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Effect of Acrylonitrile on the Procoagulant Activity of Rat Lung

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The deposition of fibrin in alveolar spaces is a pathologic feature commonly observed in acute lung injury (Saldeen, 1967 and Bachofen and Weibel, 1977). This observation may be of pathophysiologic significance because fibrin and its degradation products have been implicated in contributing to pulmonary inflammation through several mechanisms (Cuterman et al, 1977; Malik et al, 1979).

Acrylonitrile (VCN) is an important chemical which is widely used in the production of synthetic fibres, rubbers and plastics. It has also been used as a fumigant for grain (IARC, 1979). Brieger et al (1952) reported respiratory distress in rhesus monkeys exposed to a lethal concentration of VCN. Inflammation of the pulmonary system accompanied by an inflammatory exudate into bronchial lumen occur in rats following long term inhalation exposure to VCN (Knobloch et al, 1972).

Szabo et al (1980) showed that intravenously injected VCN caused 100% incidence of adrenal hemorrhage and necrosis. Electron Microscopy studies showed damage to the vascular endothelium in the adrenal cortex associated with retrograde embolisation of medullary cells and cell fragments into the cortical capillaries. Platelet aggregation and fibrin precipitation at the sites of discontinuous vascular endothelium were evident.

Pathways of inflammation and coagulation are closely interrelated at both the humoral and cellular levels (Limmerman et al, 1977 and Broze, 1982). Mononuclear phagocytes have been shown to display procoagulant

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activity (PCA) which is defined as the ability of a cell or cell products to accelerate the conversion of fibrinogen to fibrin.

Alveolar macrophages are generally thought to serve as scavengers of fibrin in lung injury because these cells phagocytose particulates and can induce fibrinolysis. (Bang et al, 1981). If AM also exhibit PCA then it is possible that these cells participate in the pulmonary deposition of fibrin as well. It appeared more interesting to us to study the procoagulant activity of alveolar macrophages, and broncho alveolar lavage (BAL) fluid in rats following exposure to acrylonitrile.

MATERIALS AND METHODS

Acrylonitrile was purchased from BDH chemicals, Bombay. Human brain thromboplastin was obtained from Sigma Chemical Company, USA. Male wistar rats were purchased from Forensic Sciences Department, Madras and kept in a room maintained at constant temperature. Rats were placed in a 53 liter rochester type inhalation system, assembled as described by P'an and Jeiger (1970) with some modifications. The VCN concentration in the chamber was calculated by the formula,

$$C = \frac{Fd \times \frac{T}{273} \times \frac{760}{p} \times 22.4 \times 10^6}{Ff \times mw}$$

where,

C = VCN concentration in ppm
F = VCN liquid delivery rate in ml/min
d = density of VCN
T = Laboratory temperature
P = Pressure of the system in mm of Hg
Ff = Total chamber air flow in litres/min and
mw = molecular weight of VCN.

Six rats were exposed to VCN chamber concentrations of 100 ppm for 5 hours a day for five days continuously. Blood of animals was drained by cutting the jugular vein. The thoracic cavity was opened and lungs, heart and trachea were removed enbloc. Trachea was cannulated. The lungs were lavaged 6 times with 5 ml cold, sterile, isotonic saline. The lavage effluent was collected in graduated centrifuge tubes kept in 250 x g for 10 min. Aliquots of the supernatant were

removed. Cells were counted in a hemocytometer chamber and viability was determined by trypan blue exclusion. Level of PCA in macrophage and BAL fluid was observed on day 1, 3, 5, 7, 14 and 28 days after VCN exposure. Procoagulant activity (method of Hvatum and Prydz, 1966) was determined in a one stage clotting system consisting of 0.1ml lavage fluid or $0.1x10^6$ cell lysate in RPMI 1640, 0.1ml citrated human plasma and 0.1ml $Cacl_2$ (30mM). Clotting times were determined in duplicate, by the manual tilt method and the mean of two measurements was used to calculate thromboplastin unit. Standard curves for thromboplastin activity were established by using a standard human brain thromboplastin preparation.

RESULTS AND DISCUSSION

Alveolar macrophage procoagulant activity was elevated from day 1 to 14 post exposure (Fig. 1). On the 28th day the levels returned to normal.

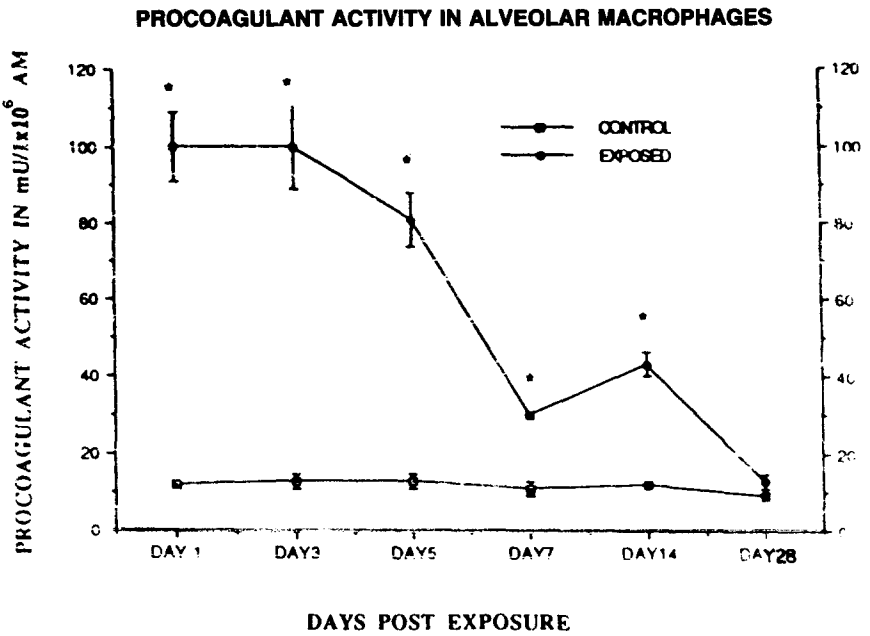

Figure 1 Procoagulant activity in alveolar macrophages obtained at various time intervals. Values are Mean ± S.D. for 6 rats. Statistically significant variations when compared to control are expressed as, * $P < 0.001$.

Lavage PCA level was found to be unaltered for first 5 days. An elevation was observed at 14th and 28th day post exposure (Fig.2).

There is a close association, although a poorly defined cause - effect relationship, between acute lung injury and abnormalities of the coagulation system. (Sitrin et al, 1983). Of particular interest are the recent observations that the formation of fibrin networks on the surfaces of stimulated peritoneal macrophages impaired their mobility and that macrophage-associated PCA appeared to promote the formation of these fibrin networks (Hopper et al, 1981). Augmentation of surface PCA may be a mechanism by which macrophages are rendered immobile and become sequestered at the sites of inflammation, where, in turn, they can cause further damage by the elaboration of proteases and toxic oxygen metabolities. Our studies on acrylonitrile showed fibrin network formation in the lung following inhalation exposure (Bhooma and Niranjali Devaraj, unpublished data).

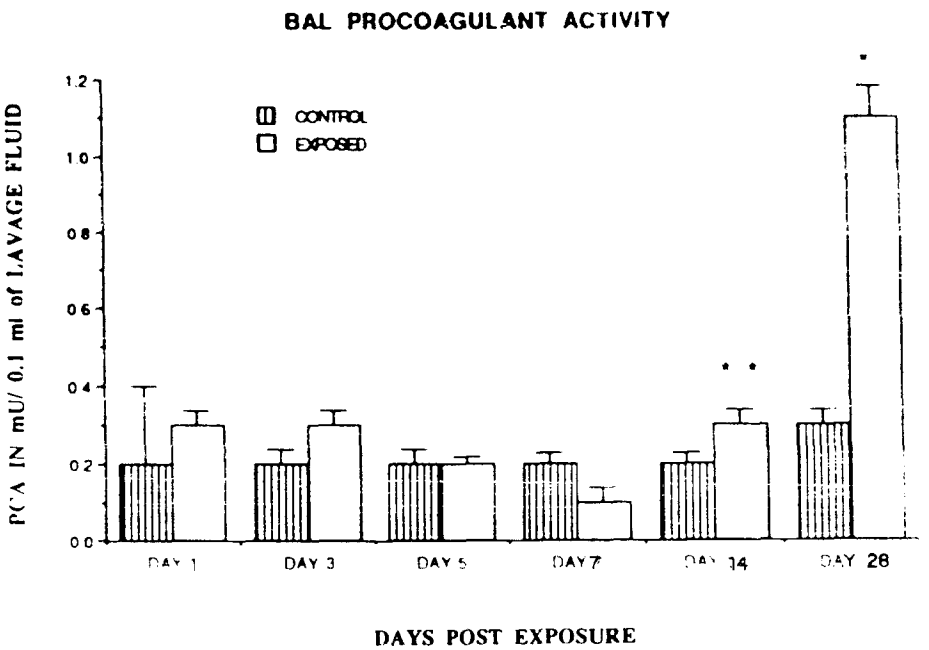

Figure 2 Procoagulant activity of BAL fluid obtained at various time intervals in control and test animals. Values are Mean ± S.D. for 6 rats. Statistically significant variations when compared to control are expressed as, * $P < 0.001$, ** $P < 0.01$.

macrophagic PCA level upto day 14 post exposure and a decrease on day 28 observed in this study shows the dynamic interplay between procoagulant activity and fibrinolytic factors.

An increase in BAL-PCA may be related to increased permeability of alveolar capillary interface. Lyberg et al (1990) showed that PCA in BAL is derived from alveolar macrophages. A significant increase observed in BAL-PCA at 28th day post exposure could have been due to the relase of macrophagic PCA into BAL facilitated by fibrin degradation products.

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