Toxicology Letters, 51 (1990) 91–97
Elsevier

TOXLET 02302

Pteridine modulation of lead inhibition of uroporphyrinogen synthesis in erythroid precursor cells

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(Received 13 June 1989)
(Accepted 29 October 1989)

Key words: Lead toxicity; Erythroleukemia cells; Uroporphyrinogen synthesis; Pteroylpolyglutamates

SUMMARY

The role of nutritional factors on heme synthesis and their influence on the development of anemia in the bone marrow during lead exposure is unclear. Previous reports suggested that pteridines could regulate the formation of uroporphyrinogen, a step midway along the heme synthetic pathway. Studies were undertaken to determine if pteridines could modulate lead inhibition of uroporphyrinogen synthesis in erythroid precursor cells. Pteroylpolyglutamates of various glutamate chain lengths were tested for the ability to protect against lead inhibition of uroporphyrinogen I (URO) synthase prepared from murine erythroleukemia cells (MELC). Pteroylpentaglutamate, the major endogenous polyglutamate form by chain length found to be present in MELC, afforded rapid and specific protection of URO synthase against lead inhibition. MELC are expected to be a useful in vitro model for studying the role of endogenous folates on uroporphyrinogen synthesis and heme formation in erythroid precursor cells following lead exposure.

INTRODUCTION

The role of various physiological factors that regulate heme synthesis and/or influence the development of anemia in the bone marrow during lead (Pb) exposure has been sparsely investigated. Previous studies in liver demonstrated that oxidized polyglutamate derivatives of folic acid protected against Pb inhibition of uroporphyrinogen I (URO) synthase (EC 4.3.1.8) [1]. As a result of the rather low levels of URO synthase activity found in liver and other tissues, it is believed that the synthesis of

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uroporphyrinogen may be potentially rate-limiting for the biosynthesis of heme. Consequently, the endogenous folate content and regulation of pteroylpolyglutamate synthesis/catabolism are anticipated to be important factors modulating the susceptibility of bone marrow heme synthesis to inhibition by Pb [2].

Most previous studies concerning the etiology of drug/toxicant-associated anemia and its relationship to the biosynthesis of heme have been conducted with the mature red blood cell, which is not actively engaged in heme synthesis. It is anticipated that more relevant information could be obtained by focusing study on the bone marrow. It is known that premature erythroid precursor cells are highly susceptible to the toxic effects of Pb. As a result of the complex cellular heterogeneity of bone marrow in vivo, controlled study of this cellular target is rather difficult. Murine erythroleukemia cells (MELC) are erythroid precursor cells which, when cultured in the presence of certain chemicals (e.g. DMSO, hexamethylene-bis(acetamide) [HMBA]) [3,4], undergo changes similar to normal erythrocyte maturation, including the synthesis of heme and hemoglobin [5]. It is anticipated that MELC will be a useful in vitro model to study drug/toxicant interactions during erythropoiesis. In the present study, this in vitro system was evaluated for its potential usefulness as a model to study folate regulation of uroporphyrinogen synthesis and heme formation in bone marrow during Pb exposure.

MATERIALS AND METHODS

Cell culture

The murine erythroleukemia cell line (MELC), clone 745A, was obtained from the Institute for Medical Research, Camden, NJ. Spinner cultures were maintained at 37°C in Iscove's minimum essential medium supplemented with 5% (v/v/) defined bovine calf serum (Hyclone Laboratories, Inc., Logan, UT). Induction of differentiation was achieved by addition of a stock solution of hexamethylene-bis(acetamide) (HMBA) (Sigma Chemical Co., St. Louis, MO) to a final medium concentration of 3 mM and maintenance of cultures for 4 days.

Subcellular fractionation

MELC pellets were lysed in distilled deionized water and homogenized in 50 mM Tris–HCl buffer (pH 8.0). After sequential centrifugation, the resulting 100000 × *g* supernatant (cytosol) served as the enzyme source. MELC cytosol was dialyzed for 24 h at 4°C against 100× volume of buffer with 4 exchanges. Protein content was determined by the method of Lowry et al. [6].

Uroporphyrinogen I (URO) synthase activity

Cytosolic URO synthase activity was measured by a modification [7] of the method of Strand et al. [8]. Cytosol was incubated with 100 μM porphobilinogen (PBG) (Porphyrin Products, Logan, UT) in 50 mM Tris–HCl/50 mM KCl (pH 7.8) at 37°C for

45 min. Following the addition of ethylacetate/acetic acid (2:1), 1.0 N HCl was added and the mixture shaken. After phase separation and standing for 30 min in room light, porphyrin fluorescence in the lower aqueous phase was determined and compared to a uroporphyrin I standard (Porphyrin Products) using a spectrophotofluorometer.

δ-Aminolevulinic acid dehydratase (ALA-D) activity

Cytosolic ALA-D activity was measured by the method of Gibson et al. [9]. The product, porphobilinogen (PBG), was measured spectrophotometrically (553 nm) after reaction with Ehrlich's reagent using a mM extinction coefficient of 61.

Effects of lead and pteroylpolyglutamates ($PteGlu_n$) on enzyme activities

Pteroylpolyglutamates in oxidized form were obtained from Dr. N.G. Nair, University of South Alabama Medical Sciences Foundation, Mobile, AL. Pb as lead acetate and pteroylpolyglutamates as free acids were added to the incubation mixtures as described in the text. Pteroylpolyglutamates exhibited 95% homogeneity by glutamate chain length as determined by high-performance liquid chromatography (HPLC) as described by Shane [10].

Pteroylpolyglutamate distribution in MELC

Endogenous pteroylpolyglutamates in MELC extracts were quantitatively cleaved to *p*-aminobenzoylpolyglutamates and separated according to glutamate chain length by HPLC as described by Shane [10].

Statistics

Data were analyzed by Student's *t*-test to determine the significance of difference between means.

RESULTS AND DISCUSSION

The effect of Pb on uroporphyrinogen synthase was studied in MELC. The results showed that URO synthase activity in untreated cytosol was unaffected by incubation with 50 μM Pb (Table I). Dialysis of cytosol rendered URO synthase activity sensitive to Pb, showing an 80% decrease in activity in Pb-treated cytosol compared to controls. Thus, MELC cytosol exhibited similarities to hepatic cytosol with respect to the presence of a dialyzable factor conferring protection on URO synthase against inhibition by Pb [1]. The dialyzable factor was postulated to be a derivative of pteroylpolyglutamic acid.

To test this possibility, various pteroylpolyglutamates were tested for the ability to protect URO synthase against inhibition by Pb in dialyzed cytosol. Although all of the pteroylglutamates tested decreased control URO synthase activity somewhat, only pteroylpentaglutamate ($PteGlu_5$) was capable of conferring protection against

TABLE I

EFFECT OF DIALYSIS AND THE ADDITION OF PTEROYLGLUTAMATES OF VARIABLE CHAIN LENGTHS ($PteGlu_n$) ON INHIBITION OF UROPORPHYRINOGEN (URO) SYNTHASE BY LEAD

Condition	URO synthase activity[a] (nmol URO/mg protein/45 min)		% Control
	Control	50 μM Pb	
No dialysis	6.70 ± 0.30	6.88 ± 0.09	103
Dialysis	6.65 ± 0.08	1.53 ± 0.16	23
Dialysis + $PteGlu_1$[b]	5.41 ± 0.23	0.72 ± 0.17	13
+ $PteGlu_2$	4.35 ± 0.09	0.75 ± 0.05	17
+ $PteGlu_4$	4.25 ± 0.05	0.84 ± 0.08	20
+ $PteGlu_5$	4.25 ± 0.05	3.42 ± 0.05*	80
+ $PteGlu_6$	3.93 ± 0.05	0.65 ± 0.05	17

[a]Values are the means ± SEM for 3 determinations.

[b]Pteroylglutamates were added to dialyzed cytosol at a concentration of 100 μM.

*Significantly different from dialysis/Pb, $P < 0.01$.

Pb inhibition (Table I). A rather high concentration (100 μM) of $PteGlu_5$ was required to protect URO synthase from Pb inhibition. One possible explanation is that the endogenous protective factor is a more potent biological derivative of $PteGlu_5$ [11]. The results in Figure 1 showed that the protection of URO synthase from Pb inhibition by $PteGlu_5$ was rapid, with near control levels of uroporphyrinogen formation observed in the first 5 min of incubation. Reduced glutathione (GSH), a dialyzable sulfhydryl compound, was also found to protect against Pb inhibition of URO synthase but only at millimolar concentrations. This concentration exceeded normal cytosolic GSH levels by at least 100-fold (data not shown).

The specificity of $PteGlu_5$ for protection of URO synthase against Pb inhibition was examined by testing the ability of $PteGlu_5$ to protect another heme biosynthetic enzyme known to be extremely sensitive to Pb, δ-aminolevulinic acid dehydratase (ALA-D) [9]. $PteGlu_5$ failed to protect against a Pb-mediated decrease in ALA-D activity (Table II), suggesting that the protective effect was specific for URO synthase and not merely a Pb-chelating function [1].

Analysis of the distribution of endogenous pteroylglutamates in MELC revealed a mixture of pteroylmono-, tetra-, penta- and hexaglutamate, with pentaglutamate predominating (> 50% of total endogenous pteroylglutamate) (Fig. 2). Thus, by glutamate chain length, the pteroylpolyglutamate which protected URO synthase against Pb inhibition was the predominant endogenous form. Identification of the exact structure of this endogenous pteridine should aid in elucidating the specific mechanism of protection of URO synthase. Such studies are currently in progress.

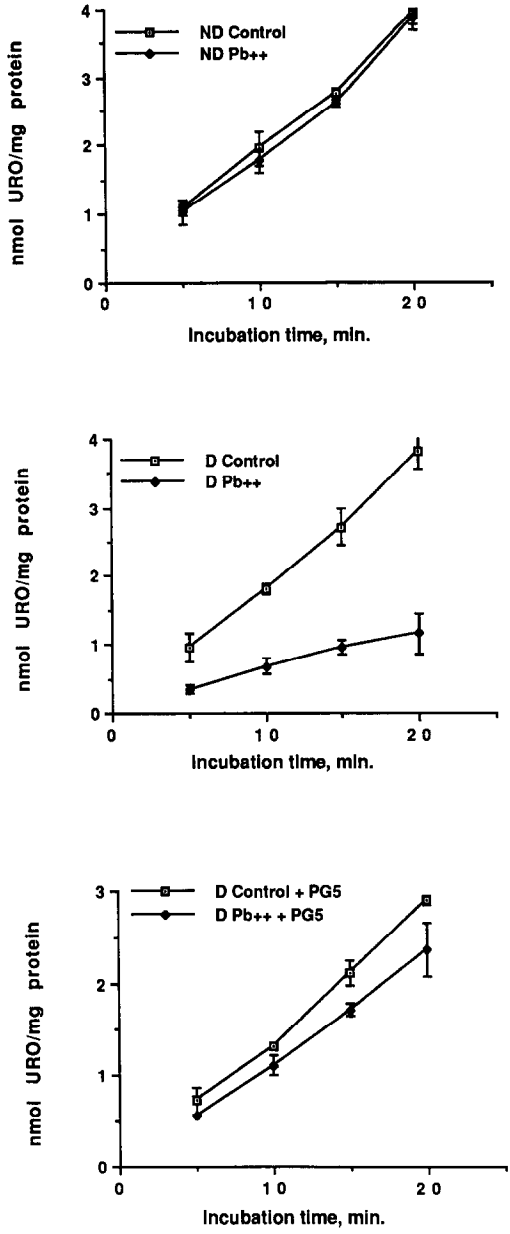

Fig. 1. Effect of dialysis and pteroylpentaglutamate addition on the time-dependent formation of uroporphyrinogen in the presence of Pb. MELC cytosol (ND = non-dialyzed; D = dialyzed; D + PG5 = dialyzed + 100 μM $PteGlu_5$) was incubated with porphobilinogen substrate at 37°C in the absence or presence of Pb acetate (50 μM) and the reaction terminated at 5, 10, 15 and 20 min. Values are the means ± SEM for 3 determinations.

TABLE II

EFFECT OF THE ADDITION OF PTEROYLPENTAGLUTAMATE ($PteGlu_5$) ON INHIBITION OF δ-AMINOLEVULINIC ACID DEHYDRATASE (ALA-D) ACTIVITY BY LEAD

Condition	ALA-D activity[a] (nmol PBG/mg protein/h)		% Control
	Control	50 μM Pb	
No dialysis	25.09 ± 0.35	11.89 ± 0.48	47
Dialysis	64.00 ± 0.40	33.75 ± 0.61	53
Dialysis + $PteGlu_5$[b]	42.67 ± 1.14	18.36 ± 1.44	43

[a]Values are the means ± SEM for 3 determinations.

[b]Pteroylpentaglutamate was added to dialyzed cytosol at a concentration of 100 μM.

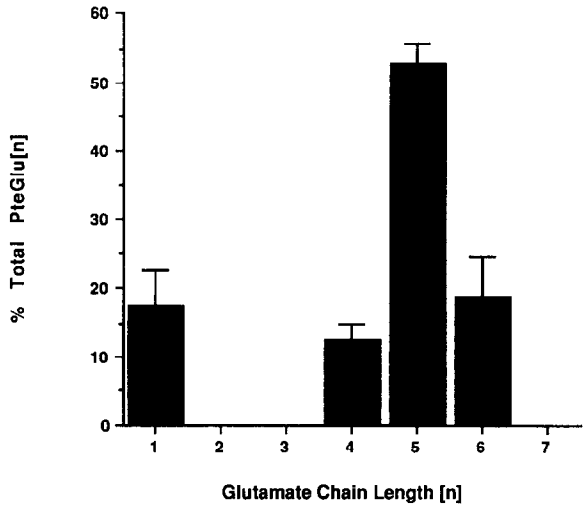

Fig. 2. Distribution of endogenous pteroylpolyglutamates ($PteGlu_n$) in differentiating MELC cultures. MELC cultures were maintained in HMBA-containing medium for 4 days. Endogenous folates in MELC extracts were quantitatively cleaved to *p*-aminobenzoylpolyglutamates and analyzed by HPLC as described by Shane [10]. Distribution of polyglutamate chain length is expressed as the percentage of total endogenous pteroylglutamate (2.23 ± 0.18 nmol/g cells). Values are the means ± SEM for 3 determinations.

These initial studies demonstrate the potential usefulness of MELC to study the role of endogenous folate content and the regulation of pteroylpolyglutamate synthesis on uroporphyrinogen synthesis and heme formation in bone marrow during Pb exposure.

ACKNOWLEDGEMENT

This research was supported by Grant ES-02424 from the National Institutes of Health.

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