Senescence-associated decrease of NADPH-induced lipid peroxidation in rat liver microsomes

Thomas P.A. Devasagayam

Biochemistry Division, Bhabha Atomic Research Centre, Bombay 400 085, India

Received 24 July 1986

Senescence is associated with decrease in the NADPH-induced lipid peroxidation in liver homogenate as well as rough and smooth microsomes of female rats. In the microsomal fractions, sensitivity to NADPH-induced lipid peroxidation is high in young adults (3-month-old), decreases in middle aged (12-month-old) and reaches lowest levels in senescent (30-month-old) rats. Increasing the concentration of co-factors or time of incubation does not alter this resistance observed in the senescent rats. Major factors responsible for this resistance in senescent rats seem to be low levels of substrate in the form of phospholipids, NADPH-cytochrome *c* reductase, cytochrome P-450 and high cholesterol:phospholipid ratios besides enhanced levels of superoxide dismutase, α-tocopherol and reduced glutathione.

Senescence *Microsomal fraction* *NADPH* *Lipid peroxidation* *Phospholipid* *Antioxidant* *(Rat liver)*

1. INTRODUCTION

Peroxidation of membrane lipids is considered to be one of the most basic deteriorative reactions involved in senescence [1,2]. Senescence has been shown to be associated with an increase in in vivo lipid peroxidation [3], in the lipid peroxide content of serum [3], brain [4] and liver [5], in the increased accumulation of lipofuscin in tissues [2,4] as well as the increased lipid peroxidation in blood platelets [6] and rat liver mitochondria [7]. The present paper, however, reports that NADPH-induced lipid peroxidation in rat liver homogenate and microsomal fractions is low during senescence.

2. MATERIALS AND METHODS

2.1. *Animals and preparation of tissue fractions*

Young adult (3-month-old), middle aged (12-month-old) and senescent (30-month-old) rats of Wistar strain (all females) were used. Rats were starved overnight and killed by cervical dislocation. Liver, uterus and kidney were removed and homogenised in 0.15 M Tris-HCl buffer, pH 7.4. Hepatic rough and smooth microsomes were prepared as in [8]. Microsomes and tissue homogenates were suspended in the above buffer at 5 mg protein/ml.

2.2. *Lipid peroxidation*

Malonaldehyde content was estimated in the freshly prepared tissue fractions using the modified thiobarbituric acid (TBA) method [8]. The assay system for the NADPH-induced lipid peroxidation was similar to that described in [8] and contained 50 μl tissue fraction, 50 μM $FeCl_3$, 4 mM ADP, 1 mM KH_2PO_4 and 0.4 mM NADPH in 0.15 M Tris-HCl buffer, pH 7.4, in a total volume of 0.5 ml. After incubation at 37°C in a shaking water bath, the malonaldehyde formed was estimated [8]. Values were corrected for endogenous malonaldehyde content.

2.3. *Factors related to lipid peroxidation*

Standard methods, as reported earlier [8,9], were used for the assay of phospholipids, degree of

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

unsaturation in lipids, cholesterol, NADPH-cytochrome *c* reductase, cytochrome P-450, ascorbic acid, reduced glutathione, α-tocopherol and superoxide dismutase.

3. RESULTS

3.1. *Lipid peroxidation in tissue fractions of young adult and senescent rats*

Table 1 presents data on the malonaldehyde content and NADPH-induced lipid peroxidation in some tissue fractions of young adult and senescent rats. Malonaldehyde content in kidney, uterus and liver homogenates of senescent rats is significantly higher when compared with that of young adults. But in the case of hepatic microsomal fractions, the malonaldehyde content is significantly high in young adults. NADPH-induced lipid peroxidation in kidney homogenates is similar in both age groups whereas in other tissue fractions studied it is significantly lower in the senescent rats.

3.2. *Time course of NADPH-induced lipid peroxidation in the microsomal fractions*

In both rough and smooth microsomes, the highest levels of NADPH-induced lipid peroxidation, as a function of time, were observed in young adults followed by middle aged rats and lowest in the senescent rats (fig.1a,b). The microsomal frac-

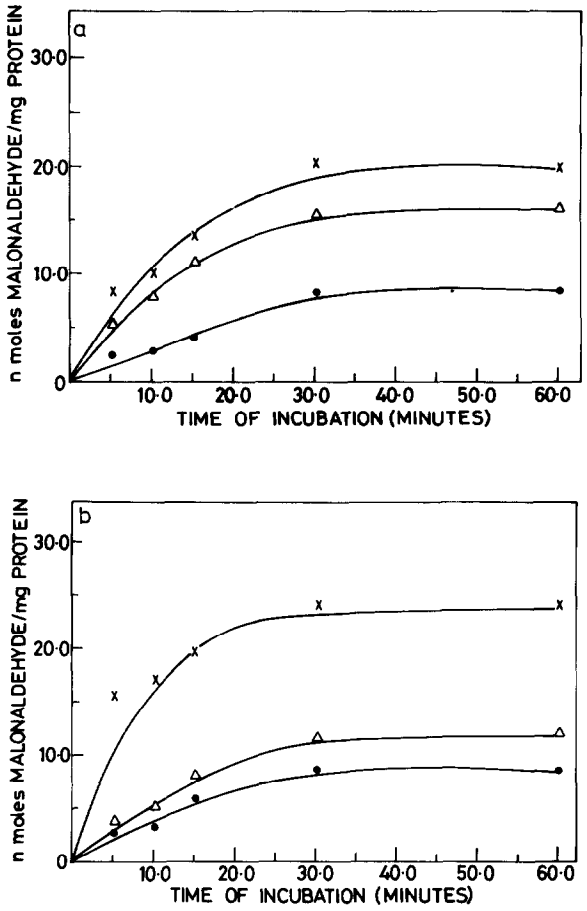

Fig.1. Time course of NADPH-induced lipid peroxidation in hepatic (a) rough and (b) smooth microsomes of (×) young adult, (△) middle-aged and (●) senescent rats. The incubations were carried out as described in section 2 using full complement of co-factors with microsomes corresponding to 0.25 mg protein. Each value represents the mean ± SE from 5 rats.

Table 1

Lipid peroxidation in tissue fractions of young adult and senescent rats

Tissue fraction	Malonaldehyde content[a]		NADPH-induced lipid peroxidation[b]	
	Young adult	Senescent	Young adult	Senescent
Kidney homogenate	1.43 ± 0.19	2.33 ± 0.25[c]	3.80 ± 0.38	4.06 ± 0.24
Uterus homogenate	0.83 ± 0.18	1.63 ± 0.26[c]	3.72 ± 0.36	1.75 ± 0.30[d]
Liver homogenate	0.36 ± 0.07	0.76 ± 0.04[d]	8.37 ± 0.43	6.63 ± 0.47[c]
Liver rough microsomes	1.85 ± 0.08	0.61 ± 0.05[e]	20.15 ± 1.40	8.21 ± 0.48[e]
Liver smooth microsomes	2.12 ± 0.15	1.18 ± 0.08[e]	24.12 ± 1.51	8.48 ± 0.59[e]

Values are means ± SE from 5 rats and are expressed as [a] nmol malonaldehyde/mg protein in the freshly prepared tissue fractions and [b] nmol malonaldehyde/mg protein after incubation at 37°C for 30 min. [c] $P < 0.05$, [d] $P < 0.01$ and [e] $P < 0.001$ as compared to young adults

tions of young adult and middle aged rats show differential response whereas those of senescent rats do not show any such trend.

3.3. *Lipid peroxidation with optimum concentration of co-factors*

Data on lipid peroxidation at optimum concentrations of co-factors in the microsomal fractions of young adult and senescent rats are given in table 2. The results show that even the increased concentration of co-factors does not alter the resistance to lipid peroxidation observed in senescent rats, as compared with that of young adults. In both age groups malonaldehyde production in the microsomal fractions is optimum with 4 mM NADPH and 1 mM Fe^{3+}.

Table 2

NADPH-induced lipid peroxidation with optimum concentration of co-factors in hepatic rough and smooth microsomes of young adult and senescent rats

Microsomal fraction	Co-factor	Optimum concentration (mM)	Lipid peroxidation	
			Young adult	Senescent
Rough	NADPH	4.0	17.85 ± 0.50	10.33 ± 0.38[a]
	Fe^{3+}	1.0	34.78 ± 2.62	17.60 ± 1.75[a]
Smooth	NADPH	4.0	34.96 ± 2.62	14.90 ± 1.41[a]
	Fe^{3+}	1.0	76.61 ± 3.90	17.49 ± 2.08[a]

Incubation mixtures contained the full complement of co-factors as described in section 2 except for the co-factor mentioned above. 0.01–4 mM co-factors were used to obtain the optimum concentrations. Lipid peroxidation values are means ± SE from 5 rats and are expressed as nmol malonaldehyde/mg protein after incubation at 37°C for 30 min. [a] $P < 0.001$ as compared to young adults

Table 3

Factors related to lipid peroxidation in hepatic rough and smooth microsomes of young adult and senescent rats

Factor	Rough microsomes		Smooth microsomes	
	Young adult	Senescent	Young adult	Senescent
Phospholipid[a]	270 ± 41	210 ± 32	402 ± 50	198 ± 41[e]
Degree of unsaturation in lipids[b]	0.95 ± 0.08	1.08 ± 0.15	1.45 ± 0.18	1.23 ± 0.14
Cholesterol : phospholipid ratio	0.13	0.94	0.18	1.11
NADPH-cytochrome *c* reductase[c]	15.14 ± 2.12	6.55 ± 0.82[f]	20.44 ± 1.85	9.73 ± 1.22[f]
Cytochrome P-450[d]	1.28 ± 0.19	0.71 ± 0.06[e]	2.25 ± 0.38	1.16 ± 0.14[e]
Ascorbic acid[a]	0.65 ± 0.09	1.55 ± 0.18[f]	1.75 ± 0.20	2.79 ± 0.24[f]
Reduced glutathione[a]	1.62 ± 0.20	4.82 ± 0.61[f]	4.73 ± 0.50	6.84 ± 0.56[e]
α-Tocopherol[a]	2.27 ± 0.19	3.51 ± 0.33[e]	2.68 ± 0.32	2.29 ± 0.31
Superoxide dismutase[c]	10.66 ± 1.70	16.70 ± 2.12	9.78 ± 1.24	17.31 ± 2.05[e]

Values are means ± SE from 5 rats and are expressed as [a] μg/mg protein, [b] μmol equiv./mg protein, [c] units/mg protein and [d] nmol/mg protein. [e] $P < 0.05$ and [f] $P < 0.01$ as compared to young adults

3.4. *Factors related to lipid peroxidation*

Table 3 presents data on the factors related to lipid peroxidation in the microsomal fractions of young adult and senescent rats. Substrate for lipid peroxidation, in the form of phospholipids, is significantly lower in the microsomal fractions of senescent rats. Other factors which enhance lipid peroxidation such as NADPH-cytochrome *c* reductase and cytochrome P-450 are significantly more in young adults whereas inhibitors of lipid peroxidation like superoxide dismutase, α-tocopherol and reduced glutathione are significantly higher in senescent rats. The cholesterol: phospholipid ratio which determines the fluidity of membranes and the amount of ascorbic acid is also higher in senescent rats.

4. DISCUSSION

Senescence is associated with an overall decline in the form and function of cells and organs [1–4]. Accumulation of products of lipid peroxidation such as lipofuscin along with enhanced lipid peroxidation has been suggested as causative factors leading to decreased efficiency of cells and organelles [1–6].

In mammalian liver, the NADPH-induced system is the major enzymatic system which induces lipid peroxidation [10]. It is mediated by NADPH-cytochrome *c* reductase [11], a component of the major drug-metabolizing system, namely the mixed-function oxidase system [12,13]. One of the major reasons suggested for the decreased capacity of senescent animals to metabolize drugs is the low levels of NADPH-cytochrome *c* reductase and other components of the mixed-function oxidase system [13]. Based on this observation one would expect a decline in NADPH-induced lipid peroxidation in senescent animals. The present report shows such a trend in hepatic microsomes as well as homogenate in female rats. Uterus homogenate, which has been assayed for comparison, also shows a similar trend.

Peroxidation of membrane lipids is regulated by several factors [14]. In the microsomal membranes polyunsaturated fatty acids of the phospholipids form the major substrates for NADPH-induced lipid peroxidation [15]. While cytochrome P-450 enhances lipid peroxidation in microsomes [14], antioxidants such as α-tocopherol and reduced glutathione besides superoxide dismutase, an inhibitor of free radical reaction, inhibit lipid peroxidation [4,14]. Fluidity of membranes, as determined by the cholesterol:phospholipid ratio, also influences the extent of lipid peroxidation [14,16]. Ascorbic acid, depending on its relative concentration with α-tocopherol, may inhibit or enhance lipid peroxidation [17].

The present study shows that in the microsomal fractions of senescent rats, the factors which contribute to the observed resistance to NADPH-induced lipid peroxidation appear to be low levels of phospholipid, cytochrome P-450 and NADPH-cytochrome *c* reductase. Higher concentrations of glutathione, α-tocopherol, superoxide dismutase and cholesterol:phospholipid ratio also seem to favour the resistance.

Player et al. [7] have observed high levels of NADPH-induced lipid peroxidation in total microsomes of 26-month-old rats. Their study also noted high levels of NADPH-cytochrome *c* reductase in old rats, contrary to other reports indicating low levels of this enzyme in senescent rats [13,18,19]. Total microsomes contain other organelles besides rough and smooth endoplasmic reticulum and as such studies using total microsomes may not truly reflect age-related changes in the endoplasmic reticulum [18,19]. Earlier studies have shown that the two microsomal fractions significantly differ from each other in their capacity to undergo lipid peroxidation [8] and in their response to ageing [18,20,21].

Lipid peroxidation potential has been inversely correlated with longevity in mammals [22]. The present study shows that the lipid peroxidation potential of microsomes as reflected by NADPH-induced lipid peroxidation in old female rats is low though the prevalence of a similar trend in the lipid peroxidation levels in old male rats remains to be ascertained. Earlier reports have also shown that lipid peroxide content as well as ascorbate-induced lipid peroxidation in hepatic microsomes of senescent rats are low [21,23,24]. It is likely that this low potential for lipid peroxidation observed is a special adaptation conferred on the microsomes to circumvent the ill effects of ageing.

ACKNOWLEDGEMENTS

The author wishes to thank Dr D.S. Pradhan, Head, Biochemistry Division, for his advice and encouragement, Dr U. Tarachand, Biochemistry Division, for his helpful suggestions and Mr T.K. Thiyagarajan, Plasma Physics Division, for executing a computer programme for graphics.

REFERENCES

[1] Bakerman, S. (ed.) (1969) in: Aging Life Processes, Charles C. Thomas, Springfield, IL.
[2] Tappel, A.L. (1973) Fed. Proc. 32, 1870–1874.
[3] Sagai, M. and Ichinose, T. (1980) Life Sci. 27, 731–738.
[4] Yagı, K. (ed.) (1982) in: Lipid Peroxides in Biology and Medicine, Academic Press, Orlando, FL.
[5] Takeuchi, N., Tanaka, F., Katayama, Y., Matsumiya, K. and Yamamura, Y. (1976) Exp. Gerontol. 11, 179–182.
[6] Vericel, E., Lagarde, M., Dechavanne, M. and Courpron, P. (1985) Thromb. Haemostas. 54, 553–554.
[7] Player, T.J., Mills, D.J. and Horton, A.A. (1977) Biochem. Biophys. Res. Commun. 78, 1397–1402.
[8] Devasagayam, T.P.A., Pushpendran, C.K. and Eapen, J. (1983) Biochim. Biophys. Acta 750, 91–97.
[9] Devasagayam, T.P.A. (1986) FEBS Lett. 199, 203–207.
[10] Hochstein, P. and Ernster, L. (1963) Biochem. Biophys. Res. Commun. 12, 388–394.
[11] Pederson, T.C. and Aust, S.D. (1972) Biochem. Biophys. Res. Commun. 48, 789–795.
[12] Lu, A.Y.H., Junk, K.W. and Coon, M.J. (1969) J. Biol. Chem. 244, 3714–3721.
[13] Schmucker, D.L. (1979) Pharmacol. Rev. 30, 445–456.
[14] Bus, J.S. and Gibson, J.E. (1979) in: Reviews in Biochemical Toxicology (Hodgson, E. et al. eds) vol.1, pp.125–149, Elsevier/North-Holland, Amsterdam, New York.
[15] Lokesh, B.R., Mathur, S.N. and Spector, A.A. (1981) J. Lipid Res. 22, 905–915.
[16] Galeotti, T., Borrello, S., Palanbini, G., Masotti, L., Ferrari, M.B., Cavatorta, P., Arcioni, A., Stremmenos, C. and Zannoni, C. (1984) FEBS Lett. 169, 169–173.
[17] Leung, H.W., Vang, M.J. and Mavis, R.D. (1981) Biochim. Biophys. Acta 664, 266–272.
[18] Devasagayam, T.P.A., Pushpendran, C.K. and Eapen, J. (1983) Mech. Age. Dev. 21, 365–375.
[19] Schmucker, D.L. and Wang, R.K. (1981) Mech. Age. Dev. 15, 189–202.
[20] Devasagayam, T.P.A. and Pushpendran, C.K. (1985) Biochem. Int. 11, 833–839.
[21] Devasagayam, T.P.A. and Pushpendran, C.K. (1986) Mech. Age. Dev. 34, 13–22.
[22] Cutler, R.G. (1985) Proc. Natl. Acad. Sci. USA 82, 4798–4802.
[23] Grinna, L.S. and Barber, A.A. (1973) Biochem. Biophys. Res. Commun. 55, 773–779.
[24] Pushpendran, C.K., Devasagayam, T.P.A. and Eapen, J. (1983) Dev. Pharmacol. Ther. 6, 55–63.