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Antioxidant Activity of Indapamide and Its Metabolite

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An antioxidant activity of indapamide (IDP) and its metabolite (OH–IDP) is demonstrated in this study. Both IDP and OH–IDP were found to scavenge 1,1-diphenyl-2-picryl-hydrazyl free radical. The scavenging effect of OH–IDP was stronger than that of IDP. Lipid peroxidation of rat liver microsomes initiated by reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine diphosphate (ADP)–Fe^{3+} was inhibited by IDP and OH–IDP with IC_{50} values of about 6 and 2 μM, respectively. The lipid peroxidation in human erythrocyte membrane, induced by 2,2′-azobis-(2-amidinopropane dihydrochloride) treatment, was also inhibited by 10 μM IDP. The antioxidant capacity of OH–IDP was at almost the same level as that of α-tocopherol, tested for comparison. The present data show that IDP and OH–IDP at micromolar concentrations are able to trap the free radicals involved in the lipid peroxidation.

Keywords indapamide; antioxidant; radical scavenger; lipid peroxidation; liver microsome; erythrocyte membrane

While oxygen is indispensable for all aerobic organisms, it is also a source of highly toxic molecules, *i.e.*, oxygen free radicals, which are generated *in vivo* as by-products of normal metabolism. During aging and in certain pathological situations such as inflammation, acute myocardial infarction and the subsequent reperfusion phase, production of oxygen free radicals increases. When increased oxygen radicals overwhelm the protective systems, they may induce membrane lipid peroxidation and subsequent cellular damage.[1–3] Many natural antioxidants are found in biological fluids and tissues, almost all of which belong to the water-soluble antioxidant family.[4–6]

Indapamide (IDP, 4-chloro-*N*-(2-methyl-1-indolinyl)-3-sulfamoylbenzamide hemihydrate) is an oral diuretic-antihypertensive drug, and is an effective and well-tolerated drug for patients with mild to moderate essential hypertension.[7,8] This is a lipophilic molecule, and is mainly bound to erythrocytes in blood.[9]

In this paper, we report a beneficial antioxidant activity of IDP on lipid peroxidation of rat liver microsomes and human erythrocyte membranes caused by oxygen free radicals. Because chemical compounds bearing a phenolic hydroxyl group in their structure may generally have a radical-scavenging activity,[10] the antioxidant capacity of 5-hydroxy-IDP (OH–IDP), which is one of the metabolites of IDP,[11] was also examined. The data on the α-tocopherol (Toc) are given for comparison.

Materials and Methods

Drugs IDP and OH–IDP were provided by Kyoto Pharmaceutical Industries, Ltd. (Kyoto, Japan). Toc, 2,2′-azobis(2-amidinopropane dihydrochloride (AAPH) and other reagents were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Stock solutions (2 mM) of IDP, OH–IDP and Toc were prepared in ethanol.

Determination of Free Radical Scavenging Capacity of the Drugs Tested by Using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) A 0.03 ml aliquot of drug stock solution was mixed with 3 ml of 0.1 mM DPPH solution (in ethanol) in a cuvette and then the time course of the optical density change at 517 nm was measured.[12]

Effect of the Drugs Tested on Lipid Peroxidation of Rat Liver Microsomes Rat liver microsomes were prepared from male Wistar rats, using 1.15% KCl solution containing 0.2% nicotinamide and 10 mM sucrose. Microsome suspension (0.5 mg protein/ml) containing 3 mM adenosine diphosphate (ADP) and 0.15 mM $FeCl_3$ was preincubated with each drug (or ethanol as a control) at 37 °C for 30 min. After addition of 0.5 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) as an initiator of lipid peroxidation,[13] the mixture was incubated at 37 °C for 20 min. The extent of lipid peroxidation was measured by the thiobarbituric acid (TBA) method,[13] in the presence of 0.15 mM butylated hydroxytoluene. Protein concentration was estimated by the method of Lowry *et al.*[14]

Effect of the Drugs Tested on Lipid Peroxidation of Erythrocyte Membrane Lipid peroxidation of erythrocyte membrane (hemoglobin-free ghosts), which was prepared from washed human erythrocytes by hypotonic hemolysis, was initiated by AAPH treatment.[15] Ghost suspension (1.0 mg protein/ml) was incubated with 100 mM AAPH at 37 °C. After 20 min, 10 μM IDP was added to an aliquot of the suspension. Incubation was continued for 60 min, then 10 μM IDP was added again to an alliquot of the suspension, which was further incubated. The amount of conjugated diene formed in each sample was measured at 233 nm[16] against a blank which was prepared immediately after addition of AAPH and/or IDP to the ghost suspension in an ice bath.

Results

Radical Scavenging Effect of the Drugs Tested DPPH, which shows a strong absorption band at 517 nm because of its odd electron, has been used as a convenient tool for the antioxidant assay of biological materials. When this compound accepts an electron or hydrogen radical to become a more stable compound, the absorption (deep violet color) vanishes.[12] As shown in Fig. 1, no decolorization of DPPH solution occured during the 20 min incubation without any drug. Addition of OH–IDP or Toc to DPPH solution induced rapid decolorization and the decrease in optical density reached a plateau level within a few minutes. IDP

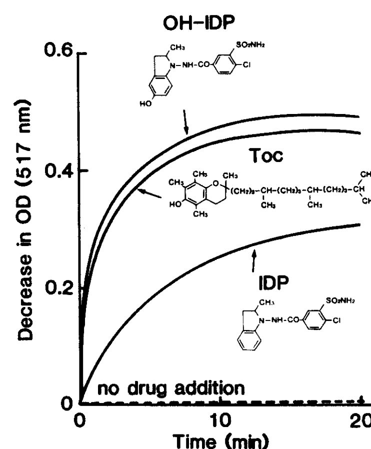

Fig. 1. Time Course of Decolorization of DPPH Solution after Addition of the Drugs Tested

DPPH solution (0.1 mM) was mixed with 20 μM IDP, OH–IDP or Toc, and the optical density change at 517 nm was monitored. The molecular structure of each drug tested is shown in the figure.

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was also able to decolorize the DPPH solution, although the decrease in optical density was slower than that by OH–IDP or Toc. The results indicate that both IDP and OH–IDP as well as Toc have a radical scavenger activity.

Inhibitory Effect of the Drugs Tested on Lipid Peroxidation of Rat Liver Microsomes Electron transport-dependent lipid peroxidation of rat liver microsomes was carried out by addition of NADPH to microsomes in the presence of ADP–Fe^{3+} complex. After incubation at 37 °C for 20 min, the extent of lipid peroxidation was measured by the TBA method. Addition of IDP, OH–IDP or Toc to the microsome suspension strongly inhibited the lipid peroxidation, as shown in Fig. 2. The inhibition by Toc, OH–IDP and IDP was dependent upon their concentration with IC_{50} of about 1, 2 and 6 μM, respectively. Since IDP and OH–IDP were found to have no inhibitory effect on the TBA reaction itself under the conditions used, it is clear that IDP and OH–IDP inhibited in a concentration-dependent manner the formation of TBA-reactive products that occurred during microsomal lipid peroxidation.

Inhibitory Effect of the Drugs Tested on Lipid Peroxidation of Erythrocyte Membrane We examined whether the drugs tested could inhibit not only chain initiation but also chain propagation of lipid peroxidation which might occur in the hydrophobic core of the cell membrane. For this purpose, we employed erythrocyte ghosts (hemoglobin-free membrane) and AAPH. AAPH is able to initiate lipid peroxidation of erythrocyte membrane without metal ion.[15] The extent of lipid peroxidation in the membrane was estimated by measurement of conjugated diene. On incubation of the ghosts with AAPH, the amount of conjugated diene formed in the cell membrane increased with prolongation of incubation time (Fig. 3). Addition of 10 μM IDP to the mixture after 20 min elicited a transitory inhibition of the lipid peroxidation. Since we employed a very low concentration of IDP (10 μM) as compared with AAPH (100 mM), lipid peroxidation started again after 80 min. However, readdition of IDP to the mixture at the time of lipid peroxidation resumption led to inhibition of lipid peroxidation again. When a higher concentration of IDP (100 μM) was used in this experiment, the suppressive effect on lipid peroxidation lasted for a much longer period of time (data not shown).

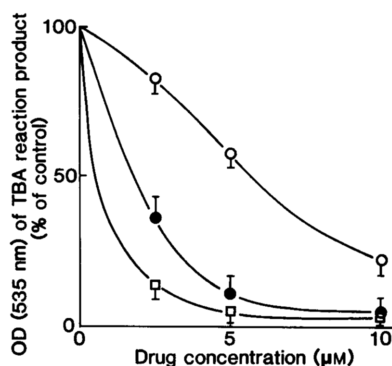

Fig. 2. Concentration-Dependent Effect of the Drugs Tested on the Lipid Peroxidation of Rat Liver Microsomes

Experimental details are described in Materials and Methods. ○, IDP; ●, OH–IDP; □, Toc.

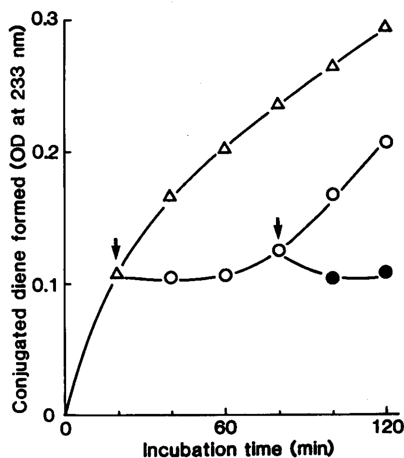

Fig. 3. Time-Dependent Effect of the Drugs Tested on the Lipid Peroxidation of Human Erythrocyte Membrane

Erythrocyte membrane suspension (1 mg protein/ml) was incubated with 100 mM AAPH at 37 °C (△). After 20 min (○) and 80 min of incubation (●), as indicated by arrows in the figure, 10 μM IDP was added to an aliquot of the suspension and incubation was continued. The amount of conjugated diene formed in each sample at each time of incubation was determined.

Discussion

In this experiment, it was found that both IDP and its metabolite (OH–IDP) at micromolar concentrations can scavenge the stable free radical of DPPH and inhibit the lipid peroxidation in rat liver microsomes and erythrocyte membranes induced by oxygen radical. The radical-trapping activity of OH–IDP was at almost the same level as that of Toc, and stronger than that of IDP.

Antioxidants can be classified into two groups; preventive and chain-breaking ones. The former (*e.g.* catalase and transferrin) can reduce the rate at which new peroxidation chains start, and the latter (*e.g.* vitamin E) can trap radicals directly.[17] Because IDP and OH–IDP inhibited not only electron transport-dependent (Fig. 2) but also electron transport-independent lipid peroxidation (Fig. 3), they appear to belong to the category of chain-breaking antioxidants, in the same manner as Toc.

While there are many antioxidants in biological fluids and tissues, such as vitamin E,[5] urate,[4] ascorbate[5] and certain enzyme systems,[2] almost all of these antioxidants are water-soluble ones, except vitamin E. Since lipid peroxidation is a chain reaction which occurs in the hydrophobic lipid domain of the cell membrane, lipid soluble compounds might be more effective as chain-breaking antioxidants. Addition of IDP to erythrocyte membrane, in which both chain initiation and chain propagation had already been started by AAPH treatment, suppressed completely further lipid peroxidation in the membrane (Fig. 3). This result indicates that IDP, because of its hydrophobicity, can also scavenge the peroxyl radical derived from lipid peroxidation in the hydrophobic region of the membrane.

Although the antioxidant activity mechanism of IDP is unknown as yet, it should be emphasized that IDP used as an antihypertensive drug[7,8] does possess an antioxidant capacity at micromolar concentrations. Long term administration of IDP may be an effective treatment against certain oxidative stresses in the human body.

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References

1) D. Harman, "Free Radicals in Biology," Vol. 5, ed. by W. A. Pryor, Academic Press, New York, 1982, p. 255.
2) I. Fridovich, *Ann. Rev. Pharmacol. Toxicol.*, **23**, 239 (1983).
3) T. F. Slater, *Biochem. J.*, **222**, 1 (1984).
4) B. N. Ames, R. Cathcart, E. Schwiers and P. Hochstein, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6858 (1981).
5) D. D. M. Wayner, G. W. Burton, K. U. Ingold, L. R. C. Barclay and S. J. Locke, *Biochim. Biophys. Acta*, **924**, 408 (1987).
6) L. A. Videla, T. Caceres and E. A. Lissi, *Biochem. Int.*, **16**, 799 (1988).
7) J. C. Demanet, J. P. Degaute and C. Hubert, *Curr. Med. Res. Opin.*, **5** (Suppl. 1), 129 (1977).
8) S. Acchiardo and V. A. Skoutakis, *Am. Heart J.*, **106**, 237 (1983).
9) S. P. Urien, P. Riant, A. Renouard, B. Coulomb, I. Rocher and J. P. Tillement, *Biochem. Pharmacol.*, **37**, 2963 (1988).
10) K. Yagi and S. Komura, *Biochem. Int.*, **13**, 1051 (1986).
11) K. Kitao, T. Sakata, Y. Ueshima, H. Muro, M. Fujitani and H. Murauchi, *Yakuri To Tiryo*, **10**, 2619 (1982).
12) M. S. Blois, *Nature* (London), **181**, 1199 (1958).
13) M. Tien and S. D. Aust, *Biochim. Biophys. Acta*, **712**, 1 (1982).
14) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
15) M. Miki, H. Tamai, M. Mino, Y. Yamamoto and E. Niki, *Arch. Biochem. Biophys.*, **258**, 373 (1987).
16) R. A. Klein, *Biochim. Biophys. Acta*, **210**, 486 (1970).
17) G. W. Burton and K. U. Ingold, *Science*, **224**, 569 (1984).

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