involved in the regulation of EOP in the emotion of anxiety.

ACKNOWLEDGMENT Ms CHEN Yu-Hua typed the manuscript.

REFERENCES

1 Lister RG. Ethologically-based animal models of anxiety disorders. *Pharmacol Ther* 1990; **46**: 321 - 40.

2 Gronzalez JP, Brogden RN. Naltrexone: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of opioid dependence. *Drugs* 1988; **35**: 192 - 213.

3 Zhang HT, Luo ZP, Qin BY. Anxiogenic effect of naltrexone in Vogel's conflict procedure in rats. *Chin J Pharmacol Toxicol* 1995; **9**: 254 - 7.

4 Zhang HT, Luo ZP. Potentiating effect of clonidine on anxiolytic action of buspirone in rats. *Acta Pharmacol Sin* 1993; **14**: 354 - 7.

5 Heal DJ, Prow MR, Buckett WR. Clonidine-induced hypoactivity and mydriasis in mice are respectively mediated via pre- and postsynaptic α_2-adrenoceptors in the brain. *Eur J Pharmacol* 1989; **170**: 19 - 28.

6 Petersen EN, Jensen LH. Proconflict effect of benzodiazepine receptor inverse agonists and other inhibitors of GABA function. *Eur J Pharmacol* 1984; **103**: 91 - 7.

7 Duka T, Stephens DN. Potentiation of the propunishment, but not the convulsant action of the β-carboline DMCM by naltrexone. *Pharmacol Biochem Behav* 1986; **25**: 595 - 8.

8 Kahn RS, Van Praag HM, Wetzler S, Asnis GM, Barr G. Serotonin and anxiety revisited. *Biol Psychiatry* 1988; **23**: 189 - 208.

在大鼠群居相互接触模型上纳曲酮的致焦虑作用[1]

张汉夏，徐志明，罗质璞，秦伯益
（军事医学科学院毒物药物研究所，北京 100850，中国）

关键词 纳曲酮；动物行为；吗啡；对氯苯丙氨酸；5-羟色氨酸；运动；焦虑症

目的：观察纳曲酮（Nal）对焦虑情绪的影响 方法：在群居焦虑模型上观察给予 Nal 等药后配对大鼠主动接触时间的变化 结果：Nal（0.1－50 mg·kg^{-1}）明显减少大鼠在强光不熟悉环境下的主动接触时间，且有剂量和时间依赖关系，并可被吗啡（5 mg·kg^{-1}）和 5-HT 合成抑制剂 Fen（150 mg·kg^{-1}×3 d）所拮抗，为 5-HT 合成前体 5-HTP（50 mg·kg^{-1}）所增强。而 Nal 对大鼠运动性活动无显著影响 结论：Nal 使动物产生焦虑状态；中枢阿片肽能神经通过其对 5-HT 能神经的紧张性抑制作用参与焦虑情绪的调控

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jul; 17 (4): 317 - 320

Effects of β-carotene on doxorubicin-induced cardiotoxicity in rats

LÜ Huan-Zhang[1], GENG Bao-Qin, ZHU Yong-Lian, YONG Ding-Guo
(*Department of Pharmacology, Zhejiang Medical University, Hangzhou 310006, China*)

KEY WORDS β-carotene; doxorubicin; lipid peroxidation; superoxide dismutase; glutathione peroxidase; free radicals; electron spin resonance spectroscopy

AIM: To study the effects of β-carotene (Car) reducing the cardiotoxicity induced by doxorubicin (Dox). **METHODS**: The pathological changes of rat myocardium were observed with photomicroscopy. The malondialdehyde (MDA) value of rat heart was measured with thiobarbituric acid method. The pyrogallol autoxidation method was used for determination of superoxide dismutase (SOD) activity. The activities of glutathione peroxidase (GSH-Px) were quantitatived with DTNB method. Electron spin resonance (ESR) technique was used to measure the level of the semiquinone free radicals. **RESULTS**: Car 10 or 30 mg·kg^{-1}·d^{-1} ig reduced the cardiotoxicity induced by Dox, diminished the myocardial MDA production ($P < 0.01$), and protected the activi-

[1] Now in *Department of Clinical Pharmacology, North Taiping Road Hospital, Beijing 100039, China*.

Received 1994-08-18 Accepted 1995-11-16

ties of SOD and GSH-Px. ESR revealed that Car scavenged semiquinone free radicals induced by Dox *in vitro*. The inhibitory rates of semiquinone free radicals formation by Car 0.02, 0.1, and 1.0 mmol·L^{-1} were 47.7 %, 76.6 %, and 82.5 %, respectively. **CONCLUSIONS**: Car, with abilities of anti-lipid peroxidation and scavenging semiquinone free radicals, possessed effects of reducing Dox-induced cardiotoxicity.

Doxorubicin (Dox) plays an important role in cancer chemotherapy. But its clinical use has been limited by its irreversible cardiomyopathy[1]. β-Carotene (Car) is a natural nutrient and has anti-oxidant effects[2]. This study was to investigate the protective effects of Car against Dox induced cardiotoxicity in rats.

MATERIALS AND METHODS

Car and Dox were the products of Shanghai Sixth Pharmaceutical Factory and Haimen Pharmceutical Factory, respectively. Car was ground with Tween-80 and then dissolved in water. Dithiobisnitrobenzoic acid and glutathione were obtained from Sigma. Sprague-Dawley rats were bred from the Experimental Animal Center of Zhejiang Medical University. The instrument of electron spin resonance (ESR) was made by Jeol Co, Japan.

1 Regimen Rats (♀ & ♂, $n = 24$, weighing 145 ± s 21 g) were divided randomly into 4 groups, each containing 6 rats. (1) Control group: injected normal saline 1 mL·kg^{-1} ip. (2) Dox group: injected Dox 1 mg·kg^{-1} ip on d 2 and d 4; 2 mg·kg^{-1} on d 6 and d 8; 3 mg·kg^{-1} on d 10 and d 12; 4 mg·kg^{-1} on d 14. During 2 wk, the cumulated dose of Dox was 16 mg·kg^{-1}. (3) Dox + Car 10 mg·kg^{-1}·d^{-1} group. (4) Dox + Car 30 mg·kg^{-1} group. Car was administered by ig.

Pathological observation Twenty-four hours after the last ip of Dox, the rats were anesthetized and blood samples were collected. Heart and liver were fixed by 10 % formalin and stained with hematoxylin and eosin. The severity of pathologic changes was graded into 0 – 3[3]. Grade 0 – normal; Grade 1 – a few cells with cytoplasmic vacuolization; Grade 2 – groups of cells with cytoplasmic vacuolization; Grade 3 – diffuse cells damage with vacuolization, mergence, frank necrosis.

Biochemical determinations MDA was measured with thiobarbituric acid method[4]. Pyrogallol autoxidation method was used for determination of SOD activity[5]. GSH-Px activity was assayed[6]. Colorimetric method (Lowry *et al* 1951) was used for determination of protein content.

2 Semiquinone free radicals measurement[7] Four normal rats, weighing 370 ± s 10 g, were anesthetized. The hearts were washed 3 times with saline, and homogenized with HEPES (50 mmol·L^{-1}) – KCl (125 mmol·L^{-1}) buffer (pH 7.4) to make a 1:4 (w:v) homogenate, which was centrifuged at 1500 × g for 20 min. The precipitate was discarded. The tubes contained 0.30 mL centrifuged homogenates, 0.05 mL Car, which was dissolved in tetrahydrofuran, at 0.02, 0.10, 1.0 mmol·L^{-1} or 0.05 mL tetrahydrofuran instead in the control group, 0.10 mL DOX at 1.28 mmol·L^{-1} and 0.05 mL NADH at 2 mmol·L^{-1}. The free radicals were assayed with ESR instrument at X band, microwave power 4 mW, modulation amplitude 2.5 G, response 0.3 s, at 25 ℃.

Statistical analysis The pathological scores were analyzed with rank sum test. Other data were analyzed with t test.

RESULTS

Effects on Dox-induced cardiotoxicity The rats of control group had no myocardial damage (Fig 1A, plate 1). In the rats received Dox alone, the marked myocardial damage occurred (Fig 1B), and the myocardial MDA value was higher than that of the control (Tab 1, $P < 0.05$). The rats given Car 10 and 30 mg·kg^{-1}·d^{-1} had less myocardial damage (Fig 1C, Fig 1D), and the pathological scores and the myocardial MDA values were lower than those of Dox group (Tab 1).

Tab 1. Effects of β-carotene on doxorubicin-induced cardiotoxicity and biological changes. $n = 6$, $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* **doxorubicin**; $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* **control**.

Dox, mg·kg^{-1}	Car, mg·kg^{-1}	Pathological scores	Malondialdehyde, μmol/g protein	GSH-Px, kU/g protein	SOD, kU/g protein heart	SOD, kU/g protein erythrocytes
0	0	0	3.93 ± 0.85	63 ± 13	8 161 ± 1 378	2 945 ± 1 147
16	0	2.1 ± 0.4^{e}	4.74 ± 0.22^{c}	47 ± 9^{e}	5 207 ± 2 177^{e}	1 140 ± 630^{f}
16	10	1.2 ± 0.3^{c}	3.99 ± 0.41^{b}	60 ± 5^{c}	6 438 ± 660	1 377 ± 411
16	30	0.8 ± 0.4^{c}	3.91 ± 0.56^{c}	60 ± 4^{c}	7 405 ± 689^{b}	2 158 ± 523^{b}

Effects on SOD activity In Dox group, the SOD activity of both erythrocytes and heart was lower than the control's ($P < 0.01$, $P < 0.05$, respectively). The SOD activity of both erythrocytes and heart of rats in the group of Dox + Car 30 mg·kg^{-1}, was higher than that of Dox group ($P<0.05$). However, the group of Car 10 mg·kg^{-1}·d^{-1} had no such effects (Tab 1).

Effects on GSH-Px activity In Dox group, the myocardial GSH-Px activity was lower than that in the control ($P<0.05$). The myocardial GSH-Px activities in Dox + Car 10 and 30 mg·kg^{-1} groups were higher than those in Dox group (Tab 2). The GSH-Px activity of liver in control group ($206 \pm s$ 50) was higher than that of heart in control group ($63 \pm s$ 13, $P<0.01$).

Tab 2. Scavenging effects of β-carotene on doxorubicin semiquinone free radicals in rat heart homogenate. $n=3$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

β-Carotene/ mmol·L^{-1}	Intensity of ESR/ signal·mm^{-1}	Scavenging rate/ %
0	22 ± 3	
0.02	12 ± 3^b	47.2
0.10	5.1 ± 0.8^c	76.6
1.0	3.2 ± 1.2^c	85.3

Effects on scavenging semiquinone free radicals The semiquinone free radicals were detected in the control group, and identified by its g-value at 2.0032 (Fig 2). Addition of Car 0.02, 0.10, and 1.0 mmol·L^{-1} reduced markedly the intensities of ESR signals (Fig 2), and their rates of scavenging semiquinone free radicals were 47.2 %, 76.6 %, and 85.3 %, respectively (Tab 2).

DISCUSSION

The experiment results showed that Dox induced severe myocardial damage, and Car 10, 30, mg·kg^{-1}·d^{-1} had protective effects against Dox induced cardiotoxicity in rats. It has been believed that the cardiotoxicity induced by Dox is caused by free radicals, which has little relation to the anticancer effects of Dox[8,9]. It is known that Car can quench singlet oxygen[2,10], but there is no report to scavenge other free radicals. In this

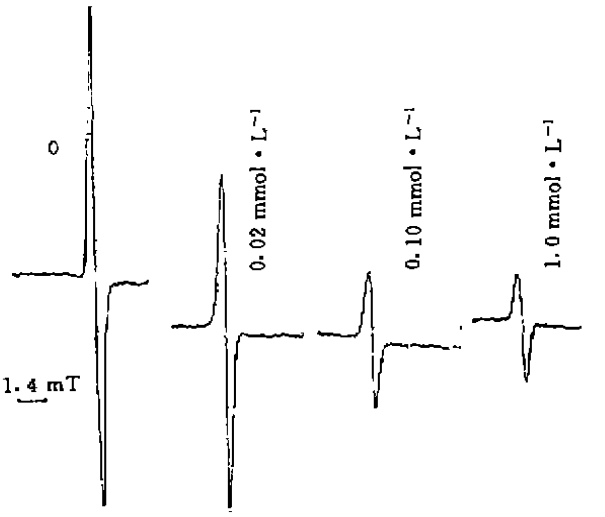

Fig 2. ESR spectra of doxorubicin semiquinone free radicals in rat heart homogenate with β-carotene.

experiment, we found that Car had a dose-dependent effects of scavenging semiquinone free radicals induced by Dox with homogenate of rat heart. The semiquinone free radicals may further transfer electron to oxygen or H_2O_2, and produce $O_2^{\cdot}$ or $OH^{\cdot}$ radicals. A deteriorative reaction caused by these active free radicals induces cell injury[11]. It is suggested that Car molecule reacts with the free radical, resulting in the formation of a new, much more stable radical. It was postulated that this is possible due to the presence of a conjugated double bond system, which facilitates a resonance condition[10].

The results showed that the escalation of myocardial MDA level, and reduction of myocardial GSH-Px activity and SOD activity of rat heart or erythrocytes occurred in Dox group. Administration of Car 10, 30 mg·kg^{-1}·d^{-1} inhibited the myocardial MDA escalation induced by Dox, and protected the myocardial GSH-Px activity. Treatment of Car 30 mg·kg^{-1}·d^{-1} showed the protection of SOD activity of rat heart or erythrocytes. These results indicated that Car possessed the effects of anti-lipid peroxidation in rats administered Dox. It appears that the reduction of Dox induced cardiotoxicity by Car may be due to its effects of anti-lipid peroxidation in rats.

We also found that there were different level of GSH-Px between heart and liver in normal rats. Doroshow *et al* reported that SOD activity of heart

was significantly lower than that of liver in mice[12]. It was suggested that lower level of myocardial antioxidase is one of the reason that Dox results in cardiotoxicity easily.

In summary, Car treatment protects rats against Dox cardiotoxicity. The mechanism of protection appears to be due, at least in part, to scavenge semiquinone free radicals and possess effects of anti-lipid peroxidation.

REFERENCES

1 Young RC, Ozols RF, Myers CE. The anthracycline antineoplastic drugs. N Engl J Med 1981; **305**: 139 - 53.

2 Burton GW, Ingold KU. β-Carotene: an unusual type of lipid antioxidant Science 1984; **224**: 569 - 73.

3 Billingham ME, Mason GW, Bristow MR, Daniels JR. Anthracycline cardiomyopathy monitored by morphological changes. Cancer Treat Rep 1978; **62**: 865 - 72.

4 Zhan H, Liu CG, Zhou JH. Protective effects of Lycium barbarum polysaccharide against physical stress and CCl_4-induced tissue lipid metabolism changes in rats and mice. Chin J Pharmacol Toxicol 1989; **3**: 163 - 8.

5 Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; **47**: 469 - 74.

6 Hafemen DG, Sunde RA, Hoekstra WG Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr 1974; **104**: 580 - 7.

7 Zhang Y, Zhang TM, Zhao BL, Han JK, Chen WC, Xin WJ. Scavenging of prombimane on semiquinone free radical formation by doxorubicin in rat heart. Acta Pharmacol Sin 1991; **12**: 20 - 3.

8 Momparler RL, Karon M, Siegel SE, Avila F. Effect of adriamycin on DNA, RNA, and protein synthesis in cell-free systems and intact cells. Cancer Res 1976; **36**: 2891 - 5.

9 Goodman J, Hochstein P. Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. Biochem Biophys Res Commun 1977; **77**: 797 - 803.

10 Levin G, Mokady S. Antioxidant activity of 9 cis compared to all-trans *in vitro*. Free Radic Biol Med 1994; **17**: 77 - 82.

11 Halliwell B, Gutteridge JMB. Free radicals in Biology and Medicine. Oxford: Clarendon Press, 1989: 188 - 266.

12 Doroshow JH, Locker GY, Myers CE. Enzymatic defence of the mouse heart against reactive oxygen metabolites: alterations produced by doxorubicin J Clin Invest 1980; **65**: 128 - 35.

317-320

β-胡萝卜素对阿霉素所致的大鼠心脏毒性的作用

吕焕章[1]，耿宝琴，朱永廉，雍定国

(浙江医科大学药理教研室，杭州 310006，中国)

关键词 β-胡萝卜素；阿霉素；脂质过氧化；超氧化物歧化酶；谷胱甘肽过氧化物酶；自由基；电子自旋共振光谱分析 心脏.毒性

目的：研究β-胡萝卜素减轻阿霉素所致的大鼠心脏毒性的作用及其机制，**方法**：应用光学显微镜技术观察心肌组织的病理变化，心肌 MDA 值用巴比妥酸法测定，SOD 活性用邻苯三酚法测定，GSH-Px 活性用 DTNB 法测定，运用顺磁共振(ESR)技术测定半醌自由基 **结果**：β-胡萝卜素 10 或 30 $mg \cdot kg^{-1}$ 可明显减轻阿霉素引起的大鼠心肌损害，保护 SOD，GSH-Px 活性，对抗阿霉素引起的心肌 MDA 水平升高，体外实验表明，β-胡萝卜素可以清除阿霉素诱导产生的半醌自由基，在 0.02，0.1，1 0 $mmol \cdot L^{-1}$ 三个浓度时的抑制率分别为 47.7 %，76.6 %，85.2 %. **结论**：β-胡萝卜可以减轻阿霉素引起的大鼠心肌损害，其机制与抗脂质过氧化和清除半醌自由基有关.

R965.1 R977.21