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Scavenging effects of tetramethylpyrazine on active oxygen free radicals

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ABSTRACT The scavenging effects of tetramethylpyrazine (ligustrazine, Lig) isolated from *Ligusticum wallichii* Franch on active oxygen free radicals were studied using spin trapping technic and chemiluminescence methods. The scavenging rate of superoxide anion ($O_2^{\cdot}$) by Lig (25 mg·ml^{-1}) was 100 % in xanthine/xanthine oxidase (Xan/XO) system, and that of hydroxyl radical ($OH^{\cdot}$) was 44 % in Fenton's reaction. The scavenging rate of lipid peroxyl radical ($LOO^{\cdot}$) by Lig (25 mg·ml^{-1}) was 80% in peroxidizing microsome system. It is possible that Lig scavenged $O_2^{\cdot}$ by catalyzing its spontaneous dismutation. The results showed that Lig had strong effects of scavenging cyotoxic oxygen free radicals ($O_2^{\cdot}$, $LOO^{\cdot}$, $OH^{\cdot}$).

KEY WORDS pyrazines; ligustrazine; electron spin resonance; chemiluminescence; antioxidants; free radicals

Active oxygen free radicals generated in cells and tissues are important mediators in various pathophysiological processes[1]. Cerebral ischemia and reperfusion would produce free radicals which caused lipid peroxidation that further caused the cerebral injury[2]. Tetramethylpyrazine (ligustrazine, Lig), an alkaloid extracted from *Ligusticum wallichii* Franch, could effectively scavenge oxygen free radicals produced by postischemic reperfusion and protect the cerebral injury better than superoxide dismutase (our unpublished data).

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In this paper, using xanthine/xanthine oxidase (Xan/XO) system to generate superoxide anion ($O_2^{\cdot}$), Fenton's reaction to generate hydroxyl radical ($OH^{\cdot}$), and peroxidizing microsome system to generate lipid peroxyl radical ($LOO^{\cdot}$), the scavenging effects of Lig were further studied by electron spin trapping technic and chemiluminescence (CL) method.

MATERIALS AND METHODS

Reagents Lig (50 mg) was dissolved in 2 ml of water as an ampule (Guang Dong Li Min Pharmaceutical Factory). 5, 5-Dimethyl-1-pyrroline-1-oxide (DMPO), α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (4-POBN), Xan and XO were purchased from Sigma Chemical Co. Luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione; Aldrich) was prepared as 1 mmol·L^{-1} stock solution in Me_2SO and diluted with phosphate buffer 50 mmol·L^{-1} (pH 7.4) before use. Other chemical reagents were of AR grade.

Instrument Electron spin resonance (ESR) spectra were recorded in flat cells at 25 C with a Varian E-109 spectrometer operating at X-band with 100-kHz modulation frequency. CL was measured with an LKB-1250 luminometer.

Measurement of $O_2^{\cdot}$ generation[2] The Xan/XO $O_2^{\cdot}$ generating reaction were measured by using ESR spin trapping technic. With or without Lig, Xan 0.32 mmol·L^{-1}, DETAPAC 0.16 mmol·L^{-1}, DMPO 80 mmol·L^{-1} and XO 100 U·L^{-1}, were mixed. ESR spectrum of DMPO-OOH were detected.

Measurement of $OH^{\cdot}$ generation[3] The Fenton's reaction was measured by ESR spin trapping technic. 1 % H_2O_2, ferrous ammonium sulfate 100 μmol·L^{-1}, DMPO 0.1 mol·L^{-1} with and without Lig were mixed. ESR spectrum of DMPO-OH were detected.

Measurement of $LOO^{\cdot}$ generation[4] The lipid peroxyl radical adduct (4-POBN-OOL) gener-

ated by Fe^{++}-induced rat liver microsome reaction was measured by trapping technic. The rat liver microsomes were prepared by using the method of Saprin *et al*[5]. The microsome mixtures mixed with and without Lig were incubated at 37 ℃ for 60 h. In addition to mixture, DETAPAC 1.2 mmol·L^{-1}, ferrous ammonium sulfate 20 mmol·L^{-1}, and 4-POBN 0.4 mmol·L^{-1} were added in proportions of 5∶1∶2∶3. The mixtures were incubated at 37 ℃ for 30 min and then detected with ESR.

Measurement of CL product[6] Using a luminol-dependent CL method, CL emission was measured in Xan/XO O_2^--generating reaction and Fenton's reaction. In the two reaction, only luminol was substituted for DMPO.

Statistical analysis Values were expressed as $\bar{x} \pm s$ and analyzed with t test.

RESULTS

Lig effect on O_2^- The spectrum of DMPO-OOH showed ESR signal intensity by calculating h values (mm) (Fig 1A). In the presence of Lig, spin-trapped O_2^- adduct was scavenged concentration-dependently (Tab 1).

Lig effect on OH^- The spectrum of DMPO-OH showed ESR signal intensity by calculating h values (mm) (Fig 1B). In the presence of Lig (25 mg·ml^{-1}), spin-trapped OH^- adduct was scavenged (Tab 1).

Tab 1. Scavenging effects of Lig on oxygen free radicals. $n=3$ experiments, $\bar{x} \pm s$, $^bP<0.05$, $^cP<0.01$ *vs* control.

	Concentration/ mg·ml^{-1}	Scavenging percentages/% O_2^-	OH^-	LOO^-
Control	0	0	0	0
Lig	8.0	13±7^b	—	59±14^b
	12.5	76±6^c	16±7	65±9^b
	25.0	100^c	44±5^c	80±5^c

Lig effect on LOO^- The spectrum of 4-POBN-OOL showed ESR signal intensity by calculating $(h_1 + h_2)/2$ values (mm) (Fig 1C). 4-POBN-OOL was scavenged concentration-dependently in the presence of Lig (Tab 1).

Lig influence on CL produce The result showed that CL was inhibited in the presence of Lig (25 mg·ml^{-1}) (Tab 2).

DISCUSSION

Experiments showed that Lig had effects on the spin trapping of O_2^- and OH^- by DMPO using two system which is water soluble. Meanwhile, the CL intensity was only

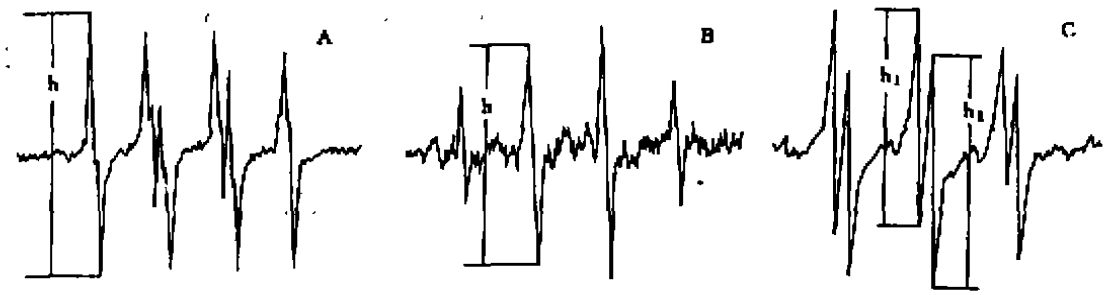

Fig 1. ESR spectra of (A) DMPO-OOH, (B) DMPO-OH, and (C) 4-POBN-OOL. Microwave power 15 mW. Scan range 20 mT. Time constant 128 ms. Modulation amplitude (A & B) 0.1 and (C) 0.2 mT. Scan speed (A & B) 5 and (C) 2.5 mT·min^{-1}.

Tab 2. Inhibition effects of Lig on CL produce. $n=3$ experiments, $\bar{x}\pm s$. $^{*}P<0.01$ vs control.

	Concentration/ mg·ml^{-1}	Xan/XO system	Fenton's reaction system
Control	0	0	0
Lig	25	52±7*	34±4*

inhibited about 52 %, but the ESR signal intensity was inhibited at 100 % in the Xan/XO $O_2^{\cdot}$ generating reaction. $O_2^{\cdot}$ can slowly undergo spontaneous dismutation at physiologic pH to generate H_2O_2 and O_2[1]. This study suggested that Lig could scavenge $O_2^{\cdot}$ by catalyzing its spontaneous dismutation which produce a lot of H_2O_2. It also showed that 4-POBN-OOL could be inhibited by Lig in a system which was lipid soluble. The scavenging effects of Lig were stronger on $O_2^{\cdot}$ and $LOO^{\cdot}$ than $OH^{\cdot}$. According to their scavenging rates, the oxygen-free-radical-scavenging activity of Lig could be arranged in the following order : $O_2^{\cdot} > LOO^{\cdot} > OH^{\cdot}$. The structure of Lig showed it has two nitrogen with a pair of electron isolated, which has reactivity. We speculate that the nitrogen of Lig can be oxidazed by active oxygen free radicals.

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四甲吡嗪对活性氧自由基的清除作用

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摘要 用电子自旋共振技术和化学发光法研究了四甲吡嗪(川芎嗪，Lig)对活性氧自由基的清除作用. Lig 25 mg·ml^{-1}对两种水溶液体系产生的$O_2^{\cdot}$和$OH^{\cdot}$的清除率分别为100 %和44%. 该作用被化学发光法证实. Lig 25 mg·ml^{-1}对一种脂溶液体系产生的$LOO^{\cdot}$的清除达80 %. 提示两性药物Lig能直接清除具细胞毒性作用的自由基($O_2^{\cdot}$, $LOO^{\cdot}$, $OH^{\cdot}$).

关键词 吡嗪；川芎嗪；电子自旋共振；化学发光；抗氧化剂；自由基