*Free Rad. Res. Comms.*, Vol. 6, No. 4, pp. 203–208
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# PEROXIDATION OF LINOLEIC ACID INDUCED BY INTERACTION WITH HAEMOGLOBIN AND HYDROGEN PEROXIDE

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*(Received July 16th 1988; in revised form September 23rd 1988)*

Peroxidation of linoleic acid was found to be induced by interaction with haemoglobin and hydrogen peroxide. The peroxidation of linoleic acid induced by this interaction was inhibited by desferrioxamine, ethylenediaminetetraacetic acid or α-tocopherol, and poorly by catalase. However, it was accelerated by ascorbic acid.

KEY WORDS: Haemoglobin, hydrogen peroxide, linoleic acid, peroxidation.

## INTRODUCTION

Lipid peroxidation (LOP) of brain synaptosomes induced by Fenton's reagent ($Fe^{2+} + H_2O_2$) has been shown to produce conjugated diene, lipid hydroperoxide and thiobarbituric acid reactive materials.[1] The peroxidation of linoleic acid has been found to be induced by addition of haemoglobin (Hb) from measurements of oxygen uptake.[2]

In this paper we demonstrate that LPO can be induced by Hb and hydrogen peroxide.

## MATERIALS AND METHODS

Ascorbic acid (AsA), catalase, ethylenediaminetetra-acetic acid (EDTA), Hb (methaemoglobin), hydrogen peroxide and linoleic acid were from Sigma Chemical Co. (St. Louis, Mo, U.S.A.), desferrioxamine was from Ciba-Geigy Co. (Basle, Switzerland). α-Tocopherol was obtained from Wako Pure Chemicals Co. (Osaka, Japan).

The standard reaction mixture contained 2.5 mM linoleic acid, $2.5 \times 10^{-1}$ mM hydrogen peroxide and $2.5 \times 10^{-1}$ mM Hb in Tris-HCl (pH 7.4) buffer in a total volume of 2.0 ml. AsA, catalase, desferrioxamine, EDTA, or α-tocopherol were also dissolved in the same buffer. The mixture was incubated at 37°C for 2 hrs throughout the experiment.

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LPO was estimated by measuring the amount of thiobarbituric acid - reactive substances (TBARS) by the thiobarbituric acid method.[3]

## RESULTS

Fig. 1 shows the time course of peroxidation of linoleic acid. TBARS generation (expressed as malondialdehyde, MDA) in the standard reaction mixture increased with time and reached a maximum (32.5 nmoles/ml) after 90 min, while in the absence of Hb or hydrogen peroxide little TBARS generation was detectable. TBARS generation was found to increase with the concentration of hydrogen peroxide, as shown in

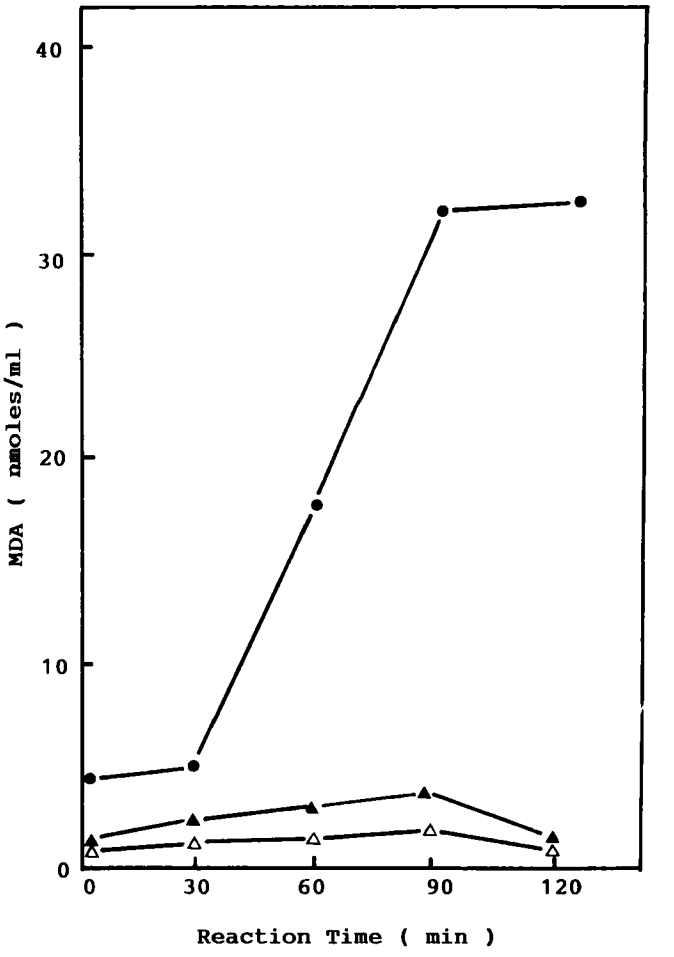


FIGURE 1 Time course of TBARS generation in the standard reaction mixture (●); Hb-absent (▲) or hydrogen peroxide-absent (△) reaction mixture.

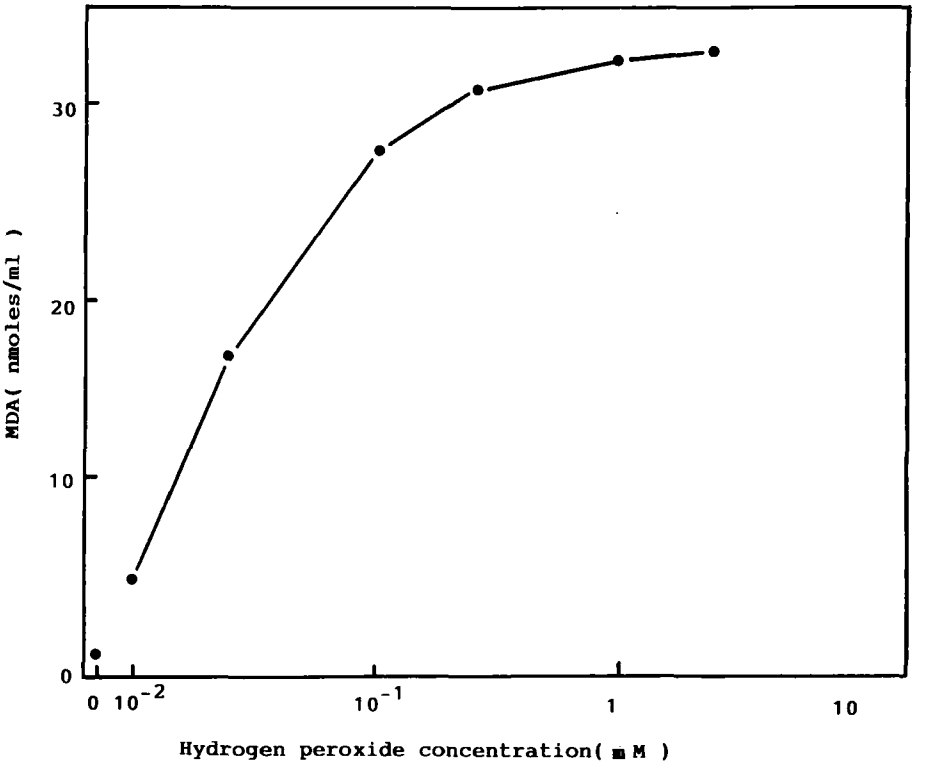


FIGURE 2 Effects of concentration of hydrogen peroxide on TBARS generation in the recent mixture.

Fig. 2, and to increase with the concentration of Hb up to a maximum (33.6 nmoles/ml) at 0.8 mM as shown in Fig. 3.

Table I shows the effects of AsA, catalase, desferrioxamine, EDTA, and α-tocopherol on TBARS generation in the standard reaction mixture. TBARS generation was inhibited by the addition of $2.5 \times 10^{-1}$, $2.5 \times 10^{-2}$, $2.5 \times 10^{-3}$ and $2.5 \times 10^{-4}$ mM EDTA respectively. On the addition of desferrioxamine or α-tocopherol inhibition was also observed. TBARS generation was inhibited poorly by addition of 25, $25 \times 10^{-1}$, $25 \times 10^{-2}$ or $25 \times 10^{-3}$ U/ml of catalase. Desferrioxamine in particular inhibited TBARS generation even at a concentration of $2.5 \times 10^{-4}$ mM. AsA did not inhibit TBARS generation, at a high concentration of $2.5 \times 10^{-1}$ mM there was stimulation of peroxidation.

## DISCUSSION

The iron-catalyzed Haber–Weiss reaction takes place by the following equations.

$$Fe^{3+} + O_2^{\cdot-} \longrightarrow Fe^{2+} + O_2$$

$$2O_2^{\cdot-} + 2H^+ \longrightarrow O_2 + H_2O_2$$

$$H_2O_2 + Fe^{2+} \longrightarrow OH^{\cdot} + OH^- + Fe^{3+}$$

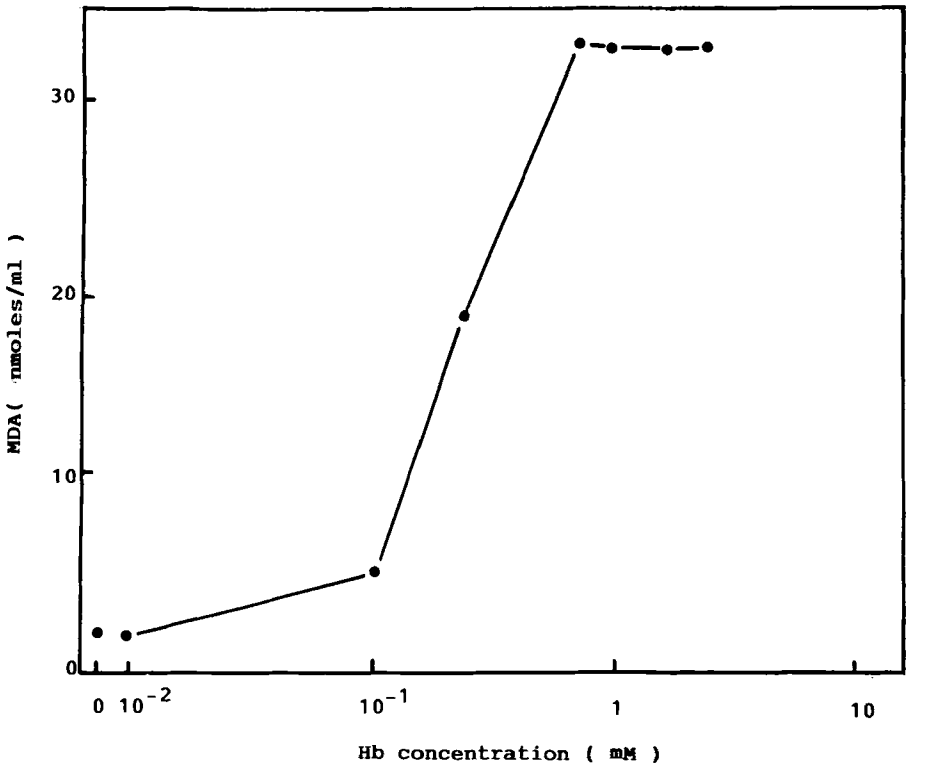


FIGURE 3 Effects of concentration of Hb on TBARS generation in reaction mixture.

Hydroxy radicals initiate LPO.[4] Girotti and Thomas[5] demonstrated that human erythrocyte ghosts are sensitive to oxidative damage. This damage is due to LPO and was completely inhibited by superoxide dismutase or catalase, indicating that $O_2^-$ and $H_2O_2$ are both required, possibly as precursors of $OH^\cdot$ via an iron-catalyzed Haber–Weiss reaction.

Halliwell gave the following equation for generation of $OH^\cdot$ radicals by iron chelates.[4]

$$Fe^{2+}\text{-chelate} + H_2O_2 \longrightarrow Fe^{3+}\text{-chelate} + OH^\cdot + OH^-$$

It is possible that Hb and hydrogen peroxide might interact in the same way as this equation to produce hydroxyl radicals.

Since in our experiment TBARS increased with the reaction time and with the concentration of Hb or hydrogen peroxide, its production results from the peroxidation of linoleic acid. Methaemoglobin was found to generate $OH^\cdot$ from $H_2O_2$.[6,7] Puppo and Halliwell reported that an excess of $H_2O_2$ degrades methaemoglobin, releasing iron ions that react with $H_2O_2$ to form a species that appears to be $OH^\cdot$.[8]

Desferrioxamine has been found to inhibit production of hydroxyl radicals in presence of free iron.[4,9] In this experiment, we demonstrated that TBARS generation was small after additions of this chelator. The addition of catalase decreased TBARS

TABLE I

Effects of ascorbic acid, catalase, desferrioxamine, ethylene diamine tetraacetic acid and α-tocopherol on TBARS generation in the standard reaction mixture

| Drugs | Concentration | MDA (nmol/ml) |
|---|---|---|
| None | | 33.45 ± 0.36 |
| Ascorbic acid (mM) | $2.5 \times 10^{-1}$ | 58.71 ± 0.70[a] |
| | $2.5 \times 10^{-2}$ | 35.40 ± 0.85 |
| | $2.5 \times 10^{-3}$ | 35.28 ± 0.32 |
| | $2.5 \times 10^{-4}$ | 34.68 ± 0.74 |
| Catalase (U/ml) | 25 | 25.22 ± 4.99[a] |
| | $25 \times 10^{-1}$ | 26.95 ± 2.81[a] |
| | $25 \times 10^{-2}$ | 25.54 ± 4.15[a] |
| | $25 \times 10^{-3}$ | 26.38 ± 3.57[a] |
| Desferrioxamine (mM) | $2.5 \times 10^{-1}$ | 4.21 ± 0.82[b] |
| | $2.5 \times 10^{-2}$ | 3.09 ± 0.31[b] |
| | $2.5 \times 10^{-3}$ | 5.98 ± 0.34[b] |
| | $2.5 \times 10^{-4}$ | 6.64 ± 1.15[b] |
| Ethylenediamine-tetraacetic acid (mM) | $2.5 \times 10^{-1}$ | 6.72 ± 1.12[b] |
| | $2.5 \times 10^{-2}$ | 8.56 ± 1.63[b] |
| | $2.5 \times 10^{-3}$ | 23.71 ± 1.03[a] |
| | $2.5 \times 10^{-4}$ | 23.47 ± 1.79[a] |
| α-Tocopherol (mM) | $2.5 \times 10^{-1}$ | 4.49 ± 0.76[b] |
| | $2.5 \times 10^{-2}$ | 4.81 ± 0.32[b] |
| | $2.5 \times 10^{-3}$ | 23.82 ± 3.13[a] |
| | $2.5 \times 10^{-4}$ | 22.47 ± 4.14[a] |

Values are shown means ± S.E. from 4 or more experiments.
[a]$P < 0.01$, [b]$P < 0.001$ compared with values for no addition.

generation, although only slightly, since catalase is a scavenger for $H_2O_2$. α-Tocopherol has been demonstrated to inhibit $O_2^-$ -or $OH^\cdot$ -induced LPO.[10] TBARS generation was also inhibited by addition of α-tocopherol in our investigation. AsA-$Fe^{2+}$ catalyzed LPO has been demonstrated by Hamilton.[11] The production of TBARS at high concentrations of AsA may be due to the interaction of AsA with the $Fe^{2+}$ of Hb with released $Fe^{2+}$.

Myoglobin, another heme-containing protein, was found to catalyze the peroxidation of arachidonic acid in the presence of hypoxanthine and xanthine oxidase and use hydrogen peroxide to form either an oxo-heme-oxidant or a caged radical that initiated peroxidation or arachidonic acid.[12]

These results indicates that the interaction with hydrogen peroxide of Hb, induces LPO.

*Acknowledgements*

The authors wish to thank Dr. Junichi Kawamata, Institute of Medical Science, Kansai Shinkyu Medical College, for his advice.

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Accepted by Prof J.V. Bannister