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Protective effect of β-carbolines and other antioxidants on lipid peroxidation due to hydrogen peroxide in rat brain homogenates

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Abstract

Tryptoline and pinoline are two β-carbolines isolated from the nervous system of mammals. We investigated the ability of these compounds to prevent lipid peroxidation induced by hydrogen peroxide in rat brain homogenates. We also compared their effects with other known antioxidants including melatonin, trolox and ascorbic acid. Lipid peroxidation was assessed by measuring malonaldehyde (MDA) and 4-hydroxy-alkenals (4-HDA) concentrations in the brain homogenates. Incubation with hydrogen peroxide (5 mM) increased MDA + 4-HDA levels, which were totally prevented by tryptoline, pinoline, melatonin and trolox in a concentration-dependent manner. By contrast, higher MDA-4-HDA concentrations compared with control experiments were found after incubation with ascorbic acid, thus reflecting an increase of lipid peroxidation induced by this compound. Although in vivo studies are needed, the data suggest that these β-carbolines may be potential neuroprotective agents because of their antioxidant activities.

Keywords: Tryptoline; Pinoline; Melatonin; Hydrogen peroxide; Lipid peroxidation; Brain homogenates

Hydroxyl radicals generated via Fenton reaction from hydrogen peroxide, which is produced in vivo in the brain [26], may initiate and propagate the degenerative reaction in the cell membranes known as lipid peroxidation. The nervous system is highly sensitive to free radical injury because neurons have an elevated metabolic rate. Additionally, the brain contains high concentrations of iron and has relatively poorly developed antioxidant defense mechanisms [23]. Several molecules including malondaldehyde (MDA) and other aldehydes are produced as a consequence of lipid peroxidation [11].

1,2,3,4-Tetrahydro-β-carboline (tryptoline) and 6-methoxy-1,2,3,4-tetrahydro-β-carboline (pinoline) are two tricyclic compounds present in the brain with a wide neuroanatomical distribution [14,20]. Although their physiological role is unclear, it has been reported that pharmacological concentrations of these compounds may inhibit the activity of the monoamine oxidase [28]. Both β-carbolines show benzodiazepine-like anxyolytic effects in animals subjected to stress [13,16,17,25], and also increase brain serotonin levels [16]. Tryptoline reduces the activity of complex I of the mitochondrial respiratory chain, the main source of free radicals in vivo [4]. Furthermore, melatonin and pinoline stabilize hepatic microsomal membranes against lipid peroxidation induced by $FeCl_3$, adenosine diphosphate (ADP) and nicotinamide adenine dinucleotide phosphate (NADPH)s [9,10].

Recently, it was proposed that β-carbolines may derive from indoles by condensation with aldehydes [5], which are generated during peroxidation of membrane lipids. In the present study we tested the effect of tryptoline and pinoline in preventing lipid peroxidation induced in vitro by the addition of hydrogen peroxide to rat brain homogenates. We also compared them with some well-known antioxidants, i.e. melatonin, trolox and ascorbic acid, under the same experimental conditions. The concentrations of MDA plus 4-hydroxyalkenals (4-HDA) were measured in the brain homogenates to assess lipid peroxidation.

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Male Sprague–Dawley rats weighing about 200 g were anesthetised with a rodent cocktail (6:4 v/v ketamine 100 mg/ml and xylazine 20 mg/ml) and subjected to intracardiac perfusion with 0.9% NaCl (4°C). The brains were removed and kept frozen at −80°C until their homogenization with 20 mM Tris–HCl buffer, pH 7.4. Aliquots of brain homogenates (3 mg protein/ml) were incubated in a water bath at 37°C for 60 min with 5 mM H_2O_2 in the presence or absence of either tryptoline (0.001, 0.003, 0.01, 0.03, 0.1, 0.3 mM), pinoline (0.001, 0.003, 0.01, 0.03, 0.1, 0.3 mM), melatonin (0.001, 0.01, 0.1, 0.3, 1, 3 mM), trolox (0.001, 0.003, 0.01, 0.03, 0.1, 0.3 mM) or ascorbic acid (0.001, 0.01, 0.03, 0.1, 0.3, 1 mM). Lipid peroxidation was stopped by placing the homogenates into ice-cold water for 10 min. Homogenates were then centrifuged at 3000 × *g* for 10 min at 4°C. MDA + 4-HDA concentration was measured in the supernatants using a colorimetric commercial assay kit from Calbiochem (San Diego, CA). Results are expressed as the

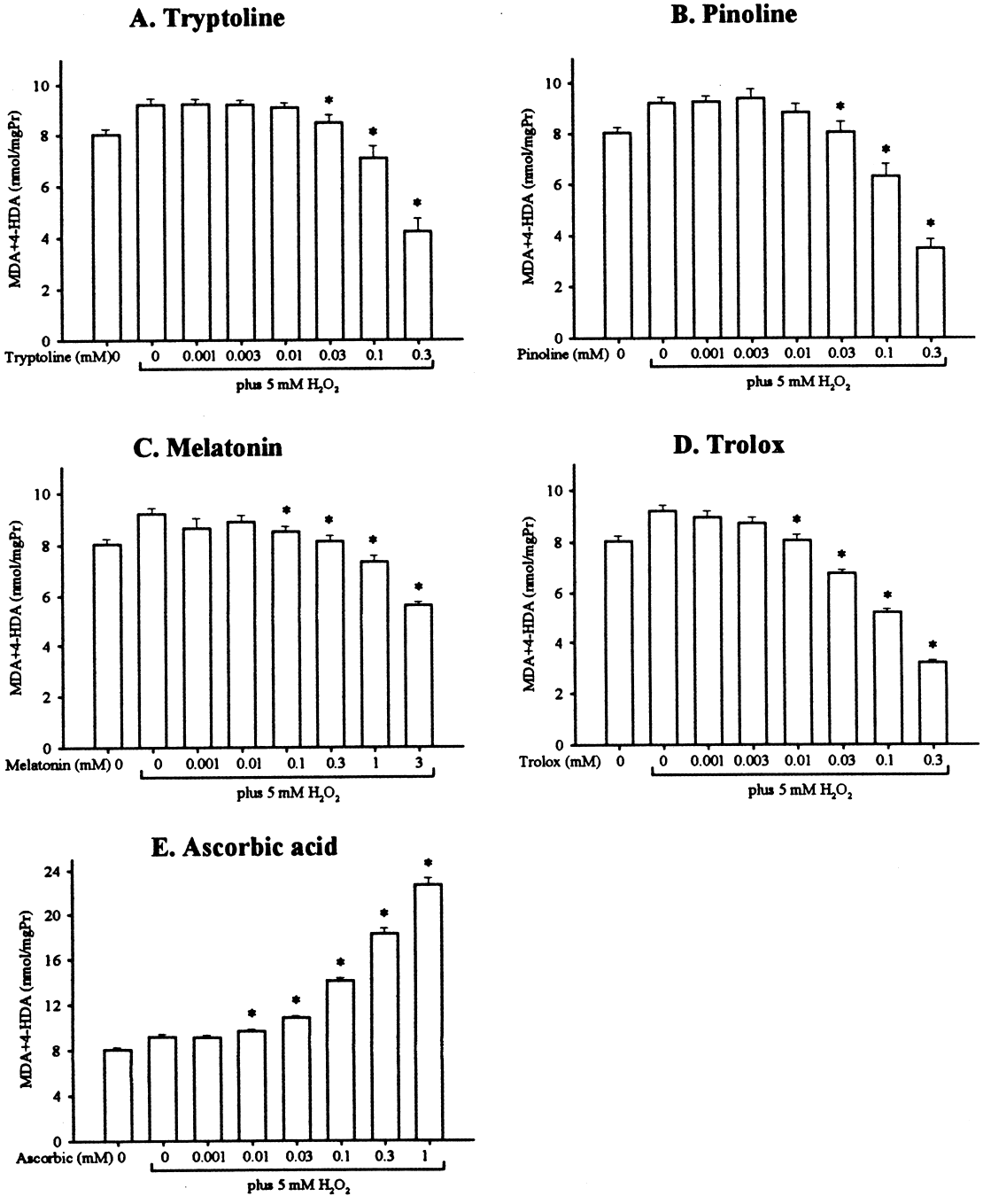

Fig. 1. The effect of several concentrations of tryptoline (A), pinoline (B), melatonin (C), trolox (D) and ascorbic acid (E) on lipid peroxidation induced by hydrogen peroxide (5 mM) in rat brain homogenates. The values represent the means ± SE obtained in six independent experiments. *$P < 0.05$ vs. hydrogen peroxide.

means ± SE of nmol MDA + 4-HDA/mg protein. The conditions used for induce lipid peroxidation in the brain homogenates were standardized in our laboratory [21]. Protein concentration was determined by the Bradford method using bovine serum albumin as a standard [3]. Statistical analysis was done using the Student's two-tailed *t*-test and the level of significance was accepted with $P < 0.05$.

As shown in Fig. 1, hydrogen peroxide significantly increased MDA + 4-HDA levels in brain homogenates ($P < 0.001$). The presence of tryptoline and pinoline reduced, in a concentration-dependent manner, the stimulatory effect of hydrogen peroxide on lipid peroxidation. Melatonin and trolox also prevented lipid peroxidation under these experimental conditions. In contrast, when ascorbic acid was added to the incubation medium, MDA + 4-HDA levels increased significantly. The highest concentration of tryptoline, pinoline, melatonin and trolox used reduced MDA + 4-HDA levels below control values.

The concentrations of tryptoline, pinoline, melatonin and trolox that reduced MDA + 4-HDA levels by 50% are presented in Table 1. The IC_{50} for pinoline was less than that for tryptoline IC_{50}. We previously reported that pinoline suppressed lipid peroxidation in brain homogenates [8,21], a protective role that may be related to its ability to reduce oxidative stress-dependent membrane rigidity. This stabilizing membrane effect of pinoline was further increased in the presence of melatonin [10]. Pinoline also reduced nitric oxide-induced lipid peroxidation in retinal homogenates [27] and this carboline is found in the human retina [14]. We have recently demonstrated that pinoline protects DNA against oxidative damage induced by chromium [22]. It is possible that pinoline scavenges hydroxyl radicals generated from hydrogen peroxide in the presence of $CuSO_4$. This scavenging action may depend on both the indolic ring and the methoxy group, which are features common to both pinoline and melatonin [19].

Herein it is shown, for the first time, that tryptoline also may reduce oxidative stress in brain homogenates. Tryptoline includes in its structure the indolic ring although it differs from pinoline and melatonin in the absence of the methoxy group at position 5. It has been shown that methoxyindoles are better antioxidants than hydroxyindoles [29] and the data reported here for β-carbolines are consistent with this observation.

Melatonin is a well-known endogenous antioxidant that may offer protection to the central nervous system because it scavenges free radicals and enhances the function of other antioxidants [1,7,9,23,24]. One molecule of melatonin is believed to scavenge two hydroxyl radicals yielding a tricyclic compound identified as cyclic 3-hydroxymelatonin; this product is proposed as a biomarker of in vivo oxidative stress [30]. Under our in vitro experimental conditions tryptoline and pinoline were more potent than melatonin in reducing lipid peroxidation. These data agree with previous studies showing that pinoline was more active in brain and retinal homogenates than melatonin [21,27]. Other in vitro experiments indicated that melatonin and pinoline are equipotent in preventing oxidative damage to DNA [22] and pinoline appears to scavenge the hydroxyl radical less actively than does melatonin [19]. In general, melatonin appears to be a much better antioxidant and free radical scavenger in vivo than in vitro.

Table 1
IC_{50} concentrations for tryptoline, pinoline, melatonin, trolox and ascorbic acid in preventing lipid peroxidation induced by hydrogen peroxide (5 mM) in rat brain homogenates

	IC_{50} (mM)
Trolox	4.2
Pinoline	15.4
Tryptoline	25.7
Melatonin	72.2
Ascorbic acid	–

Some studies have shown that synthetic derivatives from β-carbolines display higher antioxidant activity than vitamin E in preventing lipid peroxidation in potassium cyanide-treated rats [12]. However when compared with trolox, tryptoline and pinoline were slightly less active in attenuating MDA + 4-HDA damage in our experimental conditions. Trolox is a synthetic derivative of tocopherol produced to improve its access to the hydrophilic compartments of the cells, which exclude the tocopherols.

Ascorbic acid failed to prevent and, in fact, increased lipid peroxidation. One explanation for this paradoxical effect of the vitamin C may relate to the action of ascorbate in reducing transition metals that are present in brain homogenates. Thus, ascorbic acid exaggerates the Fenton reaction due to the presence of hydrogen peroxide in the incubation medium, thereby increasing lipid peroxidation [6].

Like melatonin, tryptoline and pinoline are quickly distributed to all tissues and they easily cross the blood–brain barrier when they are peripherally administered to mice [15,18]. Studies of the subcellular distribution of [^{3}H]-pinoline show that it is mainly localized in the cell nucleus [18], suggesting a potential role of this β-carboline in protecting DNA against free radical injury.

If they are to be used in vivo, another consideration with regard to these agents is the toxicity of the compounds. Carbolines are a large group of pharmacologically-active compounds and some synthetic analogs such as bromal-derived tetrahydro-β-carbolines have neurotoxic properties which impair nigrostriatal dopamine metabolism [4]. Pinoline has low toxicity and only a high intravenous dose (112 mg/kg) cause lethality in the 50% of mice [2]. In reference to melatonin no toxicity has been reported to date, and it has not been possible to define the LD_{50} for this molecule [24].

The evidence presented here suggests that tryptoline may be another molecule that reduces oxidative stress due to hydrogen peroxide. Since there are no data regarding the

in vivo effects of tryptoline and pinoline, further studies should be conducted to define the antioxidant properties of these β-carbolines.

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