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Effect of N-Acetylcysteine on Fibrin Deposition in the Rat Lung due to Intravascular Coagulation

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ABSTRACT

Intravascular coagulation was induced in rats by i.p. injection of a fibrinolysis inhibitor, tranexamic acid (AMCA, 200 mg/kg B.W.), and i.v. injection of bovine thrombin (500 NIH units/kg B.W.) and the fibrin deposition in the lungs was assessed with ^{125}I-labelled fibrinogen.

Treatment with N-acetylcysteine (NAC) partly prevented the deposition of fibrin in the lungs, and the disappearance of fibrinogen from the blood, but did not seem to influence the elimination of fibrin in the lungs. The results indicate that NAC may counteract pulmonary damage in this experimental model, by inhibiting intravascular fibrin formation.

INTRODUCTION

N-acetylcysteine (NAC) has been shown to have preventive or therapeutic effects on two types of experimental pulmonary damage (3,13).

Bernard et al (3) claimed that NAC, as a free radical scavenger, has a favourable effect in endotoxin-induced ARDS (adult respiratory distress syndrome), in sheep.

Wegener et al (13) found that in another ARDS model in the rat, NAC counteracted the pulmonary damage by diminishing the increase in lung weight and reducing of the microscopically observed interstitial and alveolar oedema. In addition, after administration of NAC less fibrin was found in precapillary arterioles by a semiquantitative method , suggesting that NAC may decrease the formation of fibrin or increase its elimination from the lungs.

The aim of this study was to quantify the fibrin deposition in the rats with pulmonary damage treated and not treated with NAC, using a method employing ^{125}I-labelled fibrinogen (10), and to determine whether NAC could diminish the trapping of fibrin in the lungs or increase its elimination.

MATERIAL AND METHODS

Animals. Male Sprague-Dawley rats (ALAB, Sollentuna, Sweden), weighing 195 to 210 g, were used. They were allowed free access to food and tap water.

Substance. Human fibrinogen KABI (grade L) and tranexamic acid (trans-4-aminomethyl-cyclohexane-carboxylic acid, abbreviated to AMCA were kindly supplied by Kabi-Vitrum AB, Stockholm, Sweden).

Bovine thrombin (Topostasine^{R}, Hoffman-La Roche, Switzerland) was used.

N-acetylcysteine (Fluimucil^{R}, 100mg/ml aqueous solution pH 6.5, Zambon SpA, Milano-Vincenza, Italy) was generously supplied by the producing company.

Pentobarbital (Inactin^{R}, Byk-Gulden, Konstanz, FRG) was used for anaesthesia.

Human fibrinogen was labelled with ^{125}I, using the iodine monochloride method (6). As human fibrinogen has been shown to give the same result as rat fibrinogen in the present context, and has greater stability (2), it was chosen for these experiments.

Procedure. Labelled fibrinogen solution (2.5 ml/kg body weight B.W.) containing about 2.5 mg of protein and 518 KBq/ml was injected into a tail vein in the rat 24 h before the experiment took place. This procedure was carried out under light ether anaesthesia.

About one hour before the experiment began, 0.5 g of Inactin was dissolved in 10 ml of sodiumchloride (9 mg/ml, isotonic solution) and 2.5 ml/kg (B.W.) was injected intraperitoneally (i.p.). Sodiumchloride was abbreviated to saline. To ensure a free airway, all animals were tracheostomized. During the experiment the body temperature was kept constant at 38 ^{o}C with an infrared lamp. Pulmonary damage was induced by an i.p. injection of AMCA, 200 mg/kg B.W., followed 10 min later by a 10-minute infusion of bovine thrombin, 500 NIH units/kg B.W., given into a tail vein by means of an infusion pump. Thrombin administration must be combined with AMCA in order to cause pulmonary damage similar to that seen in patients with posttraumatic pulmonary insufficiency (1,6,7). NAC

was administered i.v. twice in the first experiment and once in the second experiment in a dose of 125 mg/kg B.W.

The first experiment comprised 15 rats, divided into three groups. 1. Labelled fibrinogen + Thrombin + AMCA + NAC (n=6) 2. Labelled fibrinogen + Thrombin + AMCA + Saline (S) (n=6) 3. Labelled fibrinogen + Saline (n=3). Saline served as a control fluid to give equal injection volumes. The experiment was carried out as described in Table 1.

Experimental model. Table 1

	-24^{h}	0′	5′	15′ - 25′	45′	105′
1.	^{125}I-fibrinogen	NAC	AMCA	Thrombin	NAC	Killed
2.	^{125}I-fibrinogen	S	AMCA	Thrombin	S	Killed
3.	^{125}I-fibrinogen	S	S	S	S	Killed

At 105 min the animals were killed by aortic exsanguination.

The second experiment comprised 16 rats, divided into the same three groups as
described above; group 1 (n=7) group 2 (n=5) and group 3 (n=4).

Experimental model. Table 2.

	-24^{h}	0′	5′	15′ - 25′	30′
1.	^{125}I-fibrinogen	NAC	AMCA	Thrombin	Killed
2.	^{125}I-fibrinogen	S	AMCA	Thrombin	Killed
3.	^{125}I-fibrinogen	S	S	S	Killed

At 30 min the animals were killed by aortic exsanguination.

Blood was collected in plastic tubes containing either citrate or EDTA buffer. The lungs were quickly removed, perfused with isotonic saline solution, dissected free of connective tissue, cleaned with filter paper

and placed in weighed plastic tubes. The radioactivity of the left lung was determined. In the first experiment the right lung from each animal was homogenized. The homogenization procedure was made according to a method described previously by Busch et al (5) to determine how much of the labelled substance was precipitated (fibrin).

Analysis. The fibrinogen concentration in citrate plasma was determined by the method of Nilsson and Olow (9). The fibrinogen values were corrected for the influence of the erythrocyte volume fraction (EVF) upon the citrate dilution of plasma.

EVF: Aortic blood was drawn into EDTA tubes, and EVF determinations were made in triplicate in micro-EVF tubes after centrifugation at 10 000 x g for 5 min.

Radioactivity: The radioactivity in weighed samples of tissue, blood (EDTA) and labelled fibrinogen was measured in the plastic tubes using a gamma spectrometer.

Calculations. The fibrin content in the lungs was calculated from a formula presented previously by Busch et al (4) and modified by Diffang et al (6).

In short, $F = 1/Q\ (T_{exp} - T_c)$ where F is the amount of fibrin in the organ (mg/g); Q is the factor for converting ^{125}I radioactivity to mg fibrin, consisting of the mean relative specific radioactivity (cpm x 10^3/g) of plasma fibrinogen in control rats; T_{exp} is the total ^{125}I radioactivity in the tissue specimen in the experimental rat (cpm x 10^3/g); T_c is the mean ^{125}I radioactivity in the tissue specimen in control rats (cpm x 10^3/g) = (plasma + extravascular radioactivity).

Statistical methods. Conventional methods as described by Snedecor (12) were used. Differences between the groups were tested by Student's t-test and to confirm significance at the 5% level also by Wilcoxon-White's two-sample ranks test. The results are given as mean and S.D. and degrees of significance are indicated as follows: *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$.

RESULTS

The amount of fibrin in the whole lung or per g lung tissue was significantly lower ($p<0.001$) in both experiments of rats with pulmonary damage that had received NAC-treatment than in such rats not treated with NAC. The same results were obtained for homogenized lungs in the first

experiment although the mean difference was somewhat smaller (Table 3 and 4).

The fibrinogen level in non-NAC-treated animals with pulmonary damage was significantly lower (Table 3 and 4) than in those with pulmonary damage, that were treated with NAC.

Table 3. Experiment 1
Erythrocyte volume fraction (EVF) and blood and lung fibrinogen ^{125}I radioactivities and fibrin content in the lung (mean values and SD).

Groups	1 (n=6)	2 (n=6)	3 (n=3)
EVF	40 ± 5	40 ± 2	36 ± 2
Blood cpm x 10^3/g	17.6 ± 8.1	7.4 ± 2.8	35.2 ± 6.0
Whole lung cpm x 10^3/g	22.6 ± 12.7***	77.4 ± 11.7	9.7 ± 1.4
Homogenized lung, cpm x 10^3/g	18.4 ± 11.7***	54.2 ± 10.1	7.3 ± 2.0
Whole lung fibrin, mg/g lung tissue	0.5 ± 0.5***	3.0 ± 0.6	0
Homogenized lung fibrin, mg/g lung tissue	0.5 ± 0.5***	2.1 ± 0.5	0
Fibrinogen in blood, g/L	1.60 ± 0.49**	0.55 ± 0.38	2.64 ± 0.12

Level of significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 4. Experiment 2
Fibrinogen and fibrin content in the lung (mean values and SD).

Groups	1 (n=7)	2 (n=5)	3 (n=4)
Whole lung fibrin mg/g lung tissue	3.09 ± 0.34***	5.34 ± 0.69	0
Fibrinogen in blood g/L	1.07 ± 0.22*	0.43 ± 0.69 (n=3)	2.55 ± 0.15

Level of significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Fig. 1 shows that there is no difference in the elimination rate of fibrin between animals treated or untreated with NAC.

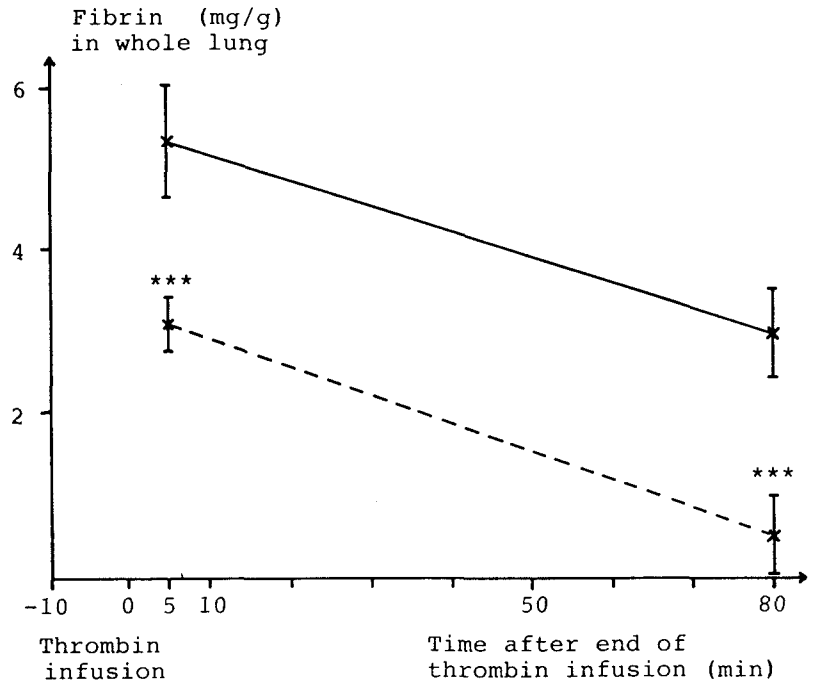

Fig. 1. Elimination rate of fibrin.
—— Isotonic sodiumchloride + AMCA + Thrombin
--- N-acetylcysteine + AMCA + Thrombin
Level of significance *** $p<0.001$

DISCUSSION

In a previous study, Wegener et al (13) found that NAC partly inhibited ARDS caused by intravascular coagulation, possibly by its scavenging effect and/or, (in this particular ARDS model), by influencing the fibrin deposition in the lung. A reduction in the deposition of fibrin could have been due to decreased fibrinogen consumption, to increased fibrin elimination or could be secondary to the scavenging effect.

Our results indicate that NAC counteracts fibrin deposition in the lung directly, since the fibrinogen level in the blood in NAC-treated animals is higher than in animals not given this treatment and the amount of fibrin in the lungs shortly after thrombin infusion was much less in NAC-treated rats. It may therefore be justified to suggest that NAC inhibits fibrin formation in the blood.

The very rapid effect on fibrin formation in the lungs after the administration of thrombin indicates that the effect of NAC on fibrin formation is not secondary to its scravenging effect but more probably due to a direct anticoagulant effect. As the elimination curves for lung fibrin did not differ between rats treated and not-treated with NAC (Fig. 1), this substance probably does not influence the removal of fibrin from the lungs. The findings of Sim et al (11) that a relatively high concentration of NAC showed no fibrinolytic activity when added to fresh human fibrin plates supports our opinion that NAC does not excert its effect by increasing fibrin elimination. Sim et al (11) also found NAC to be an active inhibitor of thrombus formation in the microcirculation of the hamster cheek pouch.

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