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Effect of Nicotinic Acid on the Posttraumatic Increase in Free Fatty Acids and Fibrinolysis Inhibition Activity in the Rat[1]

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ABSTRACT

Nicotinic acid effectively inhibited the posttraumatic increase in both free fatty acids (FFA) and fibrinolysis inhibition activity (FIA) in the blood in rats, indicating that FFA might be involved in the posttraumatic increase of FIA. The FIA in the liver was greater than that in other organs studied and was increased in the posttraumatic phase. The possible role of the liver in the posttraumatic increase of FIA is discussed.

INTRODUCTION

Delayed elimination of fibrin from the lungs due to a posttraumatic increase in the fibrinolysis inhibition activity (FIA) in the blood probably plays an important role in the pathogenesis of the delayed microembolism syndrome (12).

It has been suggested that the posttraumatic increase in FIA may be partly due to increased liver synthesis of a recently discovered inhibitor, now named the primary fibrinolysis inhibitor (PFI, α_2-antiplasmin), due to the elevated posttraumatic blood level of free fatty acids (FFA) (12).

If the above mentioned hypothesis is correct, nicotinic acid, which is known to depress FFA in the blood, should also decrease the blood level of FIA.

In the present investigation the effect of nicotinic acid on the posttraumatic increases in FFA and FIA in rats was studied.

MATERIAL AND METHODS

Chemicals: Human fibrinogen (Grade L) and trans-4-aminomethyl-cyclohexane-carboxylic acid (AMCA) (AB Kabi, Sweden); Thrombin "Topostasine^R (Hoffmann-La Roche, Switzerland); Nicotinic acid (Astra, Sweden); Palmitic acid, turpentine, propylene glycol, glycerol, isopropanol and heptane (all purum quality, chemist's shop assortment).

[1] A brief report on this investigation was published in Forensic Science 1976: 7: 90.

Animals: Sprague Dawley rats of both sexes (Anticimex Farm, Sweden) weighing 200-215 g were used. The animals were allowed free access to food (Ewos rat pellets) and tap water. All procedures were performed under ether anaesthesia.

Burn injury: The hair on the back was shaved and the rats were then immersed in water at 90°C for 20 s, which produced a third degree scald covering 14 % of the body surface area. Control rats were shaved and immersed in water at 38°C.

Chemical injury: The rats were injected intramuscularly in each thigh with 0.5 ml of either a) 100 % pure turpentine, b) 100 % pure propylene glycol, or c) 50 % pure glycerol in distilled water. Control rats were injected with saline.

Nicotinic acid treatment: 0.8 g of nicotinic acid and 0.48 g of bicarbonate were dissolved in 10 ml of saline. 0.5 ml of the solution was injected intraperitoneally 30 min before injury and every 4 h.

Determination of FIA: A clot lysis method essentially as described by Paraskevas *et al.* (9) and slightly modified by Wallin et al. (15) was used. FIA was expressed in per cent of that in normal rat serum unless otherwise stated.

The blood was allowed to clot at room temperature for 2 h and was then centrifuged for 20 min at 3,600 x g. The serum was immediately frozen (-20°C) and stored until analysed within one month. For determination of FIA in organs 1 g of frozen perfused tissue was carefully homogenized in 10 ml of physiological saline with an Ultra turrax homogenizer. After centrifugation at 3,600 x g the supernatant was frozen at -20°C.

Determination of free fatty acids (FFA): The concentration of FFA in plasma from venous blood was determined by the method of Trouth et al. (14).

Perfusion of the organs: Catheters were inserted in the aorta and inferior vena cava for perfusion with physiological saline until the perfusion fluid was macroscopically free from blood.

Statistical methods: Conventional methods as presented by Snedecor (13) were used. Differences between the groups were tested by Student's t-test. The results are given as mean $\pm$ S.D. and degrees of significance are indicated as follows: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

EXPERIMENTS AND RESULTS

Experiment I:

A burn was inflicted in female rats, which were then killed after various intervals for determination of FFA or 48 h after the injury for determination of FIA. The FFA values differed considerably between different rats (Fig. 1). FIA in the blood was 143 $\pm$ 57 % (n = 31) in the burned rats and 105 $\pm$ 8 % (n = 17) in the controls. Thus in this experimental model there were large discrepancies between different animals both in FFA and FIA values. In order to get a more standardized model other types of trauma were therefore tested in the next experiment.

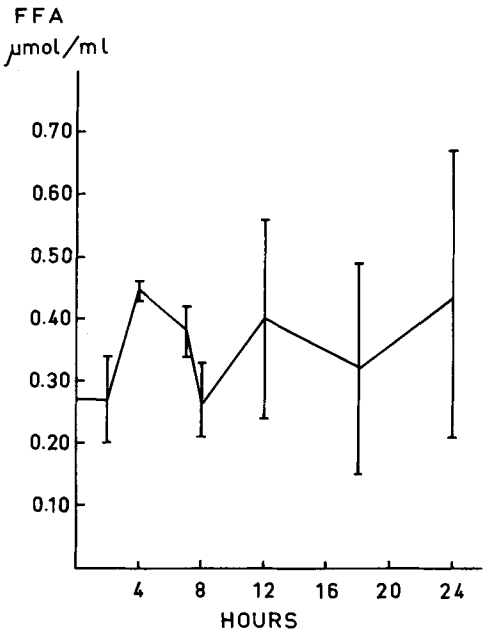

Fig. 1. Variation of FFA (mean ± S.D.) after a burn in female rats.

Experiment II:

Rats were injected with glycerol, propylene glycol (1,2-propane diol) or turpentine and killed 24 or 48 h later for determination of FIA (Table I).

Table 1. The effect of various types of chemical injury on the concentration of FIA in the blood 48 or 24 h after trauma. FIA is expressed in relation to the control values.

Sex		T	n	FIA (%)	
M	Glycerol 50 %	48	5	114 ± 30	S.D.
M	Propylene glycol	48	5	112 ± 15	S.D.
M	Turpentine	48	22	189 ± 34	S.D.***
M	Saline	48	14	100 ± 11	S.D.
F	Turpentine	24	7	94 ± 25	S.D.
F	Saline	24	4	103 ± 13	S.D.
M	Turpentine	24	7	168 ± 25	S.D.**
M	Saline	24	4	104 + 7	S.D.

Turpentine gave the highest increase in FIA and the standard deviation was smaller in these rats than in the burned rats in Experiment I. The standard deviation was smaller in male than in female rats, a finding which has since been confirmed in several similar experiments.

Thus, a turpentine injury in male rats seemed to be a convenient model for studies of posttraumatic FIA increases and this model was therefore investigated in more detail in the next experiment.

Experiment III:

Male rats were injected with turpentine and killed after various intervals for determination of FFA and FIA in the blood and FIA in different organs.

The maximal increase in FFA in the blood was noted 4 h after the injury and the maximal increase in FIA in the blood was seen 48 h after the injury (Fig. 2).

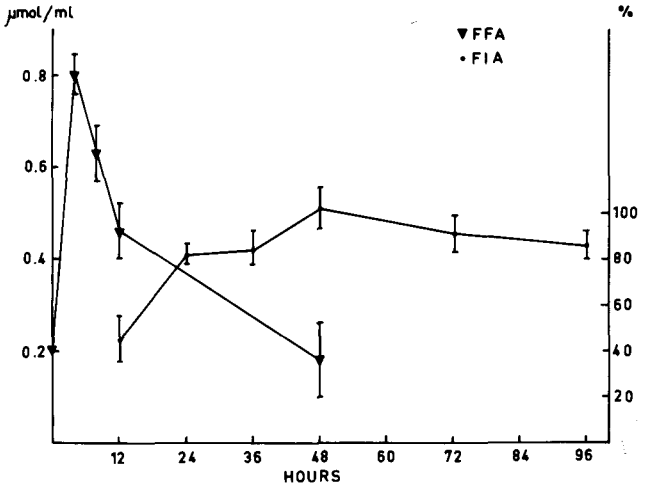

Fig. 2. Posttraumatic responses (mean ± S.D.) of FIA and FFA caused by injection of turpentine. FIA is expressed in per cent of the maximum of the mean (100 %).

In normal rats the liver had the highest amount of FIA; in the lung the mean activity was 58 % of the liver value per gram tissue, in the kidneys 57 %, in the heart 23 % and in muscle tissue 20 %. Following the turpentine trauma there was a significant increase in the mean liver FIA from the control value of 81 ± 11 % to 103 ± 18 % while the mean lung value was 66 ± 20 % in control rats and 69 ± 12 % following the turpentine trauma.

Experiment IV:

Male rats were divided into three groups (Table 2). They were treated with

Table 2. The effect of nicotinic acid on FFA and FIA in the blood at various intervals, T (hours), after intramuscular injection of turpentine. FIA is expressed in relation to the control values.

	T	n	FFA (μmol/ml)	FIA (%)
Turpentine + Saline	4	3	$0.60 \pm 0.11^{*}$	
	12	3	$0.65 \pm 0.05^{**}$	
	24	3	$0.59 \pm 0.11^{*}$	
	36	3	0.41 ± 0.15	
	48	9	0.35 ± 0.06	$180 \pm 35^{**}$
Turpentine + Nicotinic Acid	4	3	0.33 ± 0.16	
	12	3	0.32 ± 0.10	
	24	3	0.43 ± 0.20	
	36	3	0.19 ± 0.02	
	48	9	0.35 ± 0.07	106 ± 14
Controls	-	4	0.33 ± 0.07	102 ± 12

turpentine and saline, turpentine and nicotinic acid and saline alone (controls), respectively. The rats were killed at various intervals and FFA and FIA in the blood were determined.

A significant decrease in both FFA and FIA was found in the rats treated with turpentine and nicotinic acid compared to rats treated with turpentine and saline.

DISCUSSION

We have previously used burned female rats in studies of the posttraumatic increase of FIA (1, 10, 11). This trauma model has the drawback, however, that there are large differences in both FIA and FFA values between different animals. This is probably due both to the use of female rats and to the fact that this model is difficult to standardize.

The present investigation showed that turpentine injection in male rats resulted in diminished variation. This is probably due to the fact that the noxious agent is injected, the higher release of steroids in males than in females, and elimination of the variations in release of steroids caused by the menstrual circle.

Turpentine injection has been used earlier in other studies of the posttraumatic reaction (2, 3, 4, 7, 8) and no drawback of this method seem to have been reported.

The liver had a higher activity of FIA than the other organs studied, which is in agreement with our hypothesis that the FIA protein may be synthesized in the liver (12). In fact, the liver values of FIA were increased after the turpentine injury, whereas the FIA in the lungs was unchanged. However, it cannot be decided from the present study whether the FIA in the liver is due to the primary fibrinolysis inhibitor.

Administration of nicotinic acid reduced not only the FFA but also the FIA values in the turpentine-injected animals, indicating that FFA may play a role in the increase of FIA after trauma. This is compatible with the findings in other studies in our institute, which have showed that nicotinic acid treatment inhibits the increase both in FFA and in FIA after norepinephrine injection in dogs. (6) We have now shown that this is also the case in the posttraumatic phase.

Injection of nicotinic acid is known to increase the endogenous concentration of plasminogen activators in the blood. The effect of nicotinic acid observed in the present investigation should not have been directly due to an increased level of plasminogen activators, however, since these activators do not influence the employed method of determination of FIA. The clot lysis method used is mainly influenced by the primary fibrinolysis inhibitor.

It cannot be concluded from the present study whether the effect of nicotinic acid is due to decreased synthesis of the fibrinolysis inhibitor. Recent studies in our institute, however, with use of labelled precursors of the inhibitor, have indicated that nicotinic acid might exert its effect on the synthesis of the inhibitor (5). Another possible explanation for at least part of the effect is that the increased concentration of plasminogen activators in the blood induced the formation of plasmin and plasmin-inhibitor complexes and by this mechanism a decrease in the inhibitor level.

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