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Inhibition of lipid peroxidation by methylated analogues of uric acid

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Abstract—The effects of purines and methylated purine analogues on hydrogen peroxide- and ozone-induced lipid peroxidation in human erythrocyte membranes were studied in-vitro. Many purines and purine analogues showed a suppressive effect on the formation of thiobarbituric acid reactive materials. 1,3-Dimethyluric acid and 1,3,7-trimethyluric acid showed high potency in prevention of lipid peroxidation. These effects were shown to be concentration dependent and were more potent at low concentrations of hydrogen peroxide and ozone. 1,3-Dimethyluric acid and 1,3,7-trimethyluric acid may be useful as antioxidants.

The importance of oxygen radicals in tissue injury is widely recognized (Marx 1987). Mutation, cancer, inflammation, and aging are thought to be caused by oxygen radicals. Recently, the cytotoxic effect of oxygen radicals during reperfusion following ischaemia has been shown in several tissues including small intestine, heart and brain. It has also been confirmed that lipids are peroxidized by oxygen radicals leading to the damaging effects of lipid peroxide on cellular constituents. Mickel & Horbar (1973) reported that peroxidized lipids affect platelet aggregation. The concentration of lipid peroxide in the arterial wall and the severity of atherosclerosis are positively correlated (Clavind et al 1952). These findings suggest a possible role of peroxidized lipids in the pathogenesis of atherosclerosis.

The recognized important biological antioxidants are vitamin C, vitamin E and β-carotene (Fukuzawa et al 1985). Recently, uric acid has been suggested as an important physiological antioxidant against oxidative injury (Ames et al 1981; Davies et al 1986; Liu 1986; Wayner et al 1987; Simic & Jovanovic 1989). This protective role of uric acid is proposed as an important factor in the prevention of ageing and cancer. However, uric acid is an insoluble agent. High serum urate levels result in gout or kidney damage.

In the present experiment, the antioxidant effect of purine analogues has been studied.

Materials and methods

Purines and methylated analogues of purines were purchased from Sigma Chemical Corp.

Human blood was obtained from healthy donors and collected into heparinized tubes. Blood was centrifuged at 2000 *g* for 20 min at 0°C and the plasma and buffy coats were removed by aspiration. Erythrocytes were washed with isotonic phosphate buffered saline, pH 7·4, and were lysed in cold 10 mM phosphate buffer, pH 7·4. Haemolysate was centrifuged at 25000 *g* for 30 min at 0°C and then washed with 10 mM phosphate buffer until they were no longer pink.

To measure peroxidation of lipids in erythrocyte membranes, a thiobarbituric acid method was used (Yoshikawa & Hirai 1976). Erythrocyte membranes containing 3 mg protein were suspended in 0·9 mL of 10 mM phosphate buffer with or without various drugs. Reactions were started by addition of 0·1 mL of 10 mM hydrogen peroxide and were incubated at 37°C for 15 min. To generate ozone, a silent electric discharge apparatus (Nipponmicron, Tokyo) was used. The ozone/oxygen mixture was bubbled through a 1 mL sample solution for 30 s. Ozone delivery was 40 μmol min^{-1} with a gas flow rate of 1L min^{-1}.

Reactions were stopped by addition of 0·5 mL of 20% trichloroacetic acid. After centrifugation at 2000 *g* for 10 min, the supernatant (1 mL) was added to 0·5 mL of 1% thiobarbituric acid solution in 0·05 M NaOH heated in a boiling water bath for 15 min and allowed to cool. The absorbance was measured at 535 nm. Protein content was estimated by the method of Lowry et al (1951), using crystalline bovine serum albumin as standard.

Results

Treatment of erythrocyte membranes with hydrogen peroxide or ozone resulted in an increase in thiobarbituric acid-reactive material. Purine analogues and purine bases suppressed the lipid peroxidation induced by hydrogen peroxide and or ozone in human erythrocyte membranes (Table 1). The relative potencies of the inhibitory effects of 1,3-dimethyluric acid > 1,3,7-trimethyluric acid > 1-methyluric acid on a molar basis.

Table 1. Inhibitory effect of purines on hydrogen peroxide-(1 mM) or ozone-(20 μmol) induced lipid peroxidation in human erythrocyte membranes. Results are expressed as the mean ± s.d. of triplicate experiments.

Additions (500 μM)	Inhibition (%)	
	H_2O_2	O_3
Uric acid	52·5 ± 3·4	32·7 ± 4·8
1-Methyluric acid	69·2 ± 14·8	43·8 ± 5·5
3-Methyluric acid	60·2 ± 13·8	32·7 ± 9·8
7-Methyluric acid	65·3 ± 11·9	14·6 ± 3·3
9-Methyluric acid	55·4 ± 15·6	35·1 ± 10·3
1,3-Dimethyluric acid	87·4 ± 3·6	63·9 ± 5·7
1,7-Dimethyluric acid	34·9 ± 4·6	30·1 ± 5·2
1,3,7-Trimethyluric acid	76·1 ± 14·0	49·0 ± 13·1
Hypoxanthine	6·9 ± 4·1	9·2 ± 1·2
Xanthine	11·1 ± 3·0	8·2 ± 2·1
3-Methylxanthine	0	5·8 ± 4·2
7-Methylxanthine	8·9 ± 5·3	0
8-Methylxanthine	11·9 ± 4·2	22·9 ± 2·5
1,3-Dimethylxanthine (theophylline)	0	0
1,3,7-Trimethylxanthine (caffeine)	15·4 ± 2·7	0
Allopurinol	11·4 ± 9·1	16·5 ± 4·2
Oxypurinol	1·1 ± 0·8	9·2 ± 0·8
Adenine	25·8 ± 18·4	10·2 ± 4·9
1-Methyladenine	0	0
2-Methyladenine	41·5 ± 15·7	0
Guanine	16·9 ± 4·3	4·1 ± 3·6
7-Methylguanine	0	0

The inhibitory effects of varying doses of 1, 3-dimethyluric acid on lipid peroxidation by a fixed standard dose of hydrogen peroxide or ozone are shown in Fig. 1. Lipid peroxidation in human erythrocyte membranes were inhibited by 1,3-dimethyluric acid in a dose-dependent manner. Fig. 2 shows the inhibitory effect of 1,3-dimethyluric acid on lipid peroxidation by varying concentrations of hydrogen peroxide or ozone. The inhibition rate caused by 200 μM 1,3-dimethyluric acid was dependent on hydrogen peroxide or ozone concentration.

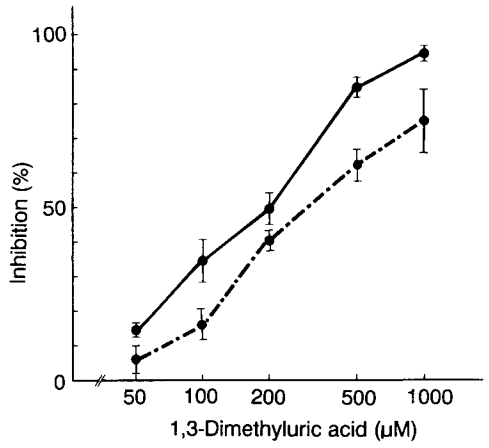

FIG. 1. Inhibitory effects of varying doses of 1,3-dimethyluric acid on lipid peroxidation by a fixed standard dose of hydrogen peroxide (●——●) and or ozone (●- -●). Results are expressed as the mean ± s.d. of triplicate experiments.

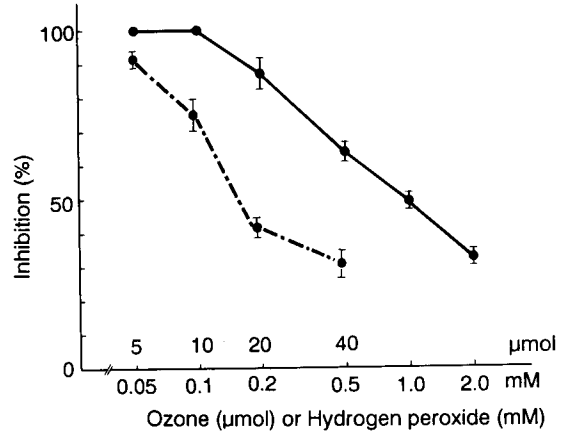

FIG. 2. Inhibitory effect of 1,3-dimethyluric acid (200 μM) on lipid peroxidation by varying concentrations of hydrogen peroxide (●——●) and or ozone (●- -●). Results are expressed as the mean ± s.d. of triplicate experiments.

Discussion

Previous experiments in-vitro have shown that uric acid protects erythrocytes against damage by singlet oxygen or t-butylhydroxide (Ames et al 1981), inhibits lipid peroxidation (Matsushita et al 1963; Smith & Lawing 1983; Meadows et al 1986; Meadows & Smith 1986, 1987; Niki et al 1986; Healing et al 1989) and protects against oxidant damage to DNA (Cohen et al 1984). Uric acid also scavenges hydroxyl radicals (Kittridge & Willson 1984) and protects certain enzymes against inactivation by reactive radical species (Aruoma & Halliwell 1989).

In the present experiment, several purine analogues were shown to have a preventive effect on lipid peroxidation, although the relationship between the relative potencies of the various derivatives and structures remains unclear. Uric acid also showed a preventive effect on lipid peroxidation. Moorhouse et al (1987) reported that allopurinol and oxypurinol were the effective hydroxyl radical scavengers. However, the inhibitory effects of allopurinol and oxypurinol on lipid peroxidation in the present experiment were weak. In addition uric acid, 1-methyl uric acid, allopurinol and oxypurinol were very insoluble (data not shown).

1,3-Dimethyluric acid and 1,3,7-trimethyluric acid were shown to have high potency in prevention of lipid peroxidation in a dose dependent manner. These antioxidant effects were more potent at relatively low concentrations of hydrogen peroxide or ozone, suggesting these agents have antioxidant effects in-vivo.

1,3-Dimethyluric acid and 1,3,7-trimethyluric acid are very soluble (data not shown). 1,3-Dimethylxanthine (theophylline) has been used in asthma drugs and 1,3,7-trimethylxanthine (caffeine) is contained in many kinds of beverages suggesting these methylxanthines have little cytotoxicity, and may be acceptible antioxidants in man.

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A novel bradykinin antagonist with improved properties

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Abstract—Acylation of the *N*-terminus of [D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7] bradykinin with 1-adamantanecarboxylic acid results in an analogue with enhanced potency of at least 33-fold. The new antagonist has potential as a pharmacological tool in the investigation of the role of endogenous bradykinin in cardiovascular regulation.

The physiological cardiovascular effects of endogenous bradykinin and its possible participation in the maintenance of normotension or the development of various hypertensive processes and cardiac diseases, as well as its contribution to the antihypertensive effects of angiotensin-converting enzyme (ACE) inhibition, have become increasingly the centre of research interest in recent years. As with many other vasoactive hormones (e.g. angiotensin II and vasopressin) the efforts towards elucidation of effects of bradykinin have acquired new impetus since the synthesis of bradykinin antagonists became possible. We now have the ability to study the various cardiovascular effects that bradykinin exerts either directly or via interaction with other vasoactive substances, by assessing the results of chronic B_2-receptor inhibition of bradykinin in terms of systemic or regional haemodynamic changes, changes in cardiac function and effects on other vasoactive systems. The fact that some bradykinin antagonists are being considered as therapeutic agents in man adds another dimension to the relevance of these studies.

Existing antagonists synthesized mostly by Stewart's group (Schachter et al 1987; Stewart & Vavrek 1991) have relatively low potency, which necessitates the use of high concentrations to inhibit the vascular effects of bradykinin. One of the most potent of these agents is [D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7] bradykinin, designated here as peptide I. Recently we reported that the acylation of the *N*-terminus of peptide I with 1-adamantaneacetic acid results in an analogue (designated peptide II) with more than ten times enhanced potency (Lammek et al 1990). As a continuation of our effort to develop more effective bradykinin antagonists, we designed a new bradykinin analogue: 1-adamantanecarboxylic acid-[D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7] bradykinin, designated peptide III. The structures of bradykinin analogues I–III are shown in Fig. 1.

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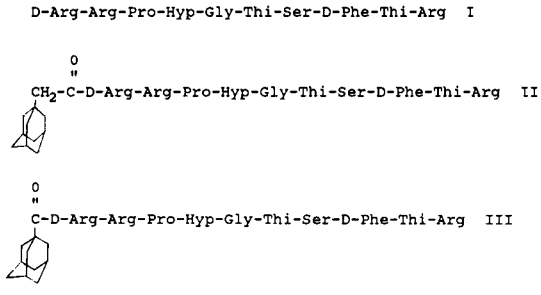

FIG. 1. Structures of bradykinin analogues.

Materials and methods

All peptides were synthesized using the solid phase method on a Merrifield resin (Stewart & Young 1984). After the protected peptides were assembled, they were cleaved from the support with simultaneous side-chain deprotection by acidolysis in anhydrous hydrogen fluoride at 0°C in the presence of 15% anisole. Crude materials were desalted by gel filtration on Sephadex G-15 and purified twice on Sephadex LJ-20. All analogues were shown to be homogeneous by thin-layer chromatography in three different solvent systems and gave the expected amino acid analysis ratios. The antagonistic potency of the analogues was assessed by their ability to inhibit the vasodepressor response to exogenous bradykinin in conscious rats (Lammek et al 1990, 1991), as follows.

Intact male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA, USA) 225–250 g, were maintained on a regular Purina chow diet, as well as tap water in a room at constant temperature (23 ± 1°C) with a 12 h dark: 12 h light cycle. One day before the experiment, the right carotid and the iliac artery were catheterized with polyethylene tubing (PE50) under light ether anaesthesia. A 'Y' type connection was attached to the carotid artery for injection of bradykinin and for infusion of the bradykinin analogues. All catheters were exteriorized subcutaneously at the back of the neck.

On the day of the experiment, the rats were conscious and unrestrained in plastic cages. Mean arterial pressure (MAP) and heart rate (HR) were monitored through a Gould-Statham P23 ID pressure transducer (Gould, Cleveland, OH, USA) connected to the iliac catheter and recorded on a Gould 2200S paper