Medical Hypotheses (2000) **54**(4), 519–522

DOI: 10.1054/mehy.1999.0889, available online at http://www.idealibrary.com on IDEAL®

Is carbonyl detoxification an important anti-aging process during sleep?

D. Yin

Department of Pathology, Linköping University, Linköping, Sweden

Summary Organisms living on the earth may undergo inevitable toxification by biological 'garbage', a variety of biometabolites. Such garbage includes a particularly large number of toxic carbonyls, such as α,β-unsaturated carbonyls created by free radicals, glycation, and other post-translational side-reactions during various stresses and diseases. The accumulation of these toxic substances and their crosslinking products leads to the formation of different age pigments, such as lipofuscin, lens cataracts, and crosslinked collagen. The diurnal fluctuation in the concentration of the pineal gland hormone, melatonin, may be responsible for the 'cleaning activities' that reverse the covalently-bound semi-toxified proteins and nucleic acids. This toxification-cleaning cycle may explain the biochemical necessity for sleep of human and animals during aging. © 2000 Harcourt Publishers Ltd

CARBONYL TOXIFICATION

An important characteristic of most stresses, diseases and aging processes is the consistent increase in the concentration of various unsaturated carbonyls, mainly toxic aldehydes, in almost all tissues and body fluids (1–5). Such carbonyls, originating from major biological macromolecules including proteins, lipids, carbohydrates, and nucleic acids, are produced mainly by different post-translational side-reactions, especially oxidative stress and glycation (4–8). The high reactivity of carbonyls, particularly α,β-unsaturated carbonyls, underlies their toxicity by crosslinking or primarily binding to important biomolecules such as structural and functional proteins and nucleic acids. This type of carbonyl-amino crosslinking is now considered to be a key process in the formation of ceroid, lipofuscin (intracellular age pigments), lens cataracts, and crosslinked collagens and elastins (*9*). It is also a critical factor in the decline of cell proliferation and various cell membrane functions, in atherosclerosis and amyloid formation, and in many other degenerative alterations during aging (*6*,*7*,10).

The amount of biochemically detectable carbonyls in different body constituents, best represented by thiobarbituric acid reactive substances (TBARS), is the most used index of the steady-state levels of the reactive and toxic carbonyls of an organism (11). Although the amount of protein carbonyls is an important index of aging, the high variability of results and the time-consuming nature of analytic techniques hinder accurate determination (11–13). On the other hand, food is another important source of carbonyls. Since most foods and beverages are unprotected during processing and cooking, or only partly protected during storage, they may contain a large amount of reactive carbonyls, which provides an unlimited and unavoidable source of such (unhealthy) substances and their derivatives. In vivo, the concentration of α,β-unsaturated carbonyls, best studied as malondialdehyde (MDA) and 4-hydroxyalkenals (4HA), is under active control of different biological enzymes, such as carbonyl dehydrogenases (14). In mammals, a first line of defense against toxic unsaturated carbonyls from food

Received 22 October 1998
Acceped 3 April 1999

Correspondence to: Dazhong Yin, Department of Pathology 2, Linköping University, S-58185 Linköping, Sweden

takes place in the digestive system, where hepatic carbonyl dehydrogenases convert carbonyls to other compounds and eventually either to carbon dioxide and water, or to soluble derivatives which are excreted in the urine (*6*).

REVERSIBILITY OF CARBONYL TOXIFICATION

It is known that the primary reactions between α,β-unsaturated carbonyls and amino compounds are reversible (Fig. 1). When organisms contain relatively high concentrations of toxic carbonyls due to daily activities, functional and structural proteins are likely to react with these substances to form primarily semi-bound protein-carbonyl compounds (*6*), e.g. amino-propanal, due to 1:1 MDA-protein reactions (Fig. 1). Although heavy stresses lead to the formation of large amounts of toxic carbonyls (as does the ingestion of oxidized food or a high caloric diet), biological defending substances, such as carbonyl dehydrogenases, glutathione and glutathione transferase (*6*,14,15) all work intensively to reduce the concentration of these toxic components and to keep the carbonyl binding and subsequent crosslinking at reasonably low levels.

POTENTIAL OF CARBONYL TOXIFICATION

Daily activities, particularly intense exercise, staying awake overnight, diseases, and major surgical operations all lead to large increases in the concentration of toxic carbonyls (5–7), thereby driving the reaction equilibrium primarily towards the formation of 1:1 amino-carbonyl compounds, although these are unstable and reversible.

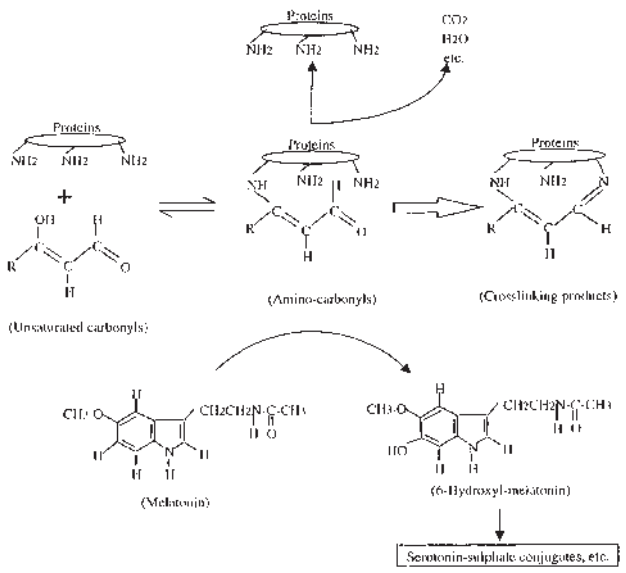

Fig. 1 Mechanisms of carbonyl toxification due to stresses, and proposed melatonin detoxification during sleep.

The detected concentration of serum TBARS mainly represents a balance between the formation of carbonyls and their elimination by defense mechanisms (probably also including the fast protection of thiol compounds in addition to enzymatic defense), which may mirror the real reaction situation between carbonyls and amino compounds in vivo. The actual concentration of amino compounds is much higher than the concentration of α,β-unsaturated carbonyls (16), e.g. the total amount of free amino acids is as high as 80 mM, although proteins are found to be more readily modified by MDA than free amino acids under physiological conditions (*6*). Since the reaction potential between α,β-unsaturated carbonyls and proteins, such as enzymes, is fairly low, the possibility of crosslinking should be even lower. Hence, the principal reaction between MDA and functional and structural proteins would be an amino-propanal reaction, rather than a crosslinking reaction, to form amino-imino-propen structures. Although the primary reaction is slow and actively reversed by several defense mechanisms, the accumulation of amino-carbonyls is progressive and affects the normal body functions, probably until the brain cannot tolerate further increase, and signals sleep (by melatonin secretion?).

SLEEP, MELATONIN AND CARBONYL DETOXIFICATION

The observation that melatonin secretion helps to keep down TBARS levels during various oxidative stresses, in addition to its known beneficial effects on sleep as well as endocrine and immune functions (17,18), leads me to raise a very interesting question: how does melatonin interfere with oxidative stress when it is secreted mostly only during sleep? Although melatonin has been shown to prevent damages from oxygen free radicals, it has also been shown that administration of melatonin often causes TBARS levels lower than in control samples (19,20) which may suggest a detoxification function of melatonin. Furthermore, the indole structure of melatonin is similar to the indole used in MDA+4HA measurement in the LPO-586 assay. It is logically unsound to suppose that melatonin has been evolved mainly as a free-radical scavenger, because oxygen free radicals, as well as most hydroperoxides, react so quickly, that they will almost immediately attack any biomaterials in their vicinity (21,22). After the rapid disappearance of oxygen radicals, only various toxic carbonyls and their derivatives remain in tissues and body fluids (3–5). Therefore, one function of melatonin may be to interact with the accumulated amino-propanal-like products produced by various stresses, in order to remove them before they form stable amino-imino-propen crosslinks. When the concentration of melatonin reaches a high level during

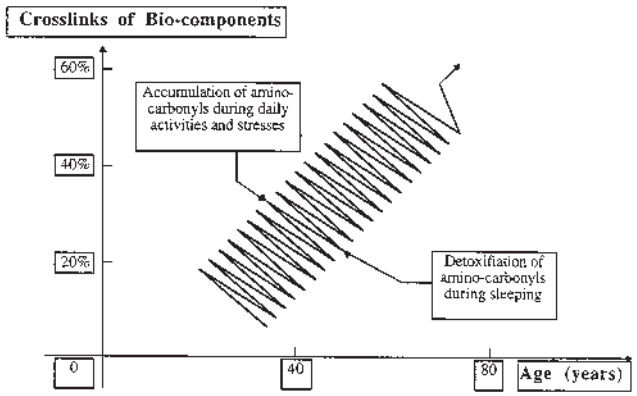

Fig. 2 Carbonyl detoxification during sleep and its hypotheszed relation to aging processes.

deep sleep, the carbonyl-amino reaction may be reversed causing a removal of toxic compounds at the expense of the hydroxylation of melatonin (23,24). A sound sleep may thus lead to an efficient clearance of carbonyl complexes, although a 100% clearance may not be attained, especially not in tissues such as collagen where less melatonin is delivered and where crosslinks seem to occur at an average speed of 0.001% of the total amount of structural protein per day in humans. When the brain is free of, or has only a very low level of such amino-carbonyl garbage, we wake up and feel refreshed and alert again, and we are ready to face a new day of activity, during which we will once again accumulate new amounts of noxious substances. This 'contamination-cleaning' cycle repeats every day even as undegradable crosslinked materials gradually accumulate, eventually becoming abundant and 'mature' in the tissues. Such fluorescent, age-related accumulates that are found either intracellularly (lipofuscin) or extracellularly are known as age pigments. When these crosslinked materials become abundant, our bodies do not function well and we enter into the aging period. Figure 2 shows a scheme for garbage accumulation, clearance during sleep and the aging process. Genetic factors are found to regulate the defending, cleaning and repairing systems (e.g. genes for superoxide dismutase, catalase and glutathione transferase, ref. 6,25,26). Differences in the efficiency of these systems would determine the life-span of different species.

This hypothesis, which links sleep and aging, is mainly based on the understanding of oxidative stress, glycation and age pigment formation. It may appear to be an oversimplification of such extremely complicated biological processes as sleep and aging (27,28). However, in the author's opinion, a sound aging theory may eventually be required to provide a comprehensive explanation to the crucial daily need for sleep.

ACKNOWLEDGMENTS

The author thanks Professors Ulf T. Brunk, Eduardo A. Porta, Liren Ma, and Zuze Wu for valuable discussions. This work was supported by the Swedish Medical Research Council, grant no. 4481 and the Sinica Academic Fund.

REFERENCES

1. Schauenstein E., Esterbauer H., Zollner H. *Aldehydes in Biological Systems*. London: Pion Limited, 1977.
2. Witz G. Biological interaction of α,β-unsaturated aldehydes. *Free Radic Biol Med* 1989; **7**: 333–349.
3. Esterbauer H., Zollner H., Schaur R. J. Aldehydes formed by lipid peroxidation, mechanisms of formation, occurrence and determination. In: Vigo-Pelfrey C. (ed.). Membrane Lipid Oxidation, vol. I. Boca Raton: CRC Press, 1990: 239–268.
4. Monnier V. M. Mechanisms of protection against damage mediated by the Maillard reaction in aging. *Gerontology* 1991; **37**: 152–165.
5. Kosugi H., Enomoto H., Ishizuka Y., Kikugawa K. Variations in the urinary thiobarbituric acid reactant in healthy humans under different physiological conditions. *Biol Pharm Bull* 1994; **17**: 1645–1650.
6. Esterbauer H., Schaur R. J., Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991; **11**: 81–128.
7. Baynes J. W., Monnier V. M. The Maillard Reaction in Aging, Diabetes, and Nutrition. New York: Alan R Liss Inc, 1989.
8. Hodge J. E. Chemistry of browning reactions in model systems. *J Agric Food Chem* 1953; **1**: 928–943.
9. Yin D. Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. *Free Radic Biol Med* 1996; **21**: 871–888.
10. Yu B. P. Aging and oxidative stress: modulation by dietary restriction. *Free Radic Biol Med* 1996; **21**: 651–668.
11. Janero D. R. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med* 1990; **9**: 515–540.
12. Levine R. L., Garland D., Oliver C. N, Amici A, Climent I., Lenz A-G., Ahn B., Shaltiel S, Stadtman E. R. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzym* 1990; **186**: 464–478.
13. Cao G., Cutler R. G. Protein oxidation and aging, I. Difficulties in measuring reactive protein carbonyls in tissues using 2,4-dinitrophenylhydrazine. *Arch Biochem Biophys* 1995; **320**: 106–114.
14. Weiner H., Holmes R. S., Wermuth B. *Enzymology and Molecular Biology of Carbonyl Metabolism 5*. New York: Plenum Press, 1995.
15. Lindahl R. Aldehyde dehydrogenases and their role in carcinogenesis. *Crit Rev Biochem Mol Biol* 1992; **27**: 283–335.
16. Yin D., Brunk U. T. Carbonyl toxification hypothesis of biological aging. In: Macieira-Coelho A (ed.). *Molecular Basis of Aging*. London: CRC Press, 1995: 421–436.
17. Reiter R. J., Pablos M. I., Agapito T. T., Guerrero J. M. Melatonin in the context of the free radical theory of aging. *Ann NY Acad Sci* 1996; **786**: 362–378.
18. Pierpaoli W., Regelson W., Fabris N. The aging clock, the pineal gland and other pacemakers in the progression of aging and carcinogenesis. *Ann NY Acad Sci vol* 719, 1994.
19. Reiter R. J. Antioxidant action of melatonin. *Adv Pharmacol* 1997; **38**: 103–117.
20. Melchiorri D., Reiter R. J., Sewerynek E., Chen L. D., Nistico G. Melatonin reduces kainate-induced lipid peroxidation in homogenates of different brain regions. *FASEB J* 1995; **9**: 1205–1210.

21. Halliwell B., Gutteridge J. M. C. Free Radicals in Biology and Medicine, 2nd edn. Oxford: Clarenton Press, 1989.
22. Yin D., Lingnert H., Ekstrand B., Brunk U. T. Fenton reagents may not initiate lipid peroxidation in an emulsified linoleic acid model system. *Free Radic Biol Med* 1992; **13**: 543–556.
23. Leone A. M., Francis P. L., Silman R. E. The isolation, purification, and characterisation of principal urinary metabolites of melatonin. *J Pineal Res* 1987; **4**: 253–266.
24. Reiter R. J. Oxygen radical detoxification processes during aging: the functional importance of melatonin. *Aging Clin Exp Res* 1995; **7**: 340–351.
25. Jazwinski S. M. Longevity, genes and aging. *Science* 1996; **273**: 54–59.
26. Orr W. C., Sohal R.S. Extension of life-span by overexpression of superoxide dismutase and catalase in drosophila melanogaster. *Science* 1994; **263**: 1128–1130.
27. Medvedev Z. A. An attempt at a rational classification of theories of ageing. *Biol Rev* 1990; **65**: 375–398.
28. Zs.-Nagy I. The horizons of an interdisciplinary synthesis in experimental gerontology. *Arch Gerontol Geriatr* 1991; **12**: 329–349.