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Anti-lipid peroxidation of gomisin J on liver mitochondria and cultured myocardial cells[1]

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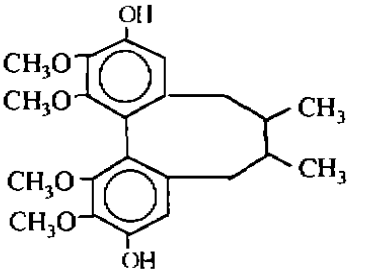
KEY WORDS lipid peroxidation; gomisin J; lignans; cultured cells; liver mitochondria; myocardium

AIM: To study the influences of gomisin J on lipid peroxidation and calcium paradox. **METHODS**: Using two *in vitro* models of rat liver mitochondria membrane lipid peroxidation (LPO) and cultured myocardial cells. **RESULTS**: Gomisin J inhibited Fe^{2+}/ascorbic acid and ADP/NADPH-induced LPO with IC_{50} (95 % confidence limits) 5.5 (4.5 − 6.7) and 4.7 (2.8 − 7.8) μmol·L^{-1}, respectively, when cultured myocardial cells preincubated with Ca^{2+}-free medium for 2 min were incubated with normal medium containing Ca^{2+}, a marked increase of malondialdehyde (MDA) formation occurred and gomisin J 10 μmol·L^{-1} protected myocardial cells through decreasing MDA formation. **CONCLUSION**: Gomisin J inhibits LPO in rat liver mitochondria and protects cultured myocardial cells from being injuried by calcium paradox.

Free radical-mediated lipid peroxidation (LPO) reactions are implicated in the genesis of tissue injury under ischemia/reperfusion, drug toxicity, and aging[1-2]. During lipid peroxidation, many aldehydes, such as malondialdehyde and 4-hydroxynonenal, accumulate and alter cellular structure and function. They may play a critical role in the evolution of free radical injury and the genesis of LPO[3].

Dibenzocyclooctadiene lignans, isolated from *Schisandra chinensis* and *Kadsura heteroclita*, protected liver from injury, induced hepatocyte microsomal cytochrome P-450, and inhibited lipid peroxidation[4-5]. A dibenzocyclooctadiene lignan, gomisin J[6], was isolated from *Kadsura interior* that is used in traditional Chinese medicine for the treatment of blood deficiency, numbness, menstrual disorder, stomachache, and infertility. In this paper, we investigated the influences of gomisin J on lipid peroxidation in rat liver mitochondria induced by Fe^{2+}/ascorbic acid and ADP/NADPH, and calcium paradox in cultured neonatal rat myocardial cells.



Gomisin J

MATERIALS AND METHODS

Materials Gomisin J was isolated from *Kadsura interior* and specified by spectral analysis. It is identical to the reported[6] and spectrum pure, dissolved in dimethyl formamide. 2-Thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane, Eagle's MEM, and HEPES were purchased from Sigma. Other reagents were AR grades produced in Shanghai Chemical Plant. ADP and NADPH were biochemical reagents obtained from Shanghai Institute of Biochemistry, Chinese Academy of Sciences.

Preparation of mitochondria Liver mitochondria were prepared from ♂ Sprague-Dawley rats weighing 250 − 300 g[7]. The liver homogenate was centrifuged at 600 × *g* at 4 ℃ for 15 min. The supernatant was centrifuged at 10 000 × *g* at 4 ℃ for 20 min and the pellet was resuspended in Tris-buffer.

Measurement of LPO The rate of membrane LPO was measured by the formation of TBA reactive substance (TBARS). Mitochondrial suspension 0.5 mL (containing 1

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mg protein) was incubated at 37 ℃ with 0.1 mL of the test compound or vehicle for 15 min. LPO was initiated by the addition of $FeSO_4$ 50 $\mu mol \cdot L^{-1}$ – ascorbic acid 0.1 $mmol \cdot L^{-1}$ 0.1 mL and the incubation continued for 45 min. The reaction was stopped by adding 2 mL HCl (0.1 $mol \cdot L^{-1}$); 0.67 % TBA was added and the mixture was heated in a boiling water bath for 30 min. The TBARS was extracted by *n*-butanol and measured at 532 nm with 722 spectrophotometer[6].

In other experiment, LPO was initiated by a ADP/NADPH system containing ADP 40 $mmol \cdot L^{-1}$ and NADPH 4 $mmol \cdot L^{-1}$[5].

The antioxidant activity of the test compound was calculated as present inhibition of the LPO elevation obtained in the absence of the inhibitor, which was assumed to be 100 %.

Tissue culture The myocardial cells were prepared from the ventricles of 1 – 3-d newborn SD rat hearts[9]. Five-day-old monolayer cultures were used. Before calcium paradox, the cultures were preincubated in Hanks' HEPES with or without the test compound for 10 min. The cells were washed twice and incubated in Ca^{2+}- and Mg^{2+}-free Hanks' HEPES for 2 min. Then the media were removed, Hanks' HEPES was added, and cells were reincubated for 10 min[10]. The media were removed, ice-cold Tris buffer was added, and the cells were released and homogenized. The fluorescent products were assayed[11]. The protein content was determined by Coomassie Brilliant Blue method.

Data analysis The fluorescent substances were calculated

$$MDA\ (\mu mol/g\ protein) = 0.5 \times \frac{f}{F} \times \frac{1}{mg\ protein}$$

f = sample tube; F = standard tube. The calibration substance was 1,1,3,3-tetraethoxypropane. The data were shown as $\bar{x} \pm s$ and evaluated by chi-square test.

RESULTS

Anti-lipid peroxidative action Gomisin J inhibited Fe^{2+}/ascorbic acid and ADP/NADPH-induced TBARS formation, more potently than vitamin E. Its IC_{50} values (95 % confidence limits) were 5.5 (4.5 – 6.7) $\mu mol \cdot L^{-1}$ in Fe^{2+}/ascorbic acid system and 4.7 (2.8 – 7.8) $\mu mol \cdot L^{-1}$ in ADP/NADPH system. There is little difference between them (Tab 1).

Effect on cultured myocardial cells When the heart cells incubated with Ca^{2+}-free medium for 2 min were reincubated with normal medium containing Ca^{2+}, a marked increase of LPO occurred. However, when incubation was done with gomisin J 10 – 100 $\mu mol \cdot L^{-1}$, MDA formation decreased ($P > 0.05$ *vs* normal control) (Tab 2). These data suggested a calcium paradox in heart cells reincubated with Ca^{2+} resulting in a product of LPO and cellular necrosis, as well as a preventive effect of gomisin J.

Tab 1. Inhibitory effects of gomisin J and Vit E (10 $\mu mol \cdot L^{-1}$) on liver mitochondria LPO. $n = 4$ rats. $\bar{x} \pm s$.

	Concentration/ $\mu mol \cdot L^{-1}$	Inhibition %	
		Fe^{2+}/ascorbic acid	ADP/NADPH
Gomisin J	50.3	117.7 ± 16.1	146 ± 42
	25.1	98.7 ± 9.5	143 ± 36
	5.0	46.6 ± 6.9	68 ± 36
	2.5	33.4 ± 7.6	25 ± 17
Vitamin E	10.0	51.9 ± 6.9	20 ± 5

Tab 2. Anti-lipid peroxidative effect of gomisin J on calcium-paradox (CP)-induced impairment of cultured myocardial cells. $n = 4$ samples (each was pooled from 40 neonatal rats and assayed in duplicate). $\bar{x} \pm s$.
[c] $P < 0.01$ *vs* normal control; [f] $P < 0.01$ *vs* CP control; [i] $P < 0.01$ *vs* CP + gomisin J 1 $\mu mol \cdot L^{-1}$.

CP	Gomisin J $\mu mol \cdot L^{-1}$	MDA formation ($\bar{x} \pm s$) $\mu mol/g$ protein
–	–	11.0 ± 1.0
+	–	28.8 ± 3.9[c]
+	1	23.7 ± 2.5
+	10	10.0 ± 2.0[fi]
+	50	8.9 ± 3.4[fi]
+	100	9.6 ± 2.3[fi]

DISCUSSION

Two commonly applied LPO inducers were used in the present study to examine the antioxidant activity of gomisin J[5,8]. Both stimulated LPO in rat liver mitochondria membranes, Fe^{2+}/ascorbic acid mainly through non-enzymatic process (Fenton reaction), while ADP/NADPH through enzymatic process by the action of cytochrome P-450. MDA is known to be the most important product of LPO, which cross-link proteins, lipids and nuclei acids to damage membrane functions and decline cellular integrity[3]. Gomisin J showed a remarkably inhibitory action on both Fe^{2+}/ascorbic acid and ADP/NADPH-initiated LPO in rat liver

mitochondria with IC_{50} values of 5.5 and 4.7 μmol·L^{-1}, respectively, which was more potent than vitamin E and also schisanhenol[4].

The other result demonstrated that gomisin J can protect myocardial cells against the deleterious effects of calcium paradox, which is associated with an irreversible loss of contractile function of heart may be caused by the generation of oxygen-derived free radials and oxidants, and by the induction of intracellular calcium overload[10,13-14]. We submit that the protective mechanism of gomisin J is in agreement with its antiperoxidative property, and perhaps it is partially relative to its chemical structure. It has been reported that schisanhenol, containing one phenolic hydroxyl group in the structure, exhibited the most effect on inhibiting LPO among lignans isolated from *Fructus Schizandrae*[4]. There are two phenolic hydroxyl groups in the structure of gomisin J and these functional groups might primarily contribute to free radical scavenging activity. On the other hand, due to certain lipophilic drugs, such as β-blockers and calcium antagonists, appeared different antiperoxidative activities with the order of their lipophilicities[12]. Lipid solubility of gomisin J might be also involved in its antiperoxidative mechanisms.

In conclusion, gomisin J, isolated from *Kadsura interior*, not only inhibited LPO initiated by oxygen-derived free radials in rat liver mitochondria, but also protected cultured myocardial cells from injury during the development of calcium paradox. Additionally, these results seem to indicate that gomisin J have potential liver and cardiovascular protective actions.

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戈米辛 J 对肝线粒体膜和培养心肌细胞的抗脂质过氧化作用[1]

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关键词 脂质过氧化；戈米辛 J；木脂素；培养的细胞；肝线粒体；心肌

A **目的**：研究来自内南五味子的戈米辛 J 对脂质过

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氧化的影响. 方法：采用离体大鼠肝线粒体膜的脂质过氧化模型和培养心肌细胞钙反常模型 结果：戈米辛 J 能抑制 Fe^{2+}-VitC 和 ADP/NADPH 所致的脂质过氧化，IC_{50} 分别为 5 5 和 4 7 μmol·L^{-1} 戈米辛 J 10 μmol·L^{-1} 对培养心肌细胞钙反常产生的丙二醛含量的增加也有抑制作用 结论：戈米辛 J 不仅能抑制大鼠肝线粒体膜的脂质过氧化而且能保护培养心肌细胞免受钙反常损伤

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Inhibitory effects of quinidine and quinine on liver microsome oxidation enzymes in man and rat[1]

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KEY WORDS quinidine; quinine; liver microsomes; pharmacogenetics; bufuralol; 7-alkoxycoumarin *O*-dealkylase; aryl hydrocarbon hydroxylases

AIM: To compare the inhibitory effects of quinidine and quinine on liver microsome bufuralol 1′-hydroxylase (BH), aryl hydrocarbon hydroxylase (AHH), and 7-ethoxycoumarin *O*-deethylase (ED) activities in man and rat. **METHODS:** A normal phase HPLC and the fluorescence spectrometry were used to assay the enzyme activities. ***RESULTS:*** *Both quinidine and quinine* produced a concentration-dependent inhibition to liver microsome BH, AHH, and ED in man and rat. Their median inhibitory concentrations (IC_{50}) on liver microsome BH, AHH, and ED low and high affinity phases were 0.2, 378, 2952, and >5000 μmol·L^{-1} for quinine in man, 290, 613, 1465, 1595, μmol·L^{-1} for quinidine in rat; 29, 207, 808, and >5000 μmol·L^{-1} for quinine in man, and 31, 54, 597, and 2508 μmol·L^{-1} for quinine in rat, respectively. **CONCLUSION:** Quinidine is a species- and stereo-selective potent inhibitor to human liver microsome BH.

The most extensively studied human genetic polymorphism of drug biotransformation is debrisoquine 4-hydroxylation, and 2 phenotypes of extensive metabolizer (EM) and poor metabolizer (PM) were identified[1]. This polymorphism is controlled by the autosomal mendelian recessive[2]. The oxidations of a number of other drugs including bufuralol 1′-hydroxylation are controlled by debrisoquine 4-hydroxylase[1,2]. On the other hand, aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin *O*-deethylase (ED), two of the other liver microsome enzymes, are not involved by the debrisoquine genetic polymorphism[3,4]. Because the frequency of PM phenotype of *debrisoquine 4-hydroxylation is low and variable in* different races[1,5], it is difficult to obtain enough PM subjects to testify whether a drug oxidation is involved by this genetic polymorphism. One reasonable approach is to look for some specific and potent inhibitor of this enzyme so that this polymorphism can be studied in some EM subjects. Quinidine was a competitive inhibitor of debrisoquine 4-hydroxylase *in vivo* and *in vitro*[6,7]. The purpose of this paper was to study the effects of quinidine and its diastereoisomer quinine on bufuralol 1′-hydroxylase (BH), AHH, and ED activities in both human and rat liver microsomes, and to determine if quinidine is a species and stereo selective inhibitor of man BH.

MATERIALS AND METHODS

Chemicals (±)-Bufuralol hydrochloride and 1′-hydroxybufuralol were generous gifts of Roche Products Plc.

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