Impaired metabolism of arachidonate in selenium deficient animals

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Summary. Arachidonate-induced respiratory distress in mice was aggravated in a selenium deficient group as compared with a selenium supplemented one. The aggravation in selenium deficient mice may be due to enhanced platelet aggregation and suppressed formation of prostacyclin (PGI_2) in the arterial . all.

It is well known that i.v. injection of arachidonate causes an obstruction of microcirculation by platelet thrombi in the lung, leading to sudden death[1]. The mechanism of the phenomenon has been thought to be the conversion of arachidonate to prostaglandins (PG) and thromboxanes[1,2], which produce severe vasoconstricti in and platelet aggregation[3,4]. Recently, the lipoxygenase pathway for arachidonate was reported to be altered in platelets deficient in selenium[5], which is an essential trace element in animals[6,7]. In the present paper, to search for a possible role of selenium in vascular thrombosis, we investigated the influence of selenium on arachidonate-induced respiratory distress.

Materials and methods. Male ddY mice or Wistar rats (3 weeks old) were divided into 2 groups. One group was fed a selenium deficient diet (selenium content: 0.012 ppm), which contained torula yeast 30%; sucrose 55.7%; lard 5.0%; cod-liver oil 3.0%; salt mixture 5.0%; vitamin mixture 1.0% and DL-methionine 0.3%. Another group was fed a selenium supplemented diet which contained 0.5 ppm selenium as sodium selenite added to the selenium deficient diet. After being fed on these diets for about 6 weeks, the animals were used for the experiments.

Respiratory distress due to arachidonate-induced vascular thrombosis was evaluated by cyanosis, interrupted breathing rate and gasping, and the duration of the effect was noted.

Platelet aggregation was measured in the aggregometer at 37 °C. Citrated blood collected from rats was used for the preparation of platelet-rich plasma (PRP, centrifugation at $230 \times g$ for 7 min) and platelet-poor plasma (PPP, centrifugation at $1500 \times g$ for 10 min). Aggregation was initiated by adding 50 µl of various aggregating agents in Tris-buffered saline (130 mM NaCl in 25 mM Tris-HCl, pH 7.4) to 0.45 ml of PRP (4×10^8 platelets/ml). The extent of platelet aggregation was expressed by the light transmittance during aggregation (PRP = 0%, PPP = 100%).

For the assay of PGI_2-like substance in arterial wall, a piece of arterial ring (transverse section, 5 mm long) was placed in 0.45 ml of Tris-buffered saline at 25 °C for 10 min, and 50 µl of the supernatant was added to the normal rat PRP 5 min before the addition of 5 µM ADP. PGI_2-like activity of the supernatant was expressed by anti-aggregatory activity, which was obtained by comparing the extent of platelet aggregation in the presence and absence of arterial ring extract.

Malondialdehyde (MDA) was determined according to the spectrofluorometrical method using thiobarbituric acid[8].

Results. As shown in table 1, i.v. arachidonate at a dose of 30 mg/kg produced severe symptoms of respiratory distress in both selenium deficient and selenium supplemented mice. The duration of respiratory distress was significantly prolonged in selenium deficient mice compared with supplemented ones. At 50 mg/kg of arachidonate, one third of the mice died in both groups. In this case, there was no difference between the 2 groups in the duration of respiratory distress of the survivors, but the survival time of mice which died was markedly shortened in selenium deficient mice.

Table 2 shows the effect of dietary selenium on platelet aggregation induced by ADP, collagen and arachidonate. Platelet aggregation induced by 1 µM ADP, 40 µg/ml collagen and 1 mM arachidonate were significantly enhanced in selenium deficient rats compared with selenium supplemented ones. In the case of collagen-induced platelet aggregation, lag time to the onset of aggregation was found to be shorter in selenium deficient rats.

Aortic rings incubated for 10 min at 25 °C released a potent inhibitor of ADP-induced platelet aggregation. This inhibitory activity was thought to be due to PGI_2 produced in the a írta, judging from the loss of the activity after boiling for 3 min and from blockade of its formation by incubating aortic rings with indomethacin (0.1 mM). The formation of PGI_2 in the aGta of selenium deficient rats was significantly depressed compared to that in selenium supplemented ones (table 3).

MDA level of the aorta assayed as an index of lipid peroxide was significantly higher in selenium deficient rats (1.77 ± 0.15, $N = 7$) than selenium supplemented rats (1.34 ± 0.11 nmoles MDA/mg protein, $N = 7$).

Table 1. Effect of dietary selenium on arachidonate-induced respiratory distress in mice

Dose (mg/kg, i.v.)	Duration of respiratory distress (min) Se(−)	Se(+)
20	No observable effect	
30	4.29 ± 0.87 (10)	2.08 ± 0.31* (10)
50	9.19 ± 1.23 (8)	9.72 ± 0.91 (8)
	Survival time (min)	
	8.31 ± 4.48 (4)	25.15 ± 8.36* (4)

Each value represents mean ± SEM for 4–10 mice. Significantly different from selenium deficient group (*$p < 0.05$).

Table 2. Effect of dietary selenium on platelet aggregation induced by ADP, collagen and arachidonate

Agent		Platelet aggregation (%) Se(−)	Se(+)
ADP	1 µM	43.2 ± 8.6 (7)	23.8 ± 6.4* (7)
	5 µM	77.6 ± 4.9 (8)	73.6 ± 5.1 (8)
Collagen	40 µg/ml	85.7 ± 5.7 (7)	70.4 ± 3.9* (8)
Lag time (sec)		27.6 ± 4.8 (7)	48.0 ± 7.2* (8)
Arachidonate	1 mM	55.9 ± 4.9 (5)	20.5 ± 5.8** (6)

Each value represents mean ± SEM of 5–8 experiments. Significantly different from selenium deficient group (*$p < 0.05$, **$p < 0.01$)

Table 3. Effect of dietary selenium on the generation of PGI_2-like substance in rat aorta

Diet	% Inhibition of ADP-induced platelet aggregation
Se(−)	31.93 ± 6.21 (8)
Se(+)	16.45 ± 3.28* (8)

Each value represents mean ± SEM of 8 experiments. Significantly different from selenium deficient group (*$p < 0.05$).

Discussion. Dietary selenium deficiency was found to aggravate the arachidonate-induced respiratory distress. Since the respiratory distress is due to platelet thrombi in the microvascular system of the lung[1], platelet function may possibly be altered in selenium deficient mice. In fact, platelet aggregation by ADP, collagen and arachidonate was stronger in the selenium deficient rats than in the selenium supplemented rats. This indicates that platelet aggregability is enhanced in selenium deficient rats. According to Bryant and Bailey[5], selenium deficient platelets showed a marked alteration in the lipoxygenase metabolism of arachidonate. In their experiment, while the conversion of L-12-hydroperoxy-5,8,10,14-eicosatetraenoic acid to L-12-hydroxy-5,8,10,14-eicosatetraenoic acid was slightly suppressed, the conversion to trihydroxy fatty acids, 8,9,12-trihydroxy-5,10,14-eicosatrienoic acid and 8,11,12-trihydroxy-5,9,14-eicosatrienoic acid was increased 3-4-fold in selenium deficient platelets as compared to selenium supplemented ones. From the recent findings that lipoxygenase metabolites may also play an important role in platelet aggregation[9], the alteration of the lipoxygenase pathway seems to contribute to the enhanced platelet aggregation in selenium deficient rats. As another mechanism of aggravated vascular thrombosis in selenium deficient mice, the formation of PGI_2 with a potent anti-aggregatory and vasodilating activity may be suppressed in the blood vessel. In the present study, the formation of PGI_2-like substances was markedly suppressed in the aorta of selenium deficient rats. Biosynthesis of PGI_2 is known to be inhibited by hydroperoxide derivatives of arachidonate[10-12], which was recently suggested to exist in vascular tissue[13]. Lipid peroxide estimated by the amounts of MDA was increased in the aorta from selenium deficient rats. Further, under the present experimental conditions, glutathione peroxidase, a seleno-enzyme[7], in the platelets and aorta of selenium deficient rats decreased to about 10% of the level found in selenium supplemented rats (data not shown). These findings suggest that lipid peroxide accumulated in the arterial wall owing to severe depletion of glutathione peroxidase suppresses the formation of PGI_2. The present findings suggest that selenium may function in vascular hemostasis and thrombosis by maintaining the metabolism of arachidonate. In the present study, however, the effect of selenium on the metabolism of endogenous arachidonate is obscure, because we did not determined the levels of arachidonate and its metabolites in the tissues. Further investigations are necessary to evaluate the role of selenium in the regulation of arachidonate metabolism.

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0014-4754/83/040405-02$1.50+0.20/0

Electron microscopic stereology of constitutive heterochromatin in *Rhinanthus minor*

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Summary. The chromocenter heterochromatin of cell nuclei in the Scrophulariacean plant, *Rhinanthus minor*, was reconstructed from ultrathin serial sections by various methods. The chromocenters are irregularly shaped blocks through which runs a ramified anastomosing system of channels. No higher order structure of chromatin organization could be recognized.

Heterochromatin remains in the condensed state through interphase[2]. Therefore, it may serve as a model system in the analysis of the higher order structure of chromatin, which is yet poorly understood. The cell nuclei of *Rhinanthus* exhibit large chromocenters, which are associated with the nucleolus, within a diffuse background composed of euchromatin and nucleolymph[3]. We have chosen this species to reconstruct the organization of heterochromatin from ultrathin sections.

Material and methods. Various tissues of *Rhinanthus minor* were fixed with glutaraldehyde (6.25%) and osmium tetroxide (1%), both in PIPES buffer, pH 7.3. After dehydration the material was embedded according to Spurr[4]. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 10 electron microscope. Serial sections were picked up with one-hole grids. Reconstruction of 17 chromocenters was made from electron micrographs of serial sections. Four chromocenters were reconstructed by the aid of an automatic image analyzing system at the Unit of Data Recording at the German Cancer Research Center, Heidelberg. The system stores the images of the micrographs, digitized into 256×256 grey level points; the connected VAX-11/780 computer and RAMEX color display unit allow visualization of the