Effects of Sub-Lethal Dose of $\gamma$-Irradiation on Levels of Acid Phosphatase in Cerebellum of Pigeons

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The changes in the activities of acid phosphatase in the sham-irradiated and $\gamma$-irradiated cerebellum of pigeons have been studied both biochemically as well as histochemically after 400 rads. The specific activity of acid phosphatase decreased significantly after 48 h and 72 h of irradiation. The histochemical observations following total body irradiation confirmed the results obtained by quantitative biochemical studies.

INTRODUCTION

An increase in enzymatic activity or metabolic rate has been observed as characteristic response of many types of tissue and cells following ionizing radiation injury. Most of the investigators concerned with effects of ionizing radiations on lysosomes, have employed technique for measuring levels of lysosomal enzymes in various subcellular fractions of homogenates prepared from lymphoid organs, liver and small intestine\(^{1-4}\). These studies have shown that following whole body exposure to irradiations, the specific and organ content are increased of some\(^{1-4}\) but not all lysosomal enzymes\(^{5-6}\). Recently, Aikman and Wills\(^7\) have confirmed the results of these earlier studies using quantitative histochemical technique.

The central nervous system (CNS) has been often considered as radioresistant organ. But the results from biochemical studies carried out on brain of adult whole body irradiated animals showed that CNS may contain a population of radiosensitive cells\(^8\). There is paucity of information on lysosomal enzymes of brain specially with low doses of irradiations. Metabolically, birds form a very closely related group with mammals being a homeothermic animal. However, so far no serious attempt has been made to study the biochemical and histochemical changes on lysosomal enzymes in brain of pigeons. The present study describes the biochemical and histochemical changes on acid phosphatase in cerebellum of pigeons subjected to total body sub-lethal dose (400 rads) of $\gamma$-irradiation.
MATERIALS AND METHODS

Pigeon (Columbia livia intermedia Strickland) of both sexes weighing approximately 260–290 g were utilized in the present study. The pigeons were exposed to total body γ-irradiation with Cobalt–60 Unit. The radiation factors were (1.17±1.33) MeV and dose rate 110 rad/min. Pigeons were irradiated individually from the dorsal side with sub-lethal dose of 400 rads. The other details of radiation procedure is described elsewhere. The adult healthy, irradiated pigeons were decapitated at different intervals and sham-irradiated pigeons were examined simultaneously as control. Cerebellum was removed, immersed in ice-cold 0.25 M sucrose and homogenised with glass homogenizer. The quantitative estimation of acid phosphatase (EC 3.1.3.2., Orthophosphoric monoester phosphohydrolase) was performed according to the method of Roels et al. Total lysosomal acid phosphatase activity was determined after treatment of homogenates with 0.1% Triton X-100. Inorganic phosphate and protein were estimated according to the methods of Fiske and SubbaRow and Lowry et al. respectively.

Holt's adaptation of the method described by Gomori was used at pH 5.0 for histochemical localization of acid phosphatase. The fresh frozen sections approximately of 15 µm were cut on freezing microtome and were fixed with calcium-formal for 24 hours. Some frozen sections incubated in medium containing sodium fluoride to give final concentration of 5 mM and in which the enzyme was inactive. Thus, it was possible to allow for any nonspecific activity which was not due to acid phosphatase.

RESULTS

The results are presented in Table 1 showing specific as well as total activity of acid phosphatase of sham-irradiated (control) and γ-irradiated (1 h, 24 h, 48 h, 72 h) cerebellum of pigeons. A minimum of six sets were performed from six animals for tissue and treatment. The statistical significance of differences between activities of control and irradiated pigeons was determined by Student's 't' test. The specific activity of acid phosphatase decreased significantly after 48 h (P<0.05) and 72 h (P<0.02) of irradiation. On the other hand, total activity of acid phosphatase did not decrease significantly.

The histochemical study of cerebellum of sham-irradiated pigeon showed nuclear as well as cytoplasmic localization of acid phosphatase (Fig. 1). After 48 h of irradiation decreased activity of acid phosphatase was observed (Fig. 2). At 72 h post-irradiation of 400 rads, acid phosphatase decreased significantly as no nuclear (replaced by granular structure) and less cytoplasmic stain was visible (Fig. 3). The decrease in acid phosphatase was clearly demonstrated in the present study by comparing the irradiated cerebellum sections with the control sections incubated for same period. These histochemical observations confirmed our biochemical data as mentioned in the previous paragraph.
EFFECTS OF SUB-LETHAL DOSE OF γ-IRRADIATION

Table 1

<table>
<thead>
<tr>
<th>Post-irradiation time (hours)</th>
<th>E. S. A.(^a)</th>
<th>E. T. A.(^b)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>10.50±1.50</td>
<td>52.0±5.3</td>
</tr>
<tr>
<td>1 hour</td>
<td>9.00±0.30</td>
<td>50.0±5.4</td>
</tr>
<tr>
<td>24 hours</td>
<td>8.40±0.83</td>
<td>48.0±4.7</td>
</tr>
<tr>
<td>48 hours</td>
<td>7.90±0.78*</td>
<td>47.0±4.6</td>
</tr>
<tr>
<td>72 hours</td>
<td>7.10±0.70**</td>
<td>45.0±4.9</td>
</tr>
</tbody>
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\(^a\)—Enzyme specific activity. \(^b\)—Enzyme total activity.

Significant at \(P<0.02\)—(**). \(P<0.05\)—(*).

Fig. 1. Normal pigeon brain (cerebellum) showing localization of acid phosphatase. Note the nuclear (arrow) and cytoplasmic localization. (×400)

DISCUSSION

Bacq and Alexander\(^{16}\) postulated that major cause of cell death following irradiation is the release of hydrolytic enzymes from damaged lysosomes, since these enzymes attack and destroy vital cellular structures. It is known that lysosomes from different cell types or even from the same tissue vary greatly in their susceptibility to damage by irradiation\(^{17}\). Although the activation of lysosomal enzymes in tissue with cells in extensive interphase death is well documented, but information on lysosomal enzymes in tissues other than lymphoid organs is scanty and often contradictory. In the present study, even at sub-lethal dose (LD 50/30 of pigeon is 950±50 rads)\(^9\)
Fig. 2. & Fig. 3. Effects of total body γ-irradiation (400 rads) on acid phosphatase activity in cerebellum of pigeon. Section prepared 48 h after irradiation showing decreased activity of acid phosphatase as few nuclear structure was visible (Fig. 2.) (×400). After 72 h of irradiation the acid phosphatase activity decreased significantly and no nuclear structure (replaced by granular structure) was visible (arrow) (Fig. 3.) (×400).

the specific activity of acid phosphatase decreased significantly after 48 h and 72 h of irradiation. On the other hand, total activity decreased insignificantly. There have been few published reports available on effects of radiation on lysosomal enzymes
specially with brain. Gerber et al. have shown that the levels of acid-phosphatase enhanced in the rat brain after 1 to 3 months of 2 krad irradiation. Histochemically, Manocha and Olkowski have also reported that in mice the activity of acid phosphatase increased significantly after 4 hours of 636 R gamma-rays. On the other hand, Kocmierska-Grodzka and Gerber have shown that the activity of acid phosphatase decreased in brain of rats after 500 and 2000 X-rays. It is also interesting to note here that in our previous study of pigeons the levels of three lysosomal enzymes viz. acid phosphatase, ribonuclease-II and deoxyribonuclease-II increased significantly in liver and spleen following 300 rads of $\gamma$-irradiation. However, in the present investigation, the cerebellum acid phosphatase decreased significantly after irradiation. We have confirmed our biochemical observations with the histochemical studies. It revealed that after 48 h and 72 h post-irradiation, the reaction of acid phosphatase decreased significantly. The decrease in specific activity of acid phosphatase may be probably due to release of inhibitors or it might be due to the decreased metabolic synthesis of phosphate esters owing to the unavailability of orthophosphate in the brain cells after irradiation. The reason for insignificant decrease in total activity of acid phosphatase in the present study could be due to the increase of total protein in homogenates after total body 400 rads. However, more work remains to be done for understanding of actual mechanism responsible for inhibition of acid phosphatase after irradiation in brain.

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REFERENCES


