Increased mast cells in paraquat lungs with special reference to their staining characteristics

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Increased Mast Cells in Paraquat Lungs with Special Reference to Their Staining Characteristics

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The staining properties of mast cells in the lungs of two autopsy cases of paraquat intoxication were studied. Both patients ingested a fatal dose of paraquat, and died of severe respiratory and renal failure in about two weeks in spite of receiving intensive therapies. The histological study revealed various changes in the lungs that had been described previously as "paraquat lung". In normal lungs the mast cells were mainly distributed in the connective tissues around bronchi and large vessels, whereas the increased mast cells in the paraquat lungs were mainly seen in the alveolar septa. The staining properties of the increased mast cells were similar to those of mucosal mast cells, and different from those of the connective tissue mast cells. However, among the increased mast cells in the alveolar septa, there were a few mast cells which also contained some granules seen in the connective tissue mast cells. We speculate that the increased mast cells were immature ones of the connective tissue mast cells around bronchi and large vessels.

Key words : mast cell, paraquat lung, histochemistry

Introduction

Paraquat is an effective herbicide but has a strong toxicity. Increasing cases of fatalities in man due to its accidental, suicidal or homicidal ingestion have been described since the first report by Bullivant(1). It causes injuries to lungs, kidneys and other organs, but, the damage to lungs, which results in severe fibrosis, is known to be most fatal. Such a condition is called "paraquat lung" (2).

While it has been reported that the pulmonary parenchymal mast cells increase in number in some fibrotic disorders (3), there have been no reports on the mast cells in the fibrotic lesions in paraquat lung. In this report we describe the distribution and histochemical characteristics of increased mast cells found in two cases of paraquat lung, and briefly discuss mast cell heter-

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ogeneity in human lungs.

Case Reports

Case 1 : A 42-year-old woman, who had a history of depression, ingested an unknown amount of Grammoxone (paraquat 20% solution) and then tried to hang herself but failed to. She vomited a few hours later and was taken to a clinic next day complaining of ulceration in the oral cavity. Three days later she was admitted to the University Hospital in an alert state. Physical examination disclosed anemia and hyperventilation. The laboratory findings were as follows : GOT, 369 u ; GPT, 244 u ; LDH, 1186 u ; serum amylase, 1180 u ; total bilirubin, 2.6 mg/dl ; BUN. 93 mg/dl ; serum creatinine, 8.1 mg/dl ; PaO_2, 18.3 mmHg ; and $PaCO_2$, 24.3 mmHg. She died of severe respiratory and renal failure in 12 days in spite of receiving intensive therapy such as hemodialysis and control ventilation.

Case 2 : A 44-year-old man ingested about 70 ml of Grammoxone for the purpose of committing suicide, and vomited 15 minutes later. He received a gastric wash in a clinic. Three days from then he was admitted to the University Hospital in a drowsy state. Physical examination revealed jaundice, oral erosion and rales of the chest. The laboratory data were as follows : GOT, 95 u ; GPT, 133 u ; LDH, 662 u ; serum amylase, 221 u ; total bilirubin, 8.2 mg/dl ; BUN, 118 mg/dl ; serum creatinine, 7.3 mg/dl ; PaO_2, 27 mmHg ; and $PaCO_2$, 27 mmHg. He received hemodialysis and control ventilation,but died of severe respiratory and renal failure in 15 days.

Materials and Methods

All organs were fixed in 10% formalin. In order to study the mast cells, some pieces of the lung of Case 2 were fixed in Carnoy's fluid : ethanol-chloroform-acetic acid (6 : 3 : 1 by vol.). All tissues were embedded in paraffin and prepared for light microscopic observation. For the lung specimens, we used the following staining methods : (1) H. E., (2) azan, (3) toluidin blue (pH 4.0), (4) alcian blue (pH 0.5 or 2.5)-periodic acid Schiff (PAS), (5) alcian blue (pH 0.5 or 2.5) -PAS-avidin-biotin-peroxidase complex (ABC) method (4), (6) PAS-ABC method and (7) berberine. For control studies, specimens of histologically normal lung and skin from other autopsy cases were fixed in Carnoy's fluid and processed as described above.

Results

Autopsy findings

Case 1 : The lungs (690/830g) were hard in consistency and markedly decreased in air content. The cut surface was reddish-brown in color. Increased diffuse fibrosis was also noticed.

Microscopically, there was diffuse and widespread hemorrhage. Fibrosis was observed in the alveolar septa, intraalveolar spaces and subpleural area. Hyaline membrane formations were also found in some alveoli. Clusters of metaplastic squamous epithelial cells were seen in some areas, but no distinct keratinization was found in them (Fig. 1, 2). There were also centrolobular necrosis of the liver,acute pancreatic necrosis and degeneration of proximal convoluted tubules of the kidneys.

Case 2 : The lungs (710/900g) were elastic hard in consistency and markedly decreased in air content. The cut surface was reddish-brown in color. The bronchi were filled with blood coagula. Increased diffuse fibrosis was noticed.

Microscopic study showed scattered focal hemorrhages, marked interstitial fibrosis and emphysema. Some alveoli were filled with loose connective tissue, suggesting the development of intraalveolar fibrosis. Hyaline membranes were

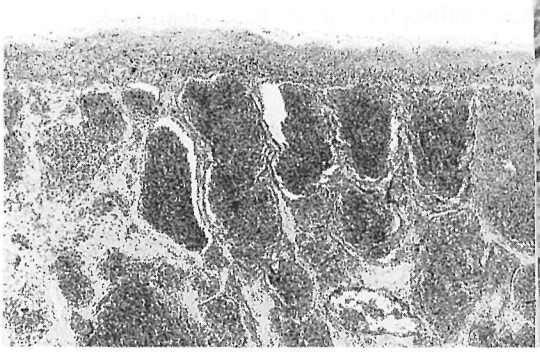


Fig. 1. Diffuse hemorrhage in the alveoli and subpleural area. Case 1. H. E. (×100)

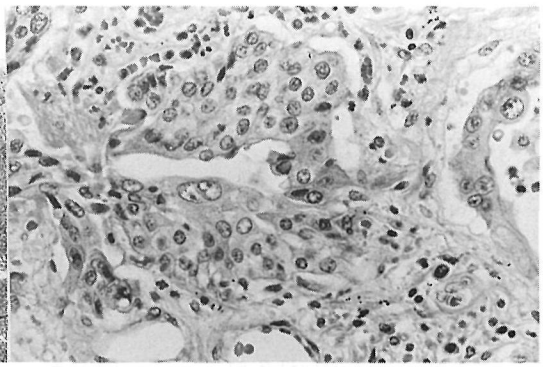


Fig. 2. Clusters of metaplastic squamous epithelial cells. Case 1. H. E. (×100)

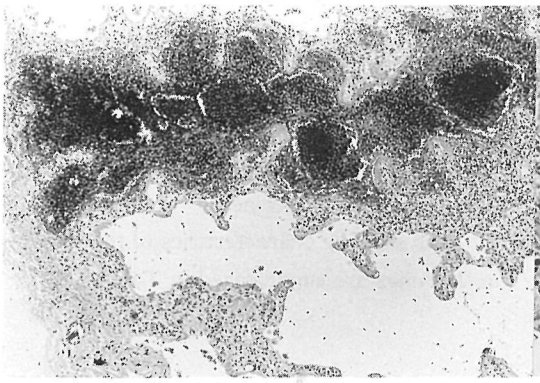


Fig. 3. A focal hemorrhage, emphysema and subpleural fibrosis (right upper portion). Hyaline membrane and interstitial fibrosis are also seen. Case 2. H. E. (×100)

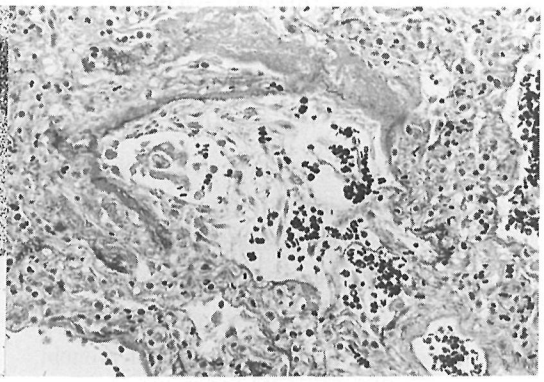


Fig. 4. An alveolus filled with loose connective tissues. Case 2. Azan. (×100)

also frequently observed on the surface of alveolar septa (Fig. 3, 4). Regenerating bronchial epithelium and metaplastic squamous cells were often observed. Other pathologic findings were petechial hemorrhages in many organs such as the gastrointestinal tract, the pericardium, the renal pelvis and the thyroid. There were also acute necrotic pancreatitis, centrolobular cholestasis of the liver, tubular degeneration of the kidneys and cardiomegaly.

Histochemical studies of mast cells in the lung

In control lungs with no detectable pathologic changes, the mast cells were mainly distributed in the connective tissues around bronchi and large vessels, while there were few mast cells in the alveolar septa.

In the lungs of Case 1 which were fixed in formalin solution and stained with toluidine blue, mast cells were found in the connective tissues around bronchi and large vessels, and also in the alveolar septa (Fig. 5).

In Case 2, we examined the staining properties of mast cells in sections fixed in Carnoy's fluid. At first, abundant mast cells showing metachromasia with toluidine blue were observed in the thickened alveolar septa as well as in the connective tissues around bronchi and large vessels. The mast cells in the septa tended to be stained red-purple, while those in the connective

Table 1. Staining properties of mast cells in the sections fixed in Carnoy's fluid. Case 2.

	Mast cells in the alveolar septa	Mast cells in the connective tissues around bronchi and large vessels
toluidine blue (pH 4.0)	+ (red purple)	+ (purple)
alcian blue (pH 0.5)	++	++
alcian blue (pH 2.5)	+	+
berberine	−	+ (fluorescent)
ABC method	−	+ (brown)
alcian blue (pH 0.5) −ABC	++ (blue)	+++ (blue+brown)
alcian blue (pH 2.5)−ABC	+ (blue)	++ (blue+brown)

tissues around bronchi and large vessels appeared purple. These cells in both regions were also stained with alcian blue as had been observed in serial sections. With the ABC method and berberine staining, ABC positive mast cells were found mainly in the connective tissues around bronchi and large vessels, and fluorescent mast cells with berberine were also mainly in these areas. In the double stainings by alcian blue-ABC method, most mast cells in the alveolar septa were positive only with alcian blue, while most mast cells in the connective tissues around bronchi and large vessels were stained with ABC as well as alcian blue. The latter cells were larger than the former in size. Among the increased mast cells in the alveolar septa, we observed a few which also contained granules positive with ABC(Fig. 6−9). Even in formalin-fixed sections, abundant mast cells showing metachromasia with toluidine blue were also observed in the thickened alveolar septa as well as in the connective tissues around bronchi and large vessels. In these sections, no longer were any of the mast cells stained with alcian blue.

In addition, all mast cells found in the histologically normal skin were stained strongly with both alcian blue and ABC methods, and also fluoresced with berberine.

The staining characteristics of the mast cells in the lungs are summarized in Table 1.

Discussion

Interstitial fibrosis was observed in both cases. In Case 2, the development of intraalveolar fibrosis accompanied by emphysema of adjoining air spaces was seen. This type of fibrosis was reported to be one of the characteristics of 'paraquat lung' (5),(6).

We confirmed that few mast cells are observed in the alveolar septa of histologically normal lungs. Therefore we can say that mast cells in the alveolar septa of the present paraquat lungs are the ones which had increased in this condition. Kawanami et al. (3) also reported that there were few mast cells in the alveolar septa of histologically normal lungs, and that mast cells increased in number in these areas of fibrotic lungs such as in those of cases with idiopathic interstitial pneumonia, sarcoidosis and some collagen diseases.

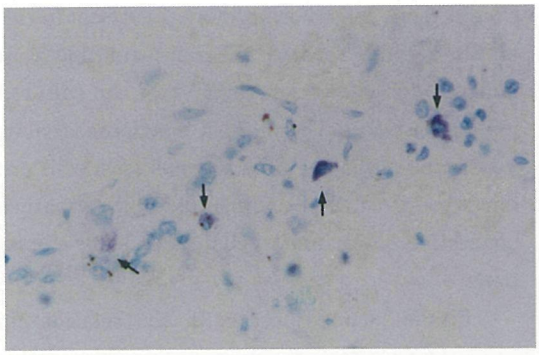


Fig. 5. Mast cells in the alveolar septa (arrows). Case 1. Toluidine blue (pH 4.0). (×200)

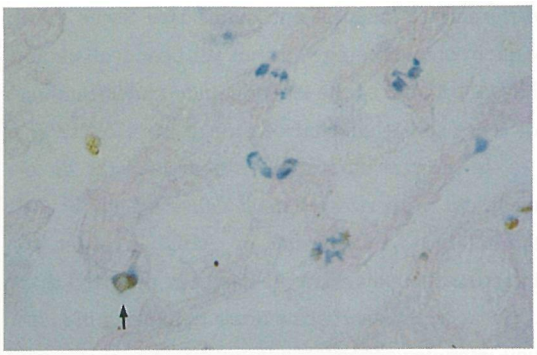
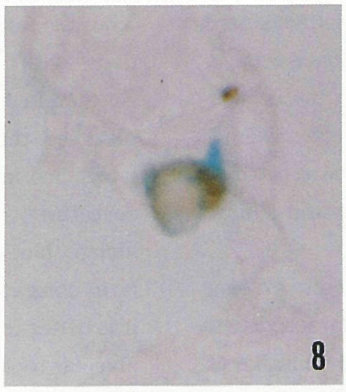


Fig. 6. Mast cells in the alveolar septa. One mast cell (arrow) is stained with both alcian blue and ABC method. Case 2. Alcian blue (pH 2.5)-ABC double stainings. (×200)



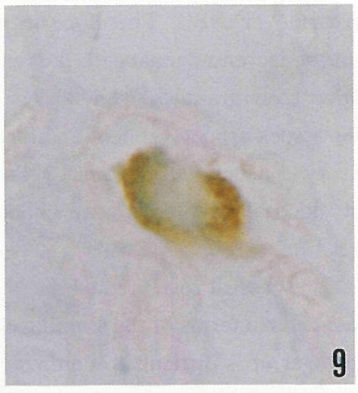


Fig. 7. Higher magnification of Fig. 6 showing mast cells positive only with alcian blue. Alcian blue (pH 2.5)-ABC double stainings. (×1000)

Fig. 8. Higher magnification of Fig. 6 showing a mast cell positive with both alcian blue and ABC methods. Alcian blue-ABC double stainings. (×1000)

Fig. 9. Mast cells in the bronchial wall strongly positive with ABC methods as well as alcian blue. Case 2. Alcian blue-ABC double stainings. (×1000)

Since the first report by Enerbäck (7), two types of mast cells have been described in rats ; the mucosal mast cell in the lamina propria of intestine, and the connective tissue mast cell in the skin and serosal cavities. These types can be differentiated by means of morphology(8), pharmacology(9)and biochemistry(10). A similar diversity of human mast cells has also been reported (11), (12), (13). Befus et al. (11) found that human mast cells, which corresponded to the rat mucosal mast cells, were present in the lamina propria of the small and large intestine. However, there were also the connective tissue mast cells in these sites. Therefore, in human beings, the mucosal and connective tissue mast cells were located in the same anatomical location. In human lungs, both types of mast cells have been reported (12), (13). However, the criteria to

differentiate mucosal and connective tissue mast cells in human beings has not yet been well established, because of the less adequate understanding of these cells than that of the rat mast cells.

In the present study, we examined the histochemical properties of mast cells, especially the stainability to berberine or ABC, in order to examine their diversity. It has been reported that ABC as well as berberine binds to heparin in mast cell granules (14), (15). In normal human lungs, most mast cells were distributed in the connective tissues around bronchi and large vessels (3), and most of them were positive for berberine and/or ABC (16). In the lungs of present cases, most of the increased mast cells were found in the alveolar septa, and these cells were usually negative for berberine or ABC. Therefore it was suggested that the increased mast cells did not have heparin within their granules, while those in the connective tissues around bronchi and large vessels did. In rats, connective tissue mast cells have heparin, but mucosal mast cells have chondroitin sulfate di-B (17).

The present results show the diversity of lung mast cells in terms of the staining characteristics. However, it is difficult to confirm the lineage of these cells only from the histochemical studies, for it has been suggested that the staining characteristics of mucosal mast cells are similar to those of immature connective tissue ones(18). For instance, by the alcian blue-safranin double stainings, mucosal mast cells stained with alcian blue, whereas connective tissue mast cells stained with safranin (4) (7). On the other hand, the immature connective tissue mast cells in rat embryos were not stained with safranin but with alcian blue, although the affinity shifted to safranin with their development (19). Matsui (20) also reported the same histochemical transition in the regenerating process of mast cells in rat ascitic fluid. Thus, at present, there is no reliable method and/or criteria to distinguish the mucosal mast cells from immature connective tissue ones except the location of these cells. Furthermore, Levi-Schaffer et al. (21) recently reported the transformation from mucosal to connective tissue type in cultured mouse mast cells. Therefore, it is supposed that at least some of the so-called mucosal mast cells are immature mast cells, and may develop to mature connective tissue mast cells under some conditions.

In the human mast cells, the relationship between the mucosal and immature connective tissue ones is likewise obscure. Nevertheless, in the present study, a few number of mast cells in the alveolar septa were alcian-blue positive and also had some ABC positive granules. The ABC reaction of these cells was usually less intense than that of preexisting peribronchial mast cells. Thus the former cells can be regarded as intermediate with respect to staining characteristics. Further, in *Nippostrongylus*-infected rats, we have observed that the proliferated lung mast cells which had initially exhibited alcian blue$^+$/ABC$^-$/berberine$^-$, shifted their staining properties to alcian blue$^+$/ABC$^+$/berberine$^+$under the long-term observation (22). Thus, in the present case, it is better to consider that these mast cells in the alveolar septa and in the peribronchial connective tissues belong to the same cell lineage rather than to different subsets of cells, and it is very likely that the increased mast cells in the alveolar septa of paraquat lungs, which have the same staining characteristics as the so-called mucosal mast cells, are immature ones which will develop to connective tissue ones with time.

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パラコート肺における肥満細胞の増加と染色特性

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我々はパラコート中毒の2剖検例における肺肥満細胞の染色特性について検索した．いずれも致死量のパラコートを服毒し約2週間の経過で強い呼吸不全と腎不全で死亡した．組織学的には「パラコート肺」として既に報告されてきたような肺の線維化，出血，気腫性変化等が観察された．正常肺では肥満細胞は主として気管支壁や血管周囲の結合組織に存在するが，パラコート肺において増加した肥満細胞は主として肺胞壁に見られた．増加した肥満細胞の染色特性はいわゆる粘膜肥満細胞と類似し，結合組織肥満細胞とは異なっていた．しかし肺胞壁に増加した肥満細胞の中に，結合組織肥満細胞に見られた顆粒をも含む肥満細胞が散見された．我々は増加した肥満細胞は気管支壁や血管周囲の結合組織肥満細胞の幼若型であると考えている．