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.

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136-139

## 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

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# 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

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of Gal-induced hepatotoxicity, and suggested that the prevention or lightening of the disturbance in intracellular $Ca^{2+}$ homeostasis may be, at least, one of the mechanisms of the protective action of $Me_2SO$ against Gal-induced hepatocyte injury.

**KEY WORDS** liver; dimethyl sulfoxide; galactosamine; cultured cells; cytosol; calcium

$Me_2SO$, usually used in the treatment of dermatologic illness, was used in rheumatism as an analgesic agent during the past years[1]. $Me_2SO$ could prolong the survival time and improve the functional state of primary cultured rat hepatocytes[2,12]. It will be significant to study whether $Me_2SO$ has protective effect against Gal-induced injury in primary cultured hepatocytes. There have been different and controversal reports about the changes in cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]c$) in early stage of Gal-induced hepatotoxicity *in vivo*[3,4], but no direct evidence about the $[Ca^{2+}]c$ change in Gal-intoxicated cultured hepatocytes has been seen. $Me_2SO$ could affect the intracellular $Ca^{2+}$ concentration of murine erythroleukemia cells to induce cell differentiation[5,6]. What is the $[Ca^{2+}]c$ change in early stage of Gal-intoxicated hepatocytes? Could $Me_2SO$ exhibit its hepato-protective action *via* an influence on the $[Ca^{2+}]c$? In present paper, we studied these theoretical problems by direct determination of hepatocyte $[Ca^{2+}]c$.

## MATERIALS

Wistar rats ♂ 190 ± *s* 10 g. Collagenase (Type Ⅳ), HEPES, DME medium, Percoll (Colloidal PVP coated silica) and digitonin were purchased from Sigma. $Me_2SO$ (AR) was made by Beijing Xudong Chemical Factory. Arsenazo III (AIII, lot № 85062) was made by Shanghai 3rd Reagent Factory. Aminophosphonate resin was a gift of Dr ZHANG Zheng-Pu, Nan-Kai University.

## METHODS AND RESULTS

**Effect of $Me_2SO$ on Gal-induced cellular injury of rat hepatocytes in primary culture** Hepatocytes were isolated from adult rats by collagenase perfusion method (*Physiol Sci* 1987; 7 : 357), with the yield of $1.5 \times 10^8 - 2 \times 10^8$ cells / liver and viability > 90% (Trypan blue exclusion). Cells were cultured with DME medium as previously reported (*Physiol Sci* 1987; 7 : 357). The plates were divided into 6 groups, and their media were changed after 8-h culture with DME containing different concentrations of $Me_2SO$ in the presence or absence of Gal 0.5 mmol · $L^{-1}$. The lactate dehydragenase (LDH) activities in medium were determined colorimetrically[7] after a further 48-h culture. $Me_2SO$ decreased the Gal-induced LDH leakage by about 50% and there was no difference in potency of this action in 0.5-2% $Me_2SO$ (Tab 1). It meant that 0.5% $Me_2SO$ is enough to cause its maximal protective effect, and that 2% $Me_2SO$ is still in the plateau region. Since 2% was said to be the optimal concentration of $Me_2SO$ for its differenciation-promoting action[12], we used $Me_2SO$ 2% in all experiments.

**Tab 1. Effects of $Me_2SO$ (vol / vol) on lactate dehydrogenase leakage from the Gal-injured monolayer-cultured rat hepatocytes. $n = 6$ (tubes), $\bar{x} \pm s$, $^{**}P < 0.05$ *vs* Gal group.**

| Gal / mmol · $L^{-1}$ | $Me_2SO$ / % | LDH / μmol · $L^{-1}$ | % |
|---|---|---|---|
| 0.0 | 0.0 | 3.11 ± 0.33 | 100 |
| 0.5 | 0.0 | 4.84 ± 0.22 | 155 |
| 0.5 | 0.5 | 3.93 ± 0.21** | 126 |
| 0.5 | 1.0 | 3.78 ± 0.36** | 123 |
| 0.5 | 1.5 | 3.92 ± 0.26** | 126 |
| 0.5 | 2.0 | 3.97 ± 0.14** | 129 |

**Influences of Gal and $Me_2SO$ on hepatocyte $[Ca^{2+}]c$**

**1 Methodology of null point titration**

**technique**

**1.1 Purification of AIII** Commercially available AIII was purified by passing through 2 successive columns of aminophosphonate ion-exchange resin in $Na^+$ form[8]. We found that the contaminative $Ca^{2+}$ of commercially available AIII (2 nmol · $L^{-1}$ / mg AIII) used was lower than the value allowed (5 μmol · $L^{-1}$ / mg AIII).

**1.2 Determination of parameters** A series of parameters was determined by colorimetric assay[9]. The values were: $\varepsilon_1 = 8 \times 10^2 \cdot mol^{-1}$; $\varepsilon_2 = 218 \times 10^2 \cdot mol^{-1}$; $K_d' = 10.75$ μmol · $L^{-1}$, the AIII concentration used for all experiments was 53 μmol · $L^{-1}$. These data tallied with those in literature ($\varepsilon_1 = 15 \times 10^2 \cdot mol^{-1}$, $\varepsilon_2 = 235 \times 10^2 \cdot mol^{-1}$)[10].

**2 Effec of Gal and $Me_2SO$ on $[Ca^{2+}]c$** After 10 min preincubation at 37℃, the suspension culture tubes were divided into 5 groups. The first group was used to determine $[Ca^{2+}]c$ immediatedly as a normal value. The other groups were incubated at 37℃ for 90 min in the presence or absence of Gal 4 mmol · $L^{-1}$, with or without $Me_2SO$ 2% vol / vol, respectively. The hepatocytes were seperated from the $Ca^{2+}$-containing DME medium and the nonviable cells were removed by low-speed isodensity Percoll centrifugation at 4℃, 50 × *g* for 10 min[11], then hepatocyte $[Ca^{2+}]c$ was determined by null point titration[10]. The results showed that in freshly isolated hepatocytes, $[Ca^{2+}]c$ was 0.19 ± 0.07 μmol · $L^{-1}$, which tallied with that in literature (0.19 ± 0.01 μmol · $L^{-1}$)[10]. Gal 4 mmol · $L^{-1}$ increased $[Ca^{2+}]c$ about 3-fold as much as the control. $Me_2SO$ itself did not decrease the $[Ca^{2+}]c$, but prevented the Gal-induced $[Ca^{2+}]c$ increase (Tab 2).

**Tab 2. Effects of $Me_2SO$ (2% vol / vol) and Gal (4 mmol · $L^{-1}$) on cytosolic $Ca^{2+}$ concentration (μmol · $l^{-1}$) of hepatocytes in suspension culture (90 min).** $n = 8$, $\bar{x} \pm s$. $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **control group.** $^{++}P < 0.01$ *vs* **Gal group.** △: **freshly isolated hepatocytes.** §: **calculated with the difference in $[Ca^{2+}]c$ between Gal and control as 100%.**

| | $[Ca^{2+}]c$ | $\Delta[Ca^{2+}]c\%$ § |
|---|---|---|
| FIH△ | 0.19 ± 0.07 | |
| Control | 0.26 ± 0.03$^{++}$ | |
| Gal | 0.75 ± 0.11$^{**}$ | 100 |
| $Me_2SO$ | 0.36 ± 0.08$^{**++}$ | 20 |
| $Me_2SO$+Gal | 0.40 ± 0.10$^{**++}$ | 30 |

## DISCUSSION

In this paper we found that $Me_2SO$ could protect hepatocytes against Gal-induced cytotoxicity (Tab 1). Our previeus work showed that $Me_2SO$ could prolong the survival time of cultured hepatocytes and improve their ability of maintaining the content of glycogen and cytochrome P-450 during long term (96 h) culture[12]. We concluded that $Me_2SO$ is of benefit to normal hepatocytes and can protect them against Gal-injury.

There have been two different suggestions about the $Ca^{2+}$ changes in hepatocytes in early stage of Gal-induced injury. Some authors thought that first thing in Gal-induced injury is membrane damage, which results in an influx of $Ca^{2+}$. Others considered that redistribution of $Ca^{2+}$ within cells first occurred which made $[Ca^{2+}]c$ increase. They interpreted indirectly that $[Ca^{2+}]c$ increase is one of the causes leading to membrane damage[4,13]. But no direct evidence about the $[Ca^{2+}]c$ change in Gal-injured hepatocytes is seen before.

We observed the change in hepatocyte $[Ca^{2+}]c$ by null point titration techenique. In the work of methodology, we verified that null point titration is an economical but reliable method. Our data showed that in the early stage of Gal-induced injury (90 min), the hepatocyte $[Ca^{2+}]c$ did increase about 3-fold. This is a first evidence of the mechanisms of Gal-induced hepatotoxicity in cell level. Because the viability of hepatocytes, observed by both trypan blue exclusion and medium

LDH activity assay, was normal in the early stage of Gal-injury (90 min), we considered that cell membrane damage might be unserious. So, the $[Ca^{2+}]c$ increase seemed to be the result of Ca redistribution within cells. Recently, it is reported[14] that lipoperoxidation in liver cell membrane increased as early as 30 min after Gal-intoxication *in vivo*, and total liver calcium increased 3 h after Gal administration. So, we think, the dynamic study of $[Ca^{2+}]c$ change in whole course of Gal-intoxication is needed to clarify the details.

The hepatoprotective action of $Me_2SO$ might be related to its differentiation-promoting action[2]. Our data in this work showed, $Me_2SO$ itself did not decrease hepatocyte $[Ca^{2+}]c$, but could prevent or lighten Gal-induced $[Ca^{2+}]c$ increase (Tab 2). This may be one of mechanisms of the hepato-protective effect of $Me_2SO$ against Gal-induced injury.

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139-142

## 二甲亚砜对 *D*-半乳糖胺损伤的培养大鼠肝细胞胞液自由钙离子的影响

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R96.2

**提要** *D*-半乳糖胺(Gal, 0.5mmol · $L^{-1}$)使培养肝细胞的乳酸脱氢酶外漏增加 55%，二甲亚砜($Me_2SO$)可使此损伤减轻约二分之一($P<0.05$)。肝细胞接触 Gal 4 mmol · $L^{-1}$ 90 min，其胞液内自由钙离子浓度($[Ca^{2+}]c$)增加到对照组的 3 倍($P<0.01$)。$Me_2SO$(2% vol / vol)可拮抗 Gal 引起的$[Ca^{2+}]c$ 增加。提示：Gal 损肝早期$[Ca^{2+}]c$ 升高，而 $Me_2SO$ 阻止细胞内钙平衡的紊乱，可能是其抗 Gal 损伤的机理之一。

**关键词** 肝脏；二甲亚砜；*D*-半乳糖胺；培养细胞；细胞溶质；钙