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INHIBITION OF LIPID PEROXIDATION BY DIHYDROQUINOLINE-TYPE ANTIOXIDANT (CH 402)

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The *in vitro* effect of a non-toxic, water soluble, low molecular weight, stable dihydroquinoline-type antioxidant, CH 402 (Na (2,2-dimethyl-1,2-dihydroquinoline-4-yl) – methane sulphonic acid) was studied on free radical reactions in brain subcellular fractions. Experiments were performed using rat and mouse brain homogenate and microsomal fractions. Non – enzymatically induced lipid peroxidation by ascorbic acid was studied in correlation with ascorbic acid and CH 402 concentrations and incubation time. Malondialdehyde production during lipid peroxidation was measured by the thiobarbituric acid test. In a concentration range of 10^{-2}–10^{-5} M CH 402 dose – dependently inhibited the ascorbic acid induced *in vitro* lipid peroxidation in mouse and rat brain subcellular fractions.

KEY WORDS: Free radicals, lipid peroxidation, dihydroquinoline type antioxidant, brain microsomes.

INTRODUCTION

Free radicals are produced enzymatically or non-enzymatically in the organism and are eliminated by the action of certain vitamins and enzymes. Microscopic alterations in connection with the production of free radicals were demonstrated both under *in vitro* and *in vivo* circumstances.[1-5] The peroxidative chain-reaction of lipids induced by accumulated oxygen free radicals might be important in the pathogenesis of certain liver, kidney, lung, brain and age-related vascular diseases.[1,2,4,6-8] The damage caused by free radicals can be prevented by antioxidants, thus complementary treatment with drugs of this group can be suggested in diseases where free radicals play a leading role.[9-11]

Free radicals arising from the reduction of molecular oxygen exert their toxic effect by altering the lipid composition in membrane structure of subcellular fractions such as mitochondria and microsomes in the brain.

In this investigation we report the effect of a new, non-toxic dihydroquinoline – type antioxidant (CH 402)[12] on ascorbic acid induced, non – enzymatic lipid peroxidation in rat and mouse brain homogenate and microsomal fraction under various experimental conditions. The malondialdehyde production during he peroxidative demage and its inhibition by the dihydroquinoline-type antioxidant were also measured.

MATERIALS AND METHODS

Male Wistar rats weighing 150–200 g and male CBA/Ca mice weighing 25–30 g were used. The animals were killed by decapitation. A 10% (w/v) homogenate from total

brain was prepared in icecold 0.15 M KCl solution. Microsomal fraction was isolated by the method of Player and Horton.[3] Lipid peroxidation induced by ascorbic acid was studied under optimal conditions described elsewhere.[13] Malondialdehyde (MDA) formation was determined by the thiobarbituric acid reaction.[14] Protein content was evaluated by the biuret method as described by Gornall *et al.*[15] Subcellular fractions from rat and mouse brain were isolated after 15 min under constant shaking in a 37°C water bath. Lipid peroxidation was studied by incubating the protein (1 mg/ml) in a medium (total volume 0.5 ml) containing 50 mM Tris maleate buffer, pH 6.8 containing 1.0 mM KH_2PO_4 and various concentrations of ascorbic acid and CH 402.

CH 402 was obtained from Chinoin (Budapest), L-ascorbic acid from Merck (Darmstadt), bovine serum albumin from Calbiochem AG (Luzern) and all other reagents from Reanal (Budapest).

Results are presented as confidence limits of the means.

RESULTS

Lipid peroxidation was found to be induced by ascorbic acid in a dose dependent manner by rat and mouse brain microsomal fractin and homogenate. The non-enzymatic peroxidation induced by 10^{-1}–10^{-7} M ascorbic acid is markedly inhibited in the presence of 5×10^{-4} M Ch 402 in rat and mouse brain microsomal fraction and homogenate (Figure 1).

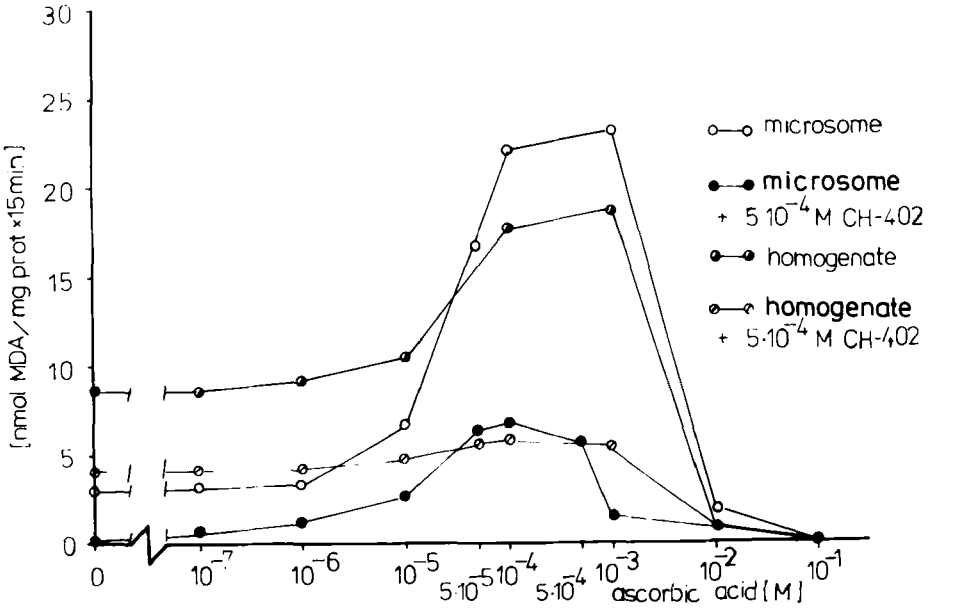

FIGURE 1 Inhibition of ascorbic acid induced lipid peroxidation by CH 402 in rat brain homogenate and microsomal fraction in 50 mM Tris maleate buffer, pH 6.8 containing 1 mM KH_2PO_4. Protein concentration was 1 mg/ml

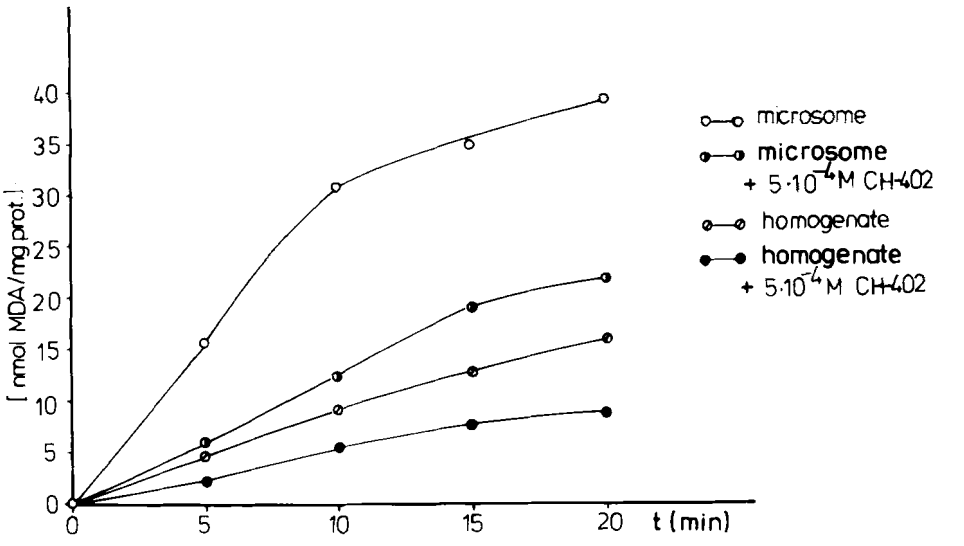

FIGURE 2 Inhibition of the time course of ascorbic acid induced lipid peroxidation by CH 402 in mouse brain homogenate and microsomal fractions. Ascorbic acid concentration: 5×10^{-5} M CH 402 concentration: 5×10^{-4} M. Medium as in Figure 1.

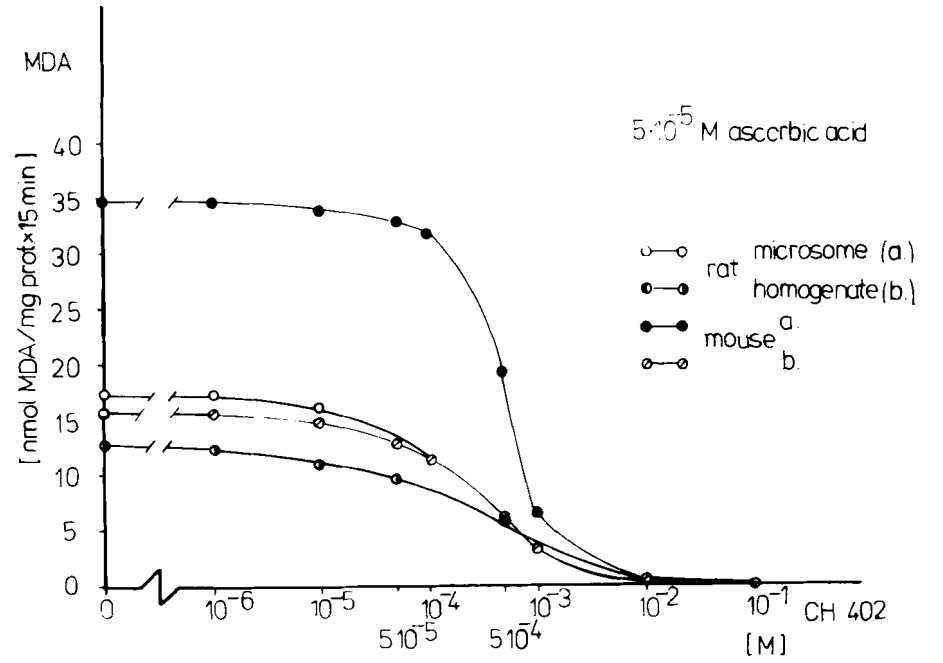

FIGURE 3 Dose dependent inhibiton of ascorbic acid induced lipid peroxidation by CH 402 in rat and mouse brain homogenate and microsomal fractions. Ascorbic acid concentration: 5×10^{-5} M, and CH 402 concentration: 10^{-1}–10^{-6} M. Medium as in Figure 1.

The time course of ascorbic acid induced lipid peroxidation and its inhibition by CH 402 was investigated at fixed concentrations of ascorbic acid and of CH 402. These were 5×10^{-5} M and 5×10^{-4} M, respectively. Lipid peroxidation increased with time but it was inhibited by CH 402 at each time it was investigated (Figure 2).

At a concentration range of 10^{-1}–10^{-6} M, CH 402 inhibited lipid peroxidation in rat and mouse brain microsomal fraction and homogenate in a dose-dependent fashion. A 50% inhibition was observed at a CH 402 concentration of 5×10^{-4} M (Figure 3).

DISCUSSION

In our previous studies we demonstrated the antioxidant and membrane stabilizing effect (10^{-4}–10^{-6} M) of (+) cyanidanol-3 (Catergen®) in subcellular fractins of rat brain.[16] Using liver and granulocyte preparations[17,18] chain reactions induced by lipid peroxidatin were found to be inhibited by two dihydroquinoline-type antioxidants: MTDQ-DA and MTDQ.[19]

In the present investigation the antioxidant effect of another dihydroquinoline-type agent CH 402 on *in vitro* lipid peroxidation is demonstrated. Species and age differences have been considered in designing the experiments. CH 402 at concentrations between 10^{-3}–10^{-5} M was found to inhibit ascorbic acid induced lipid peroxidation in brain microsomal fraction and homogenate in a dose-dependent manner.

This system offers several advantages in testing antioxidant activity since the brain studies *in vitro* has a great capacity to form lipid peroxides, having high proportion of polyunsaturated fatty acids in its various membranes, and because of continuous diffusion of oxygen through its membranes. This high capacity for lipid peroxidation can be further increased by adding ascorbic acid at low concentration to the system.[20]

On the basis of our present experiments CH 402 has been proven to be an effective antioxidant in ascorbic acid induced lipid peroxidation in rat and mouse brain. Consequently its application might be justified in various pathological disorders caused by free radicals reactions.

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