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SELECTIVE INHIBITION OF LEUKOTRIENE B_4 BIOSYNTHESIS IN RAT PULMONARY ALVEOLAR MACROPHAGES BY DIETARY SELENIUM DEFICIENCY

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Summary: Weanling male Fisher 344 rats were maintained on low selenium basal and Se-supplemented diets for 38 weeks. A several fold reduction in the glutathione peroxidase activity of the lung and liver tissues in rats maintained on low Se basal diet established their Se-deficient status. Analysis of the supernatants from resting pulmonary alveolar macrophage suspensions showed negligible extracellular release of PGE_2, TXB_2 and LTB_4 in both diet groups. A challenge with opsonized zymosan particles increased the release of the same three arachidonic acid metabolites by several fold in both diet groups. The differences between the two diet groups with respect to the secretion of the products of the cyclooxygenase pathway, PGE_2 and TXB_2 were negligible. By contrast, a significant reduction in the extracellular release of LTB_4 was observed in cells from animals on low selenium basal diet. These results suggest a selective inhibition of LTB_4 biosynthesis in pulmonary alveolar macrophages by dietary deficiency of selenium. © 1985 Academic Press, Inc.

Selenium (Se) in an essential trace element of significant importance to human health. Its deficiency has been associated with cardiomyopathy (1,2) and cancer incidence in humans (3). In recent years, evidence has accumulated to suggest that dietary Se can influence various inflammatory and immune processes in experimental animals (4). Phagocytes, which are important component of inflammation and also participate in immune reactions, have been reported to exhibit impaired functions in Se-deficient animals (5,-7).

The main phagocytes of the lung are macrophages, which protect lungs against inhaled particulates including infectious agents (8,9). These cells can secrete a diverse array of biologically active substances upon phagocytic stimulation (10). Among such substances are included arachidonic acid metabolites, which are known to possess chemotactic and

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immunoregulatory properties (11,12). In addition, the lipoxygenase products of arachidonic acid have been found to modulate the smooth muscle function and airway tones (13).

The present study was undertaken to determine the effect of Se deficiency on the ability of pulmonary alveolar macrophages (PAM) to release selected metabolites of arachidonic acid, that are derived via cyclooxygenase and 5-lipoxygenase pathways. The results suggest a selective effect of Se deficiency on the 5-lipoxygenase pathway.

MATERIAL AND METHODS

Animals and Diets: Weanling male Fisher 344 rats were obtained commercially (Harlan-Sprague Dawley, Indianapolis, IN) and were kept on Purina rat chow for one-week in quarantine rooms. The animals were randomly selected for the study and divided into two groups. The first group was maintained on low Se basal diet and the second group on the same diet supplemented with 1 ppm Se (as sodium selenite). The diets were received in small installments from Dyet Inc. PA, and were composed of torula yeast, 30%; sucrose, 59%; tocopherol-stripped lard, 5%; salt mix HMW, 5%, and vitamin mix, 1%, as in Schwartz and Fredga (14). The Se content of the low Se basal diet was 0.03 ppm

All animals were housed in hanging stainless steel wire cages and maintained under a daily light cycle of 12 hrs in environmentally controlled Bioclean rooms equipped with HEPA filters, and undergoing 40 air changes/hour. Animals had free access to food and water ad libitum.

Bronchoalveolar Lavage: After 38 weeks on low Se basal and Se supplemented diets, animals from each group were lavaged to obtain PAMs. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital and opened to expose the chest cavity. After severing the abdominal aorta to exsanguinate the animal, the trachea was cannulated and the lungs were lavaged with 8 ml aliquots of Ca^{+2} and Mg^{+2} free Hanks balanced salt solution (HBSS) 8-9 times per animal. The lavage fluids were pooled and centrifuged at 400 xg for 15 min to sediment the bronchoalveolar lavage (BAL) cells. The BAL cell pellets were washed once after a brief hypotonic shock to lyse the erythrocytes and resuspended in HBSS. Small aliquots were used for the determination of total, viable and differential cell counts. Viability was determined by trypan blue exclusion and differential cell counts were made on giemsa stained cell smears. Pieces of lung and liver tissues from each animal were immediately frozen in liquid nitrogen and stored at -80° for later analysis of tissue glutathione peroxidase (GSH-Px) activity.

Extracellular Release of Arachidonic Acid Metabolites: Cell suspensions were adjusted to 10^6 cells/ml in HBSS containing Ca^{+2}/Mg^{+2} and glucose. All experiments were performed within 2 hrs of animal sacrifice using siliconized glass tubes containing 0.5 ml cell suspension. Release of the metabolites was measured under resting and zymosan challenged states. Zymosan particles were opsonized by incubation in rat serum for 30 min at 37°C. The cell suspensions were incubated alone and with opsonized zymosan particles (1:10) for 30 min at 37°C and the tubes transferred to ice bath. Supernatants were collected after centrifuging the suspensions at 400 xg for 10 min. The cell pellets were used to

determine protein content by Lowry method (15). The supernatants were immediately frozen in liquid nitrogen for later determination of various arachidonic acid metabolites.

Assay of PGE_2, TXB_2 and LTB_4: Levels of PGE_2 and TXB_2 were determined by specific radioimmunoassays described previously (16,17). Concentration of LTB_4 was analyzed by a specific radioimmunoassay recently developed in our laboratory. Cross-activities of LTB_4 antibodies with other arachidonate metabolites are less than 0.1% with the exception of 12-HETE (0.8%).

Liver and Lung Tissue GSH-Px Assay: Lung and liver tissues were homogenized in 0.15 M KCl buffer and centrifuged at 20,000 xg for 20 min. The supernatants were collected and used to assay the activity of GSH-Px using t-butyl peroxide as substrate (18).

RESULTS

The body weights of experimental animals on Se supplemented and deficient diets averaged 352 ± 7 and 301 ± 24 g, respectively. The recovery of BAL cells in the two groups was similar (Table 1). Greater than 95% of the recovered cells in both diet groups were viable as indicated by trypan blue exclusion. Differential cell analysis showed that over 97% of the cells in both groups were macrophages as judged by morphology, phagocytosis of latex particles and nonspecific esterase staining. Enzymatic analysis of the hepatic and pulmonary tissue homogenates showed a significant reduction in the activity of Se-dependent GSH-Px in rats maintained on low Se basal diet (Table 1)

Fig 1, and 2 illustrate the extracellular release of PGE_2, TXB_2 and LTB_4 by BAL cells from rats maintained on low Se basal diet and Se supplemented diet for 38 weeks. Under resting conditions, the release of the three arachidonic acid metabolites by these cells was negligible.

Table 1

Dietary Selenium	(N)	BAL Cell Recovery (X 10^6/rat)	GSH Px Activity (nmoles/min/mg protein) Liver	Lung
Se^-	(4)	2.8 ± 0.3	25 ± 2	14 ± 1.1
Se^+	(4)	3.0 ± 0.1	1058 ± 76	504 ± 36
	P	NS	< 0.01	< 0.01

Bronchoalveolar Lavage (BAL) cell recovery and glutathione peroxidase (GSH-Px) activity in liver and lung tissue from Se deficient (Se^-) and supplemented (Se^+) rats.

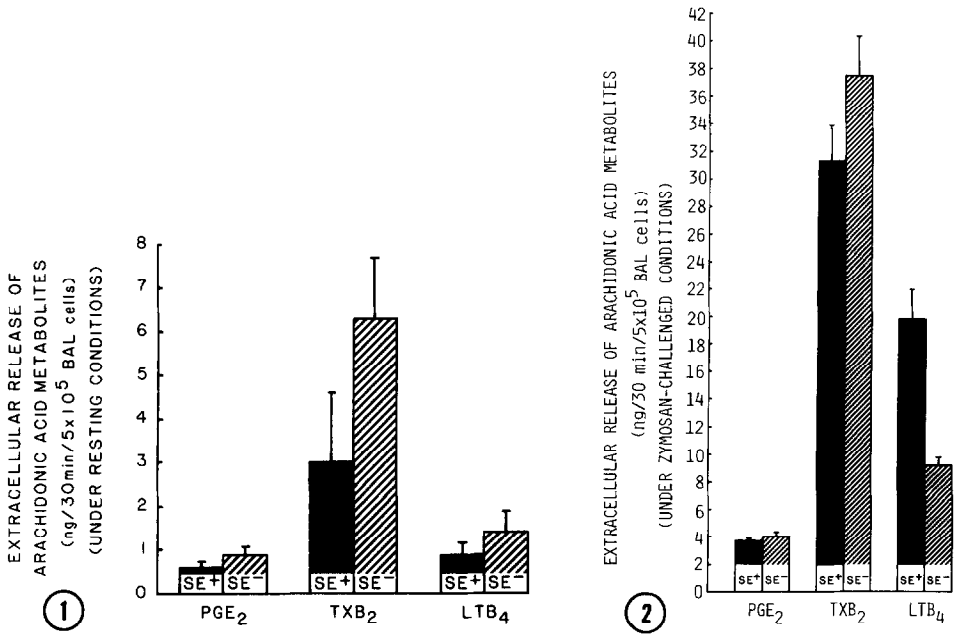

Figure 1. Extracellular release of PGE_2, TXB_2 and LTB_4 by pulmonary alveolar macrophage suspensions under resting conditions.

Figure 2. Extracellular release of PGE_2, TXB_2 and LTB_4 by pulmonary alveolar macrophage suspensions under zymosan-stimulated conditions.

Challenge with zymosan particles increased the macrophage secretion of PGE_2, TXB_2 and LTB_4 by an average of 7.8, 17.5 and 22.9 fold in Se-fed group and by 4.5, 10.1 and 6.52 fold in low Se basal diet group. The release of PGE_2 and TXB_2 by BAL cells in resting or Zymosan challenged states was not significantly altered by the dietary Se-deficiency. In contrast, the release of LTB_4 was significantly reduced in Se-deficient group under phagocytically stimulated conditions. TXB_2 was the predominant arachidonic acid metabolite produced by PAMs under phagocytically challenged state. The levels of LTB_4 and PGE_2 were approximately 50% and 25% of TXB_2, respectively.

DISCUSSION

The main aim of the present study was to examine if dietary Se deficiency causes any changes in the ability of PAMs to secrete arachidonic acid metabolites into the extracellular environment. The

Se-deficient status of the animals was established by demonstrating a several fold reduction in the activity of GSH-Px in lung and liver tissue of animals maintained on low Se diets. Deitary Se is known to regulate the activity of this enzyme in different tissues (19). Although we did not measure the GSH-Px activity in PAMs, it was presumed to be similarly reduced, as reported earlier (20).

In view of the evidence that dietary Se may influence various inflammatory and immunoregulatory processes in the experimental animals (4) and that arachidonate metabolites may play important role in inflammatory and immune reactions (11,12), we investigated the effect of Se deficiency on the ability of PAMs to secrete PGE_2 and TXB_2 (two end products of cyclooxygenase mediated metabolism of arachidonate) and LTB_4 (a product of 5-lipoxygenase pathway). The secretion of PGE_2, TXB_2 and LTB_4 was studied in the absence of added arachidonate. Therefore, the secreted metabolites were presumably synthesized from the endogenously generated arachidonate. Because the secretion of PGE_2 and TXB_2 by PAMs in two diet groups was similar, it would appear unlikely that the reduced level of LTB_4 secretion by Se-deficient PAMs was due to an impairment of the release of arachidonate from membrane phospholipids. An inhibition of the synthesis of LTB_4 from arachidonate appear to be a more likely explanation for the decreased release of the metabolite in the Se-deficient group.

Dietary Se regulates the cellular activity of GSH-Px, an enzyme that catalyzes the transformation of lipid peroxides to less toxic alcohols (21). A relationship betweem GSH-Px activity and altered formation of hydroxy fatty acids of the 12-lipoxygenase pathway has also been demonstrated in rat platelets by Bryant and Bailey (22). These workers reported that platelets from Se-deficient rats synthesized 30% less 12-HETE from added labeled arachidonate but exhibited increased formation of 12-HPETE derived THETES. In contrast to such altered levels of 12-lipoxygenase mediated products, Se deficiency did not affect the

synthesis of TXB_4. These observations indicated a differential effect of dietary Se on cyclooxygenase and lipoxygenase pathways in platelets. Our results in PAMs also exhibited a selective effect on 5-lipoxygenase pathway but in a different manner. If GSH-Px is also involved in the reduction of 5-HPETE to 5-HETE, Se deficiency should divert 5-HPETE to the formation of LTA_4 and thereby LTB_4 unless LTA_4 synthase and/or LTA_4 hydrase are also Se dependent enzymes. So far the cofactor requirements of the latter two enzymes are not known although LTA_4 hydrase has been purified recently to homogeneity (23). The decreased formation of LTB_4 in PAMs of Se-deficient rats suggests that LTA_4 synthase and/or LTB_4 hydrase may be Se dependent enzymes.

The significance of our findings can be several fold. The cofactor nature of leuketriene biosynthetic enzymes is yet to be defined. Our results suggest that either LTA_4 synthase or LTB_4 hydrase or both may be Se dependent enzymes as indicated above. LTB_4 has been shown to be a major metabolite of 5-lipoxygenase pathway in PAMs of several species (23,24) and is implicated to play an important role in inflammatory process in the lung (25). Decreased synthesis of LTB_4 in PAMs may in part account for the reported impaired functions of phagocytes. Furthermore, the interaction of LTB_4 with other humoral factors such as complements will also be altered leading to other undesirable consequences.

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REFERENCES

1. Johnson, R.A., Baker, S.S., Fallon, J.T., Maynard, N., Ruskin, J., Wen, Z., Ge, K. and Cohen, H. (1981). N. Engl. J. Med., 304:1210-1212.
2. Collip, P.J. and Chen, S.Y. (1981). N. Engl. J. Med., 304:1304-1305.

3. Clark, L.C. (1985). Federation Proc. 44:2584-2589.
4. Spallholz, J.E. (1981). Adv. Exp. Med. Biol., 135:43-62.
5. Boyne, R. and Arthur, J.R. (1979). J. Comp. Path., 89:151-158.
6. Serfass, R.E. and Ganther, H.E. (1975). Nature 255:640-641.
7. Serfass, R.E. and Ganther, H.W. (1976). Life Sci., 19:1139-1144.
8. Brain, J.D., Golde, D.W., Green, G.M. (1978). Am. Rev. Resp. Dis., 118:435-443.
9. Brain, J.D. (1980). Environmental Health Persp. 35:21-28.
10. Takemura, R. and Werb, Z. (1984). Am. J. Physiol. 246 (Cell Physiol.) C1-C9.
11. Goodwin, J.S. and Webb, D.R. (1980). Clin. Immunol. Immunopathol 15:106-122.
12. Nathan, C.F., Murray, H.W. and Cohn, Z.A. (1980). N. Engl. J. Med., 303:622-626.
13. Hammarstrom, S. (1983). Annu. Rev. Biochem., 50:355-377.
14. Schwartz, K. and Fredga, A. (1969). J. Biol. Chem., 244:2103-2110.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). J. Biol. Chem. 193:265-275.
16. Chang, D. and Tai, H.H. (1982). Arch. Biochem Biophys. 214:464-474.
17. Tai, H.H. and Yuan, B. (1978). Anal. Biochem. 87:343-349.
18. Levander, O.A., DeLoach, D.P., Morris, V.C., and Moser, P.B. (1981). J. Nutr., 113:55-63.
19. Lawrence, R.A. and Burk, F. (1976). Biochem. Biophys. Res. Comm., 71:952-958.
20. Parnham, M.J., Winkelmann, J. and Leyck, S. (1983). Inter. J. Immunopharmacol. 5:455-461.
21. Sunde, R.A. and Hoekstra, W.G. (1980). Nutr. Rev. 38:265-273.
22. Bryant, R.W. and Bailey, J.M. (1980). Biochem. Biophys. Res. Comm. 92:268-276.
23. Radmark, O., Shimiau, T., Jornvall, H. and Sammuelsson, B. (1984). J. Biol. Chem. 257:12339-12345.
24. Davies, P. Bonney, R.J., Humes, J.L. and Kuehl, F.A. (1980). In "Mononuclear Phagocytes, Functional Aspect" Ed. R. VanFurth, Martinus Nijhoff Publishers, The Hague, 1980. p-1317.
25. Martin, T.R., Altman, L.C., Albert, R.K. and Henderson, W.R. (1984). Am. Rev. Respir. Dis., 129:106-111.