EFFECT OF CYCLOPHOSPHAMIDE ON ERYTHROCYTE AND PLASMA ACETYLCHOLINESTERASE ACTIVITY IN SHEEP

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SUMMARY

Twenty-seven 4- to 6-month-old crossbred ewe and ram lambs were assigned at random within sex to dosages of 10, 20 or 30 mg of cyclophosphamide (CPA), a chemical defleecing agent, per kilogram of body weight. Erythrocyte and plasma acetylcholinesterase (ACHE) activities were measured in jugular blood before CPA treatment and 1, 5, 9, 13, 17, 21 and 26 days thereafter. As in man, both plasma and erythrocyte AChE activities were depressed following CPA treatment, reaching their lowest levels on days 9 and 1, respectively. No physical signs of depressed AChE activity were observed in any of the sheep. The depression of neither the plasma or the erythrocyte enzyme was observed in any of the sheep. The depression of neither the plasma or the erythrocyte enzyme can be used presumptively to identify sheep that have been treated with CPA before that fact is evident from the wool itself.

(Key Words: Sheep, Wool, Chemical Defleecing, Cyclophosphamide, Acetylcholinesterase.)

INTRODUCTION

In human patients treated with cyclophosphamide (CPA) as a non-tumor agent, blood acetylcholinesterase (AChE) activity is depressed. This depression of activity has been observed both in vitro (Limongi et al., 1966; Wolff, 1966) and in vivo (Mone and Mathie, 1967; Wolff, 1965, 1966). After a single dose of CPA, the depression of AChE activity is evident within 24 hr (Priesching et al., 1967; Wolff, 1966). The degree of depression is directly dependent on CPA dosage (Wolff, 1965), and may be as much as 75% of the pretreatment AChE level (Priesching et al., 1967; Wolff, 1966).

In sheep treated with CPA as a defleecing agent, the wool loosens gradually over time at rates which are dependent on the sex of the sheep, the particular location of the wool on the sheep's body, and most importantly, on CPA dosage (Reynolds et al., 1972a,b). Whatever the rate of wool loosening, there is a period of at least 3 days, and usually longer, immediately after CPA treatment during which CPA treated sheep cannot be distinguished from untreated sheep by the looseness of their wool.

The objective of the study presented here was to determine whether CPA at defleecing dosage affects the levels of AChE plasma and erythrocytes, and, if so, whether this effect might be used presumptively to identify CPA treated sheep before such treatment is evident by the looseness of the fleece.

MATERIALS AND METHODS

A total of 27 crossbred ewe and ram lambs, 4 to 6 months of age, were randomized within sex to three groups. Following a preliminary bleeding to determine pretreatment AChE levels, 10, 20 or 30 mg of CPA per kilogram of live weight was administered to the three respective groups. The CPA solution, freshly prepared in tap water, was given by stomach tube. The sheep were kept as a single group on a raised screen floor and fed dehydrated alfalfa throughout the study.

Jugular blood was drawn over sodium heparin before CPA treatment and 1, 5, 9, 13, 17, 21 and 26 days thereafter. Both plasma and erythrocyte (RBC) AChE activities were assayed on the day of sampling by the electrometric method of Michel (1949) using a Beckman Model G pH meter fitted with a glass-calomel electrode pair. The data are expressed as Δ pH/hr, that is, the change in pH of the lightly buffered assay medium resulting from the

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hydrolysis of acetylcholine to choline and acetic acid, and were corrected for the non-enzymatic hydrolysis of acetylcholine, as described by Michel (1949).

Analyses of variance of the AChE activities were fitted (Harvey, 1960) to the following model:

\[ Y_{ijk} = \mu + S_i + T_j + ST_{ij} + e_{ijk} + D_k + SD_{i\ell} + TD_{j\ell} + e_{ijk\ell} \]

where \( \mu \) is a constant common to all subclassifications, \( S_i \), \( T_j \) and \( D_k \) are the effects of the \( i \)th sex, the \( j \)th dosage of CPA and the \( k \)th day after CPA treatment, respectively, \( ST_{ij} \), \( SD_{i\ell} \) and \( TD_{j\ell} \) are interactions among the above described factors, \( e_{ijk} \) is a residual attributable to the \( k \)th sheep of the \( i \)th sex treated with the \( j \)th dosage level of CPA, and \( e_{ijk\ell} \) is a residual attributable to, in addition to the factors already described, the \( \ell \)th day after CPA treatment. The model was fitted to RBC and plasma data separately. Dunnett's (1955) two-sided comparison was used to compare CPA dosage mean AChE levels on each observation day after CPA treatment with the pretreatment value.

RESULTS

The AChE activities of rams and ewes were not different (\( P>.10 \)) nor were there significant (\( P>.05 \)) interactions of sex with CPA dosage or with time after CPA treatment.

Plasma enzyme activity (table 1) decreased slowly after treatment at all CPA dosage levels and was less (\( P<.05 \)) than the pretreatment level on day 9 after treatment. By day 13, however, activity was similar to the pretreatment levels. Thereafter, at 20 and 30 mg per kilogram, activity declined at days 21 and 26 to less than the pretreatment levels. At 10 mg per kilogram, however, this subsequent decline after the day 13 recovery was not observed.

Little consistent effect of CPA dosage was observed in the plasma enzyme.

Erythrocyte enzyme activity (table 1) had declined (\( P<.01 \)) on day 1 after treatment at all dosage levels. Thereafter, at 20 and 30 mg per kilogram, activity gradually increased to levels which, by day 13, were greater (\( P<.01 \)) than pretreatment levels. At 10 mg per kilogram, the depression of activity persisted (\( P<.01 \)) through day 9, after which, like those at 20 and 30 mg per kilogram, activity increased to greater than pretreatment levels. Generally, after day 13, erythrocyte AChE levels were greater than pretreatment levels in all dosage groups. Except for the prolonged depression of AChE activity at 10 mg per kilogram through day 9, little consistent effect of CPA dosage was observed on erythrocyte AChE activity.

The wool was not manually removed. In the sheep treated with 20 or 30 mg of CPA per kilogram, the wool came off in the course of the sheep's activities. At 10 mg per kilogram, the wool perceptibly loosened, but generally did not come off during the observation period.

Discussion

The data presented show that sheep respond to CPA at dosages of 10 to 30 mg per kilogram of body weight by a brief, transitory decline of both plasma and erythrocyte AChE activity.

This response, in general, is similar to that observed in man following a single dose of CPA (Limongi et al., 1966; Wolff, 1965, 1966; Priesching et al., 1967). In man, however, the degree of the depression is directly related to CPA dosage. To the extent that a consistent dosage dependence was observed in sheep in the present study, the depression was inversely related to dosage. No explanation or rationalization of such an inverse relationship is apparent.

No physical signs of depressed AChE activity, such as lethargy, incoordination and excessive salivation, were observed in the sheep of the present study, or in those used in previous CPA studies in this laboratory (Dolnick et al., 1969; Reynolds et al., 1972a,b). No observations have been reported to our knowledge in younger sheep, but older sheep (mature ewes) can withstand a considerably greater depression of erythrocyte AChE activity for a considerably longer time than those observed in the present study without displaying the signs of depressed AChE activity (Reynolds et al., 1971). Physical signs of depressed AChE activity are not, therefore, a part of the overall response of sheep to CPA at defleecing dosages.

It would be useful to be able to identify sheep that have been treated with CPA before that fact is at least presumptively evident from the looseness of the fleece itself, or from the complete absence of the fleece, if it has been removed, or from the appearance of the wool regrowth, on which shearing marks are characteristically absent. Such a means of identification as applied to live sheep, i.e., as distinct
TABLE 1. EFFECT OF CPA DOSAGE AND TIME AFTER CPA TREATMENT ON PLASMA AND ERYTHROCYTE ACETYLCHOLINESTERASE ACTIVITY

<table>
<thead>
<tr>
<th>Days after CPA treatment</th>
<th>Plasma CPA dosage, mg/kg</th>
<th>Erythrocyte CPA dosage, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>.070</td>
<td>.074</td>
</tr>
<tr>
<td>1</td>
<td>.058</td>
<td>.068</td>
</tr>
<tr>
<td>5</td>
<td>.052c</td>
<td>.062</td>
</tr>
<tr>
<td>9</td>
<td>.024b</td>
<td>.047b</td>
</tr>
<tr>
<td>13</td>
<td>.075</td>
<td>.077</td>
</tr>
<tr>
<td>17</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>21</td>
<td>.063</td>
<td>.059c</td>
</tr>
<tr>
<td>26</td>
<td>.060</td>
<td>.058c</td>
</tr>
</tbody>
</table>

*Each tabulated value is the average of nine observations. Standard deviations, computed from least squares estimates of $e_{ijk}$ and $e_{ijk}$ in the analyses of variance were ± .046 and ± .014 Δ pH/hr for the red cell enzyme and ± .013 and ± .010 for the plasma enzyme, respectively.

bSignificantly different (P<.01) from the pretreatment value by Dunnett’s (1955) two-sided comparison.

Significantly different (P<.05) from the pretreatment value by Dunnett’s (1955) two-sided comparison.

dThe day 17 plasma samples were not assayed until 3 days after collection. The value seemed low, suggesting that the enzyme was not stable in storage, and the data were omitted.

from the problems of possible residues of CPA and its metabolites in meat and other products, might even be a presumptive one, i.e., one not necessarily specific for CPA. But it would need to be rapid, simple and inexpensive. The AChE determination, as applied in the present study, is simple and rapid to perform and might be simplified further for field use. It is, of course, not at all specific for CPA.

Plasma AChE levels reached their lowest values on day 9 after CPA treatment (table 1). By this time, except perhaps at lower dosages, CPA treatment is generally evident from the wool itself.

The depression of the erythrocyte enzyme, on the other hand, is evident by day 1 after CPA treatment, when it would be useful, and evidently recovers over several days thereafter. The magnitude of the erythrocyte enzyme depression is of the order of .030 to .035 Δ pH/hr (table 1). The standard deviation among sheep in erythrocyte AChE activity, computed from the least squares estimate of $e_{ijk}$ in the analysis of variance, was ± .046 Δ pH/hr. Thus, the depression of erythrocyte AChE activity in response to CPA administration, averaged over several sheep, is less than one standard deviation representing the variation in activity of the enzyme from one sheep to another. Thus, one could not expect to distinguish successfully the depression of erythrocyte AChE activity resulting from CPA treatment from normal variation in activity from one sheep to another. The conclusion is, therefore, that sheep treated with CPA cannot be identified as such by reference to their plasma or erythrocyte AChE activities.

LITERATURE CITED


Reynolds, Paul J., E. H. Donick, G. M. Sidwell and C.

