quantitative microassay for activity. *J Immunol* 1978; **120** : 2027–32.
14 Diamantstein T, Osawa H, Graf L, Schirrmacher V. Studies on interleukin 2 receptor expression and IL–2 production by murine T cell lymphomas. *Br J Cancer* 1985; **51** : 23–30.
15 Gilmore W, Weiner LP. β–Endorphin enhances interleukin–2 (IL–2) production in murine lymphocytes. *J Neuroimmunol* 1988; **18** : 125–38.

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甲硫氨酸脑啡肽对白细胞介素 2 产生及其受体表达的影响

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提要 甲硫氨酸脑啡肽(met–enk)在 10 nmol · L^{-1}–100 μmol · L^{-1} 浓度下与 Con A 能协同增强淋巴细胞产生 IL–2，并呈剂量依赖关系：ip 0.01、0.1 及 1 mg · kg^{-1} 后，IL–2 产生与脾淋巴细胞增殖平行加强；但 met–enk 10 nmol · L^{-1} 对胸腺细胞、脾细胞及肠淋巴结细胞上 IL–2 受体表达无影响，提示 met–enk 的免疫调节作用是通过促进 IL–2 产生而不是增加其受体表达而实现的.

关键词 甲硫氨酸脑啡肽；白细胞介素 2；白细胞介素 2 受体；迟发型超敏感性；淋巴细胞转化

BIBLID: ISSN 0253–9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Mar; 13 (2) : 136–139

Effects of copper and selenium on electric parameters of cultured myocardial cells damaged by xanthine–xanthine oxidase

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ABSTRACT Addition of xanthine 0.42 mmol · L^{-1} and xanthine oxidase 5.3 nmol · L^{-1} (X–XO) to the culture medium increased the amplitude of ESR spectra of myocardial cells, demonstrating an increase in free radical contents; diminished the action potential parameters significantly and reduced the input impedances from 0.34 ± 0.11 to 0.24 ± 0.1 MΩ, expressing a typical electrical appearance of membrance damage. Supplying Cu 62.5 ng · ml^{-1} and / or Se 173 ng · ml^{-1} to the medium brought all of the electric parameters and the free radical content of myocardial cells back to normal. The results indicate that both the two trace elements are able to scavenge free radicals, thus antagonizing X–XO, which induces damage to myocardial cells.

KEY WORDS cultured cells; myocardium; copper; selenium; free radicals; action potentials; xanthine oxidase

Received 1990 Nov 19 Accepted 1991 Nov 29

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Cu and Se are the active centers of superoxide dismutase (SOD)[1] and glutathione peroxidase (GP)[2] respectively, which are powerful scavengers of free radicals in the body. In this experiment, we studied the antioxidative action of the two trace elements, taking the action potential, input impedance, and free radical content of cultured myocardial cells as indices.

METHODS

Cardiomyocyte culture[3] Dispersed cardiomyocytes from neonatal Wistar rats were cultured in a carbon dioxide incubator (36.5℃, pH 7.2), and divided into 5 groups, according to the different compositions of the culture media:

Control group: 80% Dulbecoo's modified Eagle medium (DMEM) and 20% fetal bovine serum (FBS).

Enzyme group: 80% DMEM+20% FBS+xanthine 0.42 mmol · L^{-1} and xanthine oxidase 5.3 nmol · L^{-1} (X–XO)

Enzyme+Cu group: 80% DMEM +20% FBS+X–XO+Cu 62.5 ng · ml^{-1} (Cu)

Enzyme+Se group: 80% DMEM +20% FBS+X–XO+Se 173 ng · ml^{-1} (Se)

Enzyme + Cu + Se group: 80% DMEM + 20% FBS + X–XO + Cu + Se

X–XO was added to the culture media 16 h before observing on the 3 indices. The concentration of Se and Cu was maintained by adding Na_2SeO_3 and $CuCl_2$ to the media at the beginning of culture and throughout the whole course of the experiment.

Recording of action potential The top wall of polytyrene culture vessel was removed and a layer of liquid paraffin was put onto the culture medium to maintain the circumstances of cardiomyocytes at 36.5℃, pH 7.2. Action potentials were recorded inside the cell with glass microelectrode (tip 0.5 μm, electric resistance 20–40 MΩ).

Measurement of membrane input impedance[4] Two microelectrodes identical to that used in action potential recording were separately penetrated into two different cells, 100–120 μm apart, in the same beating cluster. One electrode was used to inject current 10 nA(I), while the other one was used to record the membrane potentials. The inputimpedance (R_{inp}) was calculated from the change of potential (Δ V): $R_{inp} = \Delta V / I$.

Free radical measurement The cultured cardiomyocytes were put into the ESR quartz sample tube at 18–20℃. The content of free radicals was measured in evaporating liquid N_2 environment with a ER200D – SRC electron spin resonance spectroscopy (ESR). Test conditions: temperature −177℃, microwave frequency 9.53 GHz, microwave power 17 db 4.1 mW, modulation frequency 100 kHz, modulation amplitude 32 G, gain 2.5×10^5.

RESULTS

Action potential of cultured cardiomyocyte During the period of d 4 – d 10 explantation, action potentials were recorded. Seven parameters were analyzed with an APPLE II microcomputer on–line: action potential amplitude (APA), over–shoot (OS), threshold (TP), maximal diastolic potential (MDP), maximal rate of depolarization (V_{max}), action potential duration at 90% repolarization level (APD_{90}), and the discharging frequency (F). Compared with the control group, all of the electric parameters of the enzyme group decreased significantly. On the other hand, the APA, OS, MDP, TP, and F of enzyme+Cu, enzyme+Se, and enzyme+Cu+Se groups were restored to the control levels or even greater. When Cu was used in combination with Se, the effects were more notable than either of them alone (Tab 1, Fig 1).

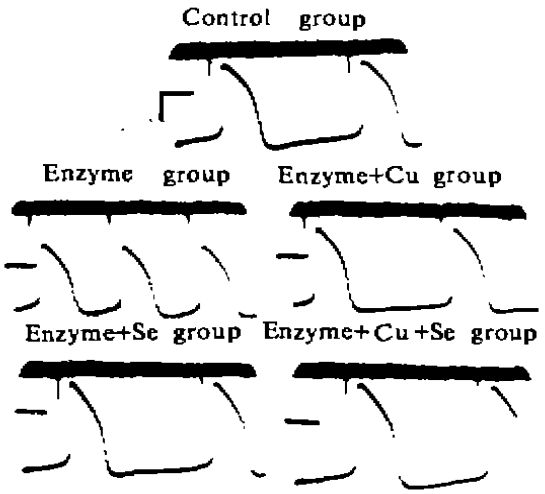

Fig 1. Typical action potential recordings of cultured rat myocardial cells. Upper tracing: *dV/dt*; Lower tracing: action potential. Vertical scale: 20 mV, 10 V · s^{-1}; Horizontal scale: 100 ms

Tab 1. Effects of X–XO, Cu, Se on action potentials and membrane input impedances of cultured rat heart cells. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **enzyme group.**

	Cells	APA / mV	OS / mV	MDP / mV	TP / mV	V_{max} / $V \cdot s^{-1}$	APD_{90} / ms	F / bpm	Cells	R_{inp} / MΩ
Control	25	71 ± 14**	24 ± 8**	47 ± 10**	29 ± 8**	15 ± 5**	139 ± 22**	157 ± 80**	23	0.338 ± 0.11**
Enzyme	33	58 ± 8	20 ± 4	39 ± 10	22 ± 6	7 ± 2	89 ± 22	280 ± 41	23	0.244 ± 0.10
Enzyme +Cu	28	69 ± 8**	23 ± 4**	46 ± 6**	28 ± 6**	13 ± 5**	141 ± 22**	177 ± 46**	15	0.320 ± 0.12**
Enzyme +Se	21	71 ± 6**	24 ± 5**	47 ± 9**	28 ± 10**	13 ± 4**	161 ± 23**	189 ± 30**	23	0.414 ± 0.11**
Enzyme +Cu+Se	24	74 ± 10**	25 ± 7**	50 ± 9**	31 ± 7**	17 ± 9**	166 ± 36**	176 ± 46**	18	0.427 ± 0.17**

Input impedance of cardiomyocytes The column 8 in Tab 1 shows the input impedances estimated during the period of d 4 – d 10 explantation. X–XO reduced the input impedance significantly, while Cu and / or Se supplement turned the input impedance back to the control value or beyond. Again, the supplement of Cu and Se was more effective than either of them alone.

Free radical content of cardiomyocytes After 4 d culture, the ESR spectra of the cultured myocardial cells were recorded. The shapes and durations of the spectra in the 5 groups were similar to each other and the g values of all were the same (2.0006), indicating the identity of the free radicals. The spectral amplitude of enzyme group was higher, while those of the enzyme+Cu, enzyme+Se, enzyme+Cu+Se groups were lower than the control group, showing that X–XO increased while Cu and Se decreased the free radical contents of cardiomyocytes (Fig 2).

DISCUSSION

In this experiment, the difference of the ESR spectra among the groups demonstrated conclusively that X–XO did increase the free radical content of the cultured rat heart cells, while Cu and Se were able to decrease the content of free radicals effectively. These results were consistent with the reports that X–XO system induces superoxide anion free radical[5], and Cu and Se are the active centers of SOD[1] and GP[2], respectively.

The increase in free radical content led to the degradation of membrane lipid and formation of nonspecific ionic channels[6], which resulted in the disorder of ion distribution across

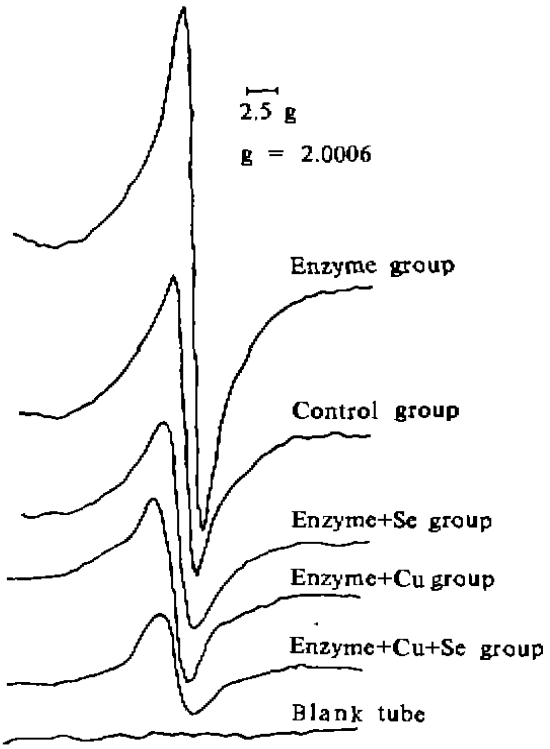

Fig 2. ESR spectra of cultured rat myocardial cells.

the membrane, thus depolarizing the membrane. The decrease in MDP, in turn, reduced the rate and amplitude of 0 phase depolarization, thus decreasing V_{max}, APA, OS, TP. The increase of $[Ca^{2+}]_i$ inactivated the Ca^{2+} channel and activated the K^+ channel[7], thus shortening the duration of the action potential. Cu and Se might restore the electrical parameters by way of scavenging the free radicals and protecting the cardiac cell membrane.

The results of this experiment demonstrate that Cu and Se played an antioxidative role in scavenging the free radicals. As a result, the heart cells were protected.

REFERENCES

1 Wang WJ. Superoxide free radical and superoxide dismutase. *Prog Physiol Sci* 1985; **16**: 196–202.

2 Flohe L, Günzer WA, Schock HH. Glutathione peroxidase: A selenoenzyme. *FEBS Lett* 1973; **32**: 132–4.

3 Jiang Y, Zhao P, Zhang WJ. Influence of Se on the survival of cultured heart cells. *Chin J Endemiol* 1986; **5**: 163–6.

4 Schanne OF, Ceretti E. *Impedance measurement in biological cells* 1 st ed. NY: John Wiley, 1978: 202–12.

5 McCord JM. Oxygen derived free radicals in postischemic tissue injury. *N Engl J Med* 1985; **312**: 158–63.

6 Meerson FZ, Kagan VE, Kozlow YP, Belkina LM, Arkhipenko YV. The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of heart. *Basic Res Cardiol* 1982; **77**: 465–85.

7 Ruiz-Petrich E, de lorenzi F, Chartier D. Role of the inward rectifier I_{k1} in the myocardial response to hypoxia. *Cardiovasc Res* 1991; **25**: 17–26.

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铜硒对 X-XO 致损的培养心肌细胞电参数的影响

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R963

提要 向培养基中加入黄嘌呤 0.42 mmol · L^{-1} 与黄嘌呤氧化酶 5.3 nmol · L^{-1}(X-XO)，心肌细胞的自由基含量增高；显著降低动作电位参数，输入阻抗由 0.34 ± 0.11 降到 0.24 ± 0.1 MΩ. 向培养基中加入 Cu 62.5 ng · ml^{-1} 与/或 Se 173 ng · ml^{-1}，心肌细胞的所有电参数恢复，心肌细胞的自由基含量回升. 提示铜、硒都能清除自由基，对抗 X-XO 所致的心肌细胞损伤.

关键词 培养的细胞；心肌；铜；硒；自由基；动作电位；黄嘌呤氧化酶

BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Mar; 13 (2): 139–142

Effect of dimethyl sulfoxide on cytosolic calcium in cultured rat hepatocytes injured by *D*-galactosamine[1]

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ABSTRACT *D*-galactosamine (Gal 0.5 mmol · L^{-1}) made lactate dehydrogenase (LDH) leakage from the hepatocytes in monolayer-culture increase by 50%. Dimethyl sulfoxide (Me_2SO 2% vol/vol) decreased the LDH leakage ($P<0.05$). The cytosolic free Ca^{2+} concentration ($[Ca^{2+}]c$) of rat hepatocytes exposed to Gal 4 mmol · L^{-1} for 90 min in suspension culture increased about 2-fold ($P<0.01$). Me_2SO (2%) antagonized this $[Ca^{2+}]c$-increasing effect of Gal. These results verified directly that the $[Ca^{2+}]c$ of hepatocytes was increased in the early stage

Received 1990 Sep 11 Accepted 1991 Dec 26
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