FEBS Letters 583 (2009) 1011–1016

journal homepage: www.FEBSLetters.org

pH-Dependent Fe (II) pathophysiology and protective effect of an organoselenium compound

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ARTICLE INFO

Article history:
Received 30 November 2008
Revised 8 February 2009
Accepted 11 February 2009
Available online 21 February 2009

Edited by Aleksander Benjak

Keywords:
pH
Lipid peroxidation
Organoselenium compound

ABSTRACT

Influence of pH on the extent of lipid peroxidation and the anti-oxidant potential of an organoselenium compound is explored. Acidosis increased the rate of lipid peroxidation both in the absence and presence of Fe (II) in rat's brain, kidney and liver homogenate and phospholipids extract from egg yolk. The organoselenium compound significantly protected lipids from peroxidation, both in the absence and presence of Fe (II). Changing the pH of the reaction medium did not alter the anti-oxidant activity of the tested compound. This study provides in vitro evidence for acidosis-induced oxidative stress in brain, kidney, liver homogenate and phospholipids extract and the anti-oxidant action of the tested organoselenium compound.

1. Introduction

The role of iron in catalyzing oxygen-derived free radical production is well known, and there is evidence that free radicals may be a primary cause of cerebral damage during ischemia and post ischemic reperfusion [1]. The pH of tissue could modulate the ability of iron to participate in detrimental lipid peroxidation reactions. It has been suggested that metabolic changes induced by ischemia, such as acidosis [2] lead to intracellular iron delocalization [3] providing a source of iron in a form capable of catalyzing free radical production. Thus, the fall in intracellular pH that is associated with ischemia cannot only influence metabolic processes, but it can also potentate or act sinergically with oxidative stress, contributing to increased cellular injury. Iron is more soluble at lower pH values; therefore, we hypothesized that decreasing the pH of the reaction medium will lead to increased lipid peroxidation. For this purpose we have studied the effect of pH on Fe (II)-mediated lipid peroxidation in rat's brain, kidney, liver homogenates and phospholipids extract from egg yolk by measuring thiobarbituric acid-reactive species (TBARS).

Organoselenium compounds have been described to possess very interesting biological activities. Reports have shown that these selenium-containing organic compounds are generally more potent anti-oxidants than classical anti-oxidants and this fact serves as an impetus for an increased interest in the rational design of synthetic organoselenium compounds [4,5]. From a hypothetical point of view, the formation of stables selenolate (Se^{-1}) ions can increase the reducing properties of these moieties on the organochalcogenides and hypothetically can increase their anti-oxidant properties. However, there is no data in the literature supporting this assumption. Thus, to get a deeper insight into the potential use of an organoselenium compound 2-((1-(2-(2-(2-(1-(2-hydroxybenzylideneamino) ethyl) phenyl) diselanyl) phenyl) ethylimino) methyl) phenol (Compound A) (Fig. 1) as a possible pharmacological agent, we have determined for the first time the influence of pH on protective effect of Compound A in vitro at different pH ranging from low (acidic) to physiological values in rat's brain, kidney, liver and phospholipids extract from egg yolk.

2. Materials and methods

Compound A (Fig. 1) was synthesized according to literature methods [6,7] with little modifications. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that the compound obtained (with 99.9% purity) presented analytical and spectroscopic data in full agreement with their assigned structure. All other chemicals were purchased from standard suppliers.

Brain, kidney and liver was removed from adult male wistar rats, while phospholipids were extracted from eggs yolk by a solution 3:2 of hexane-isopropanol in the proposition of 1 g of egg yolk to 10 ml of this solution. The mixture was filtered and put in the rotary evaporator with the maximum temperature of 60 °C. Extract was weighed and dissolved in distilled water in the proposition of

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0014-5793/$36.00
doi:10.1016/j.febslet.2009.02.020

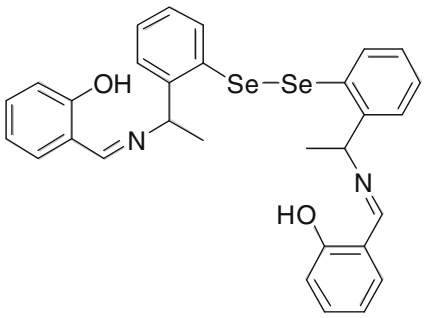

Fig. 1. Chemical structure of Compound A, i.e. 2-((1-(2-(2-(2-(1-(2-hydroxybenzylideneamino) ethyl) phenyl) diselanyl) phenyl) ethylimino) methyl) phenol.

25 mg of extract to 10 ml of water which was used for (TBARS) assay. Lipid peroxidation was determined by measuring (TBARS) as described by Ohkawa et al. [8] with a slight modification that pH of the incubation medium was changed from 5.4 to 7.4 (i.e. 5.4, 5.8, 6.4. 6.8 and 7.4).

3. Results and discussion

Two-way analysis of variance (ANOVA) of Fe (II)-induced TBARS levels in rat's brain, kidney, liver and phospholipids extract from egg yolk revealed a significant main effect of pH and Fe (II) and also a significant Fe × pH interaction ($P < 0.05$). Indeed, lipid peroxidation in the absence of Fe (II) is enhanced upon a shift in the pH of the incubation solutions from physiological conditions (pH 7.4) to acidic ones (pH 5.4) as shown in (Fig. 2). Similarly, the amount of TBARS produced by incubation of homogenate and phospholipids extract with Fe (II) alone at pH 7.4 was lower. However, as the pH of the solution was decreased from 7.4 to 5.4, Fe (II)-dependent TBARS production markedly increased as shown in (Fig. 2).

The enhancement of pH dependent lipid peroxidation can be attributed to mobilized iron, which may come from reserves where it is weakly bound. It has been shown that the protein transferrin carries two iron ions, although only about one third of it is normally saturated with iron [9]. Transferrin loses its bound iron at acidic pH. The initial 10% of iron in saturated human transferrin is lost at a pH of 5.4 and the final 10% at a pH of 4.3 [10]. On the other hand, if transferrin is bound to its receptor, essentially all the iron is released at pH 5.6–6.0 [11]. The pH-dependent affinity of transferrin for iron decreases under acidic conditions leading to dissociation of iron from transferrin and other proteins like ferritin and lactoferrin [12]. Once mobilized, free iron likely binds non-specifically to a variety of small molecular moieties and augments the ordinarily small low molecular weight (LMW) non-protein-bound tissue pool. In cortical homogenates, striking increases in LMW iron are observed at pH 6.0 when pH is reduced from 7.0 by direct addition of lactic acid. Furthermore, brain from decapitated hyperglycemic rats shows elevated LMW iron relative to normoglycemic controls [13]. The mobilized iron Fe (II) can interact with enzymatically and/or non-enzymatically generated superoxide ($O_2^{\cdot -1}$) (Haber–Weiss reaction) and/or hydrogen peroxide (H_2O_2) (Fenton reaction) [14,15] producing reactive oxygen species. In fact, $O_2^{\cdot -1}$ and H_2O_2 may be produced directly from dissolved oxygen (O_2) in aqueous media in the Fe (II)-mediated basal/autooxidation reactions as follows:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\cdot -1} \quad (1)$$

The dismutation of superoxide to hydrogen peroxide and oxygen has been shown to be faster at acidic pH [13]

$$HO_2^{\cdot} + O_2^{\cdot -1} + H^+ \rightarrow H_2O_2 + O_2 \quad (2)$$

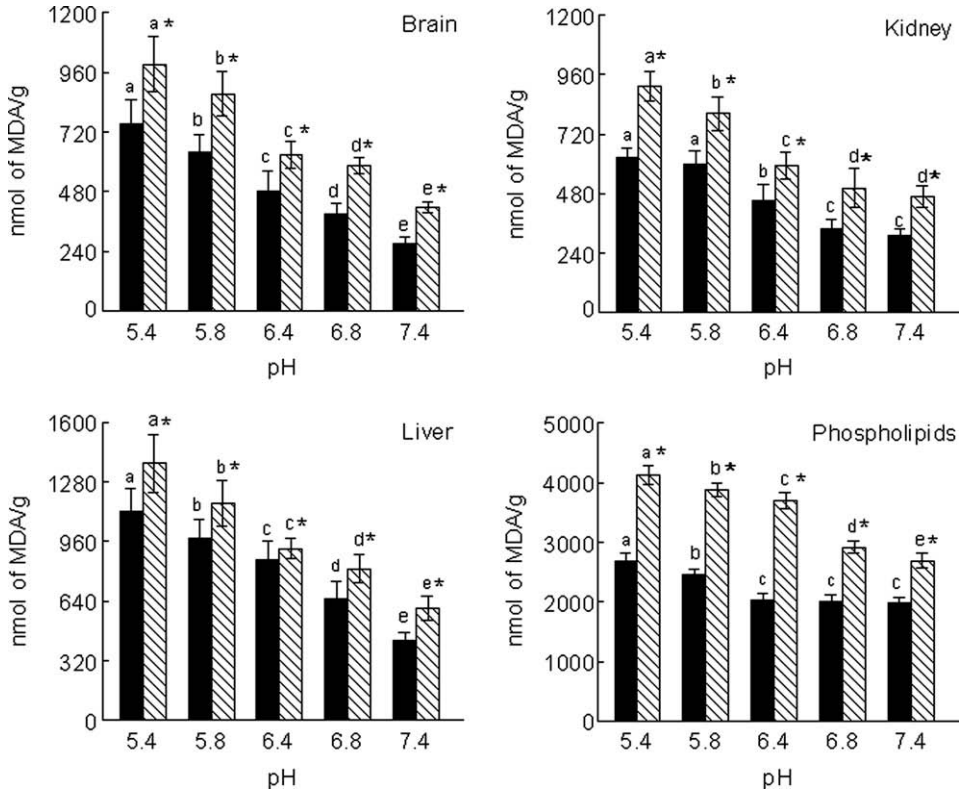

Fig. 2. Effect of pH on basal (shaded bar) or Fe (II)-induced (bar with lines) TBARS production in rat's brain, kidney, liver homogenate and phospholipids extract from egg yolk. The values are expressed as nmol of MDA per gram of tissue. Data are expressed as means ± S.E.M. ($n = 5–7$). Different letters shows significant difference from each pH group while asterisk shows significant main effect of Fe (II) at $P < 0.05$.