be evaluated whether NAC is or not effective in cases of poisoning caused by aflatoxins and, if it is, it will be determined in which doses and intervals it could be used. This subject will be discussed also in many aspects, considering the activity of antioxidant enzymes (superoxide dismutase and catalase) and malondialdehyde level.

Material and Methods

In the study, 42 rabbits, 6-8 weeks of age, were used. The rabbits were divided into 6 equal groups.
While the first group was kept as the control, the dose of 0.4 mg/kg/bw of AF (78.70% of AF B1, 10.80% of AF B2, 6.80% of AF G1, 3.70% of AF G2), that is oral LD<sub>50</sub> (4) for AF B1, was administered in dimethyl sulfoxide (DMSO) (2 mg of AF/ml of DMSO) by a catheter directly into the stomach of the animals in groups 2, 3, and 4. Likewise, DMSO, which did not contain AF, was given in the same manner to animals of groups 1, 5, and 6. Twenty-four hours after AF treatment (on day 1), NAC was administrated intramuscularly at the dose of 250 mg/kg/bw/d to groups 3 and 5 and at a dose of 500 mg/kg/bw/d to groups 4 and 6 for 6 d. At the beginning of the trial i.e. on day 0 and then on days 1, 4 and 7 following the administration of AF, blood was sampled into heparinized tubes. The blood samples were centrifuged at 3000 rpm for 10 min. The plasma was used for the determination of malondialdehyde (MDA) level and precipitated erythrocytes, rinsed three times by a phosphate buffer containing 140 mM of NaCl, were used for the determination of haemoglobin content and superoxide dismutase (SOD) and catalase (CAT) activities (38). The determinations of MDA, haemoglobin, and CAT and SOD activities were done according to the methods of Yoshoiko et al. (39), Fairbanks and Klee (13), Luck (22), and Sun et al. (36), respectively. The production of AF used in the study was based on Shotwell et al. method (35), which was modified by Demet et al. (7). Moreover, the species analysis of aflatoxin and their rates were determined according to the method by Nabney and Nesbit (25). As a result of this detection, AF rates which were detected previously were found to be 78.70%, 10.80%, 6.80%, and 3.70% for B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>, respectively. The analysis of total aflatoxin (B<sub>1</sub>+B<sub>2</sub>+G<sub>1</sub>+G<sub>2</sub>) extracted in rice flour was performed by ELISA reader, using Ridascreen® label kit and the suggested method in the kit procedure. Solvents of elute containing AF which was extracted (25) from rice flour was evaporated. Then, the refined AF was dissolved in DMSO. The data were presented as arithmetic means and standard deviation. The one-way variance analyses were used and Duncan test was performed to demonstrate the significance of differences between groups using SPSS 10.0 for Windows Package Program.

### Results

No significant differences in all three parameters (MDA, SOD, CAT) among the groups were found in blood samples taken on day 0 (P>0.05). An increase in the severity of oxidative damage was detected only in the group given AF (group 2) on the days 1, 4 and 7. Depending on this, the MDA level gradually increased but SOD and CAT activities decreased. These changes were statistically significant on days 4 and 7 (P<0.05). Following the administration of AF, a decrease in the severity of oxidative damage in the groups which were given different doses of NAC (250 and 500 mg/kg/bw/d) (groups 3 and 4) was observed. This was obvious from the changes in the studied parameters (decrease in MDA and increase in SOD and CAT activities). These changes were statistically significant only when compared to the groups given AF on day 7 regarding the MDA level (P<0.05). It was understood that this compound did not cause any statistically significant difference only in the groups given NAC (groups 5 and 6), compared to control, in terms of MDA, SOD and CAT parameters through all periods (P>0.05) (Tables 1-3). Four rabbits in group 2, 2 rabbits in each of groups 3 and 4 died throughout the study.

#### Table 1

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Group 1</td>
<td>20.20±6.43</td>
</tr>
<tr>
<td>Group 2</td>
<td>17.04±7.38</td>
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<td>Group 3</td>
<td>19.68±10.08</td>
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<tr>
<td>Group 4</td>
<td>16.00±8.24</td>
</tr>
<tr>
<td>Group 5</td>
<td>16.45±5.60</td>
</tr>
<tr>
<td>Group 6</td>
<td>20.43±8.46</td>
</tr>
</tbody>
</table>

<sup>a, b</sup> Differences between means within the same column with different letters are statistically significant (P<0.05).

* Group 1 - control; group 2 - AF; group 3 - AF+NAC (250 mg/kg/bw/d); group 4 - AF+NAC (500 mg/kg/bw/d); group 5 - NAC (250 mg/kg/bw/d); group 6 - NAC (500 mg/kg/bw/d).
Table 2
Erythrocyte SOD activity in control and trial groups (U/g Hb)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>212.32±43.13</td>
<td>219.68±57.61</td>
<td>211.24±30.53a</td>
<td>204.93±39.96a</td>
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<tr>
<td>Group 2</td>
<td>198.07±33.67</td>
<td>190.95±26.77</td>
<td>154.55±37.52b</td>
<td>135.84±39.15b</td>
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<tr>
<td>Group 3</td>
<td>205.76±16.76</td>
<td>195.99±56.03</td>
<td>181.76±17.81ab</td>
<td>169.27±35.37ab</td>
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<tr>
<td>Group 4</td>
<td>206.87±39.11</td>
<td>194.92±27.02</td>
<td>186.84±22.36ab</td>
<td>161.51±38.57ab</td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>201.29±23.19</td>
<td>215.33±46.56</td>
<td>214.69±31.02a</td>
<td>200.03±18.51a</td>
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</tr>
<tr>
<td>Group 6</td>
<td>194.90±33.99</td>
<td>211.55±41.29</td>
<td>203.18±41.27a</td>
<td>194.58±20.75a</td>
<td></td>
</tr>
</tbody>
</table>

a, b Differences between means within the same column with different letters are statistically significant (P<0.05).

Table 3
Erythrocytes CAT activity in control and trials group (k/100mg Hb)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>56.06±15.50</td>
<td>55.35±18.25</td>
<td>60.21±11.86a</td>
<td>62.84±17.72a</td>
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<tr>
<td>Group 2</td>
<td>52.26±19.56</td>
<td>43.83±14.38</td>
<td>31.02±7.62c</td>
<td>25.26±9.30c</td>
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<tr>
<td>Group 3</td>
<td>47.41±26.28</td>
<td>40.84±10.27</td>
<td>38.94±15.43bc</td>
<td>33.03±9.54c</td>
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<tr>
<td>Group 4</td>
<td>59.58±22.64</td>
<td>49.49±12.02</td>
<td>44.02±15.81abc</td>
<td>40.83±17.31bc</td>
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</tr>
<tr>
<td>Group 5</td>
<td>52.16±15.17</td>
<td>58.60±16.46</td>
<td>62.38±11.85a</td>
<td>56.98±8.28ab</td>
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</tr>
<tr>
<td>Group 6</td>
<td>51.17±12.29</td>
<td>55.36±16.22</td>
<td>55.99±20.39ab</td>
<td>60.00±11.55a</td>
<td></td>
</tr>
</tbody>
</table>

a, b, c Differences between means within the same column with different letters are statistically significant (P<0.05).

Discussion

There are the cell defence mechanisms that prevent the oxidative damage caused by reactive products. If any compound exhibit toxic effects or not, usually it depends on the balance between bioactivation and detoxification (3, 14, 16). SOD and CAT play a very important role in the conversion of highly effective endogenous compounds into less harmful or ineffective compounds which cause lipid peroxidation, in turn, lead to cell membrane damage (18, 20, 24). The extent of oxidative damage which develops in biomolecules can be assessed by measuring the density of the end products which result from lipid peroxidation and antioxidant enzyme activity (16-19).

The numerous in vitro and in vivo studies indicate that aflatoxins are cytotoxic compounds (1, 23, 32, 37). The most accurate approach of the studies, aiming to reduce the toxic effects of these compounds (efficacy of NAC was assessed in this study) may be to consider the toxic effect mechanism (this may be lipid peroxidation). The groups were assessed, in terms of the specified enzyme activity and MDA levels, prior to starting the trial and no the statistically significant differences regarding these parameters between the groups (on day 0) were found. This indicated that the animals used in the study were physiologically identical to each other. The analysis of blood samples, taken at the 24th h, following the administration of AF (groups 2, 3 and 4) showed that there was no statistically significant differences regarding any of these parameters. Yet, if we made an evaluation individually, we observed an increase in MDA level in group 2, compared to control with a tendency to decrease in SOD and CAT activities. This indicated that aflatoxin started to affect animals from the 24th h. We could observe the same situation in groups 3 and 4, because NAC application had not started yet in these groups. On day 4 of the study the statistically significant increase was detected only in the MDA level of the group which received AF alone (group 2), compared to control. The statistically significant decrease was found in SOD and CAT activities (group 2), compared to control, which indicated that AF started to affect rabbits. As indicated above, it was obvious that the formation of free radicals accelerated and the cell membrane underwent peroxidation and in turn, the MDA levels increased. The formation of free radicals was above the level that could be compensated by cells. It was clearly explained by the increase in MDA level and decrease in SOD and CAT activities which are responsible for conversion of these radicals into less harmful and less effective metabolites (tended to deplete as they were responsible for detoxification). During this period, the groups which received NAC following the administration of AF (groups 3 and 4), showed their enzyme activities above those of the group given AF alone. Yet, these values never approached the values of control group (group 1). Thus, it was clear that the applied NAC was effective in reducing the adverse effects caused by AF. It was considered that the compound, as an intracellular glutathione precursor (5), played an important role in this pathway. It was also clear that this compound was effective in maintaining the level of glutathione which...
accelerated the removal of free radicals, formed in the cells during oxidative stress, from the body by binding them. Moreover, the activities of relevant parameters (SOD and CAT) did not completely return to the values of control group enzyme activity, which indicated that this effect was not complete regarding the groups in which this compound was used (groups 3 and 4). It was also possible to reach the same result, when the assessment was made in terms of MDA. There was no statistically significant difference regarding these parameters between the groups in which only NAC was used (groups 5 and 6), compared to the values of control group (group 1).

The statistically significant differences were also found regarding the received parameters of the group which received AF alone, compared to control, at the last period of the study (on day 7), as occurred on day 4. The obtained data revealed that the severity of the toxic effect caused by AF gradually increased in the animals at the last period of the study. It was possible to observe the same effect in the groups in which NAC was administered (groups 3 and 4) on day 7. There was no statistically significant difference of the studied parameters in the groups which were treated only with NAC (groups 5 and 6). The fact that the most of deaths were observed in the group 2 (the group given only AF) during the experimental periods shows that the animals were seriously affected by AF. The decrease in the range of deaths in the groups given AF plus NAC (groups 3 and 4) shows that NAC partly weakens the effects of the toxin. On the other hand, seeing no death in groups given only NAC (groups 5 and 6) may indicate that NAC in the used doses had no toxic effects in animals.

Finally, it was concluded from the evaluation of some lipid peroxidation parameters in the rabbits with aflatoxin poisoning that the animals were considerably affected by this compound. It was possible to suggest that NAC used at two different doses (250 and 500 mg/kg/bw/d) was, at least partly, effective in the prevention of this effect. However, it was quite difficult to say that the extent of this effect was correlated with the applied dose of the compound. It seems, after all, that NAC could be given as a supportive agent in addition to other curative applications in the cases of poisoning by AF. Furthermore, this study revealed that NAC applied at specified doses did not have an adverse effect. In other words, it was considered that the doses, especially 250 mg/kg/bw/d, might be safely used in the treatment of aflatoxicosis.

References