Vol. 117, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1983 Pages 183-189

HYDROPEROXY FATTY ACID FORMATION IN SELENIUM DEFICIENT RAT PLATELETS: COUPLING OF GLUTATHIONE PEROXIDASE TO THE LIPOXYGENASE PATHWAY

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Received October 17, 1983

SUMMARY: After incubation with [1-^{14}C]-arachidonic acid, washed platelets from selenium deficient rats produced a sevenfold greater amount of 12-hydroperoxytetraenoic acid than platelets from control animals. When stimulated with either arachidonic acid or t-butyl-hydroperoxide, antimycin-A1 treated platelets from the deficient rats also converted markedly lower amounts of [1-^{14}C]-glucose to [^{14}C]-CO_2 than platelets from control rats. These results indicate a significant role for platelet selenium-dependent glutathione peroxidase in the enzymatic reduction of platelet-produced hydroperoxides.

Platelets contain an active lipoxygenase (1,2) which converts arachidonic acid (AA) to L-12-hydroperoxy-5,8,11,14-eicosatetraenoic acid (12-HPETE). In platelets the major route for 12-HPETE metabolism is reduction to L-12-hydroxy-5,8,11,14-eicosatetraenoic acid (12-HETE). This reductive step involves glutathione peroxidase (E.C.1.11.1.9) and reducing equivalents from the hexose monophosphate (HMP) shunt (3,7). Glutathione peroxidase activity occurs in both selenium dependent (4) and independent (5) forms. Platelets from rats fed a selenium deficient diet show a lowered glutathione peroxidase

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ABBREVIATIONS: AA, arachidonic acid; 12-HPETE, 12-hydroperoxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; THETE; trihydroxyeicosatrienoic acid; HMP shunt, hexose monophosphate shunt; PPP, platelet poor plasma; PRP, platelet rich plasma; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis-(-aminoethyl ether)N,N'-tetraacetic acid; TRIS, tris(hydroxymethyl)aminomethane; TLC, thin layer chromatography; HHT, 12-hydroxyheptadecatrienoic acid.

This work was supported by USDA grant 59-2113-0-1-487-0 and NIH grants HL-24838 and HL-22527.

0006-291X/83 $1.50

activity and raised levels of the 12-HPETE rearrangement products, 8,9,12- and 8,11,14-trihydroxy-eicosatrienoic acids (THETES). This paper reports experiments which demonstrate that platelets from selenium deficient rats convert a significantly greater amount of added AA to 12-HPETE when compared to controls. In addition, conversion of [1-^{14}C]-glucose to [^{14}C]-CO_2 in response to added t-butyl-hydroperoxide and AA in antimycin-A1 treated platelets from both selenium deficient and supplemented rats was determined. Platelets from the deficient rats had a markedly lower conversion of the labeled glucose than platelets from the control group. Taken together, these results demonstrate the direct involvement of selenium-dependent glutathione peroxidase in the reduction of 12-HPETE in the rat platelet.

MATERIALS AND METHODS

Weanling male Wistar rats were housed in hanging wire cages and supplied with tap water ad libitum. The rats were fed either a selenium deficient basal diet (8), or the basal diet supplemented with 0.5 ppm of Se as Na_2SeO_3 to serve as the control. Both diets were prepared by ICN Nutritional Biochemicals. The basal diet also contained 500 ppm dl-alpha-tocopheral acetate and 0.3% dl-methionine.

[1-^{14}C]-AA and [1-^{14}C]-D-glucose were obtained from Amersham Corp, Deerfield, IL, and unlabeled AA was purchased from Nu Chek Prep, Elysian, MN. Antimycin-A1 and t-butyl-hydroperoxide were obtained from Sigma Chemical Co., St. Louis, MS. TLC plates were purchased from Analtech, Inc., Newark, DE, and X-ray film was Kodak X-omat-AR. All other chemicals used were of reagent grade and were purchased from Fisher Scientific Corp, Pittsburg, PA.

Rats were anesthetized with diethyl ether and blood was withdrawn from the abdominal aorta. The blood (8-10 mL) was added to 1/10 volume of 90 mM EGTA, 0.35% NaCl anticoagulant. One half volume of 0.9% NaCl was added to the whole blood to increase platelet yield (9). Erythrocytes were sedimented at 220 x g for 8 min. The PRP was diluted 1:1 with ice cold 12 mM TRIS, 1.5 mM EDTA, 135 mM NaCl, 5 mM D-glucose, pH=7.4 buffer and then centrifuged at 900 x g for 10 min at 4°. The platelet pellet was resuspended in 5 mL of the same buffer and the suspension was centrifuged as above. The washed platelets were finally resuspended in modified Krebs-Henseleit buffer (10) without calcium but containing 1 mM D-glucose. Platelets were pooled from individual rats in both the selenium deficient and selenium supplemented groups to a cell count of (8-11) x 10^5 platelets/ L. Glutathione peroxidase assays were performed on both the platelet suspension and platelet poor plasma for each rat as previously described (6).

Platelet suspension for the [14-C]-AA metabolism studies had D-glucose added to give a final concentration of 5 mM. 0.5 ML aliquots of the platelet suspension were incubated for 5 min at 37° with 0.1 Ci of 56 Ci/ mole [1-^{14}C]-AA which had been diluted to a specific activity of 6 Ci/ mole with unlabeled AA. The AA metabolites were extracted from the incubations using cold diethyl ether and separated by thin layer chromatography as previously described (3). The radioactive

regions of the TLC plate corresponding to each metabolite were identified by autoradiography, and quantified by scraping the region from the plate and determining radioactivity by liquid scintillation counting.

For measurement of [1-^{14}C]-glucose metabolism, the washed platelets in 1 mM D-glucose were supplemented with antimycin-A1 (1 g/ml) before addition of [1-^{14}C]-glucose (59 Ci/ mole) to obtain 0.5 Ci/mL in the platelet suspension. After a 30 min incubation at 37^{0} in metabolic flasks, 1.5 L of t-butyl-hydroperoxide (1.75%) or AA (5 g/mL) in ethanol was added to each 0.75 mL platelet incubation for 15 min. [^{14}C]-CO_2 was trapped using Protosol (New England Nuclear) as previously described (3). The appropriate amount of ethanol was added to controls for the stimulating agents.

RESULTS

Groups (n=10) of male weanling Wistar rats were raised on either a basal selenium deficient diet or basal diet supplemented with sodium selenite (0.5ppm as Se). After five weeks the platelet poor plasma (PPP) from rats fed the selenium deficient diet showed a glutathione peroxidase activity of 6 + 1% or less of the PPP from rats fed the supplemented diet. Platelet levels of glutathione peroxidase in the deficient group were less than 15 + 3% of the control group values.

Washed platelets from the two groups were incubated with [14-C]-AA. The products were separated by thin layer chromatography, and the distribution of radioactivity in the products was visualized by autoradiography (Fig. 1). A distinctive difference was observed in the AA metabolite pattern between the two groups. Platelets from selenium deficient rats showed much greater conversion of added AA to 12-HPETE (lane 2) than platelets from control rats (lane 1). This difference was statistically significant as shown in Table 1, where the amount of 12-HPETE formed by the platelets from selenium deficient rats was 750 + 250% than formed by platelets from selenium supplemented rats. The small decrease in 12-HETE formation by platelets from selenium deficient rats was sufficient to account for the increased accumulation of 12-HPETE observed.

Stimulation of the HMP shunt by t-butyl-hydroperoxide and AA was measured in platelets from selenium deficient and selenium supplemented rats (Table 2). The platelets from selenium deficient rats showed a

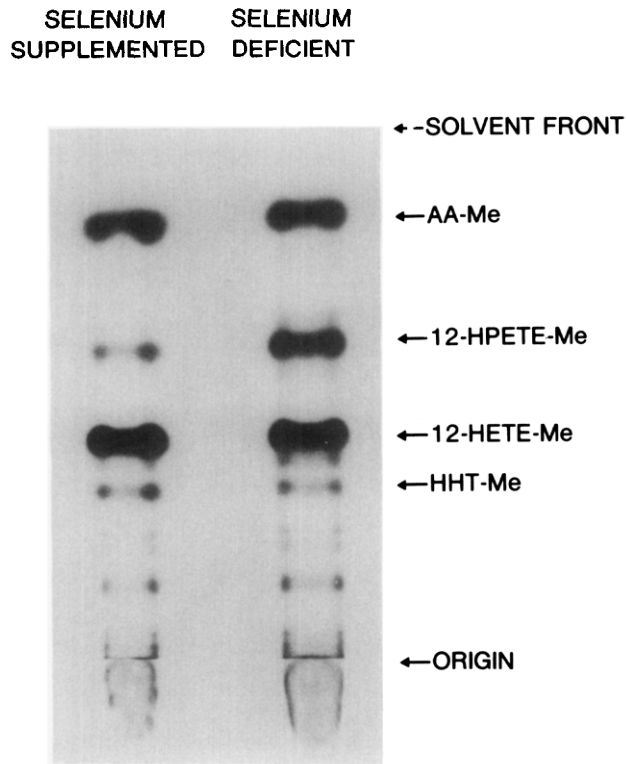

FIGURE 1. Stimulation of 12-HPETE formation from added [1-^{14}C]-arachidonic acid in selenium deficient platelets. Washed platelets isolated from selenium deficient (lane 2) or selenium supplemented (lane 1) rats were incubated with [1-^{14}C]-arachidonic acid. The products were extracted, converted to methyl esters, and analyzed by TLC as described in Materials and Methods.

significant decrease in AA and t-butyl-hydroperoxide coupled HMP shunt activity compared to controls for both t-butyl-hydroperoxide (40% of control) and AA (56% of control).

TABLE 1. Increased formation of 12-HPETE in platelets from selenium deficient rats. Nine animals per diet group were used, pooled in groups of three. 12-HPETE and 12-HETE were extracted following incubation of washed platelets with [1-^{14}C]-arachidonic acid and isolated by TLC separation as described in Materials and Methods. Values are mean ± S.D., *based on three pooled groups.*

metabolite	Per Cent Conversion of Added Arachidonic Acid	
	Se-Supplemented	Se-Deficient
12-HPETE	0.4 ± 0.1	3.0 ± 1.1*
12-HETE	75.0 ± 2.7	69.0 ± 3.5

* Indicates $p < .01$.

TABLE 2. Impaired stimulation of the HMP shunt by t-butyl-hydroperoxide or arachidonic acid in platelets from selenium deficient rats. Washed platelet suspensions were preincubated at 37° for 30 min in the presence of [1-^{14}C]-glucose and antimycin-A1, and for an additional 15 min with the stimulants added dissolved in ethanol (1.5 L). The appropriate amount of ethanol was added to controls. [^{14}C]-CO_2 was trapped as described under Materials and Methods. Nine animals per diet group were used, pooled in groups of three. Values are mean ± S.D. based on three pooled groups. Figures in parentheses represent selenium deficient values as a percent of selenium supplemented controls.

Stimulating Agent Added	Release of $^{14}CO_2$ from 1-^{14}C-Glucose[a] (dpm/15 min/6 x 10^8 Platelets)	
	Se-Supplemented	Se-Deficient
Control	1,290 ± 270	1440 ± 230 (112%)
t-Butyl Hydroperoxide (348 μM)	10,480 ± 5250	4240 ± 1650 (40%)*
Arachidonic Acid (33 μM)	5,710 ± 2,360	3180 ± 1110 (56%)*

* Represents $p < .05$.

DISCUSSION

We have previously reported that enzymatic reduction of 12-HPETE to 12-HETE in platelets is tightly coupled to the HMP shunt through a glutathione peroxidase (3). This lipoxygenase-glutathione peroxidase couple is illustrated in Figure 2. Support for this hypothesis comes from the observation of a stoichiometric relationship of two moles of 12-HETE formed per mole of CO_2 released via the HMP shunt in platelets stimulated with AA (3). Results reported in this paper further support this hypothesis in that decreased levels of the selenium-dependent

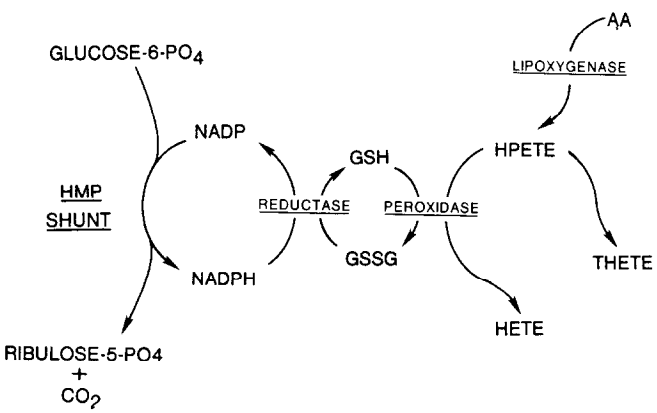

FIGURE 2. Lipoxygenase-glutathione peroxidase couple. This diagram illustrates the enzymatic coupling of the platelet lipoxygenase pathway to glutathione peroxidase and the HMP shunt which thus provides the NADPH required for enzymatic reduction of 12-HPETE to 12-HETE.

glutathione peroxidase were associated with increased platelet accumulation of 12-HPETE as well as reduced glucose metabolism via the HMP shunt in platelets incubated with AA. Taken together, these two observations demonstrate that selenium dependent glutathione peroxidase provides a major route of 12-HPETE reduction in rat platelets.

It should be noted that the data suggests another source of reducing equivalents is present in these cells in addition to the selenium-dependent glutathione peroxidase. The 44% decline in AA-stimulatd cycling of the HMP shunt in selenium-deficient platelets (Table 2) would be expected to result in a corresponding decline in reduction of 12-HPETE to 12-HETE if no other source of reducing equivalents is available. The actual changes in 12-HPETE and 12-HETE formation were tenfold smaller than expected. This alternative system either does not use glutathione as a reductive agent, or if it is a selenium-independent glutatione peroxidase, a separate source of reduced glutathione besides the HMP shunt would be required.

The possible release of 12-HPETE by platelets in dietary selenium deficiency has potential physiological significance. 12-HPETE has been demonstrated to be a potent inhibitor of vascular prostacyclin synthesis (11), indicating release of this compound could adversely affect platelet-endothelial cell interactions. In addition, Maclouf *et al* (12) have shown that 12-HPETE generated *in situ* by platelets greatly enhances the conversion in human blood leukocytes of AA to leukotriene B4, a potent chemotactic agent. The consequences of platelet release in selenium deficiency of the lipoxygenase-derived THETES is not yet clear, since no metabolic functions have yet been ascribed to these compounds. 8,11,12-Trihydroxy eicosatrienoic acid has also been observed in arachidonic acid incubations of rat lung tissues (13).

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