·论 著·

IL-6下调血管内皮细胞组织因子抑制物表达

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[摘要] **目的**：观察IL-6对脐静脉内皮细胞(HUVECs)表达组织因子途径抑制物(TFPI)的影响。**方法**：应用胰酶消化HUVECs并进行传代培养，用生长良好的第2、3代细胞进行试验。同时应用CCK-8测定不同浓度的IL-6(0.125～2.0 ng/ml，实验组)刺激后细胞活性变化，对照组给予培养液；应用逆转录聚合酶链反应(RT-PCR)法检测细胞内TFPI mRNA水平。**结果**：与对照组相比，IL-6(0.125～0.5 ng/ml)对细胞活性没有显著影响($P>0.05$)；IL-6增加到1 ng/ml后，作用12 h后细胞活力开始下降。IL-6(0.5 ng/ml)作用6～24 h显著下调细胞TFPI mRNA表达($P<0.05$)，6 h时抑制效果最强(TFPI mRNA/GAPDH mRNA均值仅为0.191)，以后抑制效应渐减弱，48 h达到正常水平(TFPI mRNA/GAPDH mRNA均值为0.399)。**结论**：提示IL-6(0.5 ng/ml)对HUVECs的活性不造成直接的影响，同时IL-6(0.5 ng/ml)在6～24 h内可抑制HUVECs的TFPI mRNA表达而诱发凝血系统失衡，这可能与急性炎性反应时相的血液凝固和血栓形成有关。

[关键词] 白细胞介素6；组织因子抑制物；脐静脉；内皮细胞；逆转录聚合酶链反应
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IL-6 inhibits tissue factor pathway inhibitor expression in human umbilical vein endothelial cells

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[**ABSTRACT**] **Objective**: To elucidate the effects of interleukin-6(IL-6) on tissue factor pathway inhibitor (TFPI) expression in human umbilical vein endothelial cells. **Methods**: In experimental group, cultured human umbilical vein endothelial cells(HUVECs) were treated with IL-6 at 0.125 ng/ml, 0.5 ng/m and 1.0 ng/ml for different hours. HUVECs in control group were treated with culture medium. Cell viability was then determined by cell counting kit-8(CCK-8). Cytoplasmic RNA was prepared using the TRIzol method and TFPI mRNA levels was assayed by reverse transcript polymerase chain reaction(RT-PCR). **Results**: IL-6(0.125-0.5 ng/ml) did not produce cell toxicity compared to the control group according to LDH determination in culture media, but IL-6(≥1.0 ng/ml) elicited a significant cytotoxic effect. TFPI mRNA level decreased after HUVECs were exposed to IL-6(0.5 ng/ml) from 6 h($P<0.05$). TFPI mRNA level reached the lowest in 6 h(TFPI mRNA/GAPDH mRNA=0.191), then gradually restored to the normal level till 48 h(TFPI mRNA/GAPDH mRNA=0.399). **Conclusion**: IL-6 at 0.1-0.5 ng/ml does not show signs of cell toxicity, but it can inhibit the expression of TFPI mRNA. The decrease of TFPI mRNA expression in HUVECs induced by IL-6 may play an important role in the modulation of coagulation processes in blood circulation and coagulantion system change during the acute inflammation period.

[**KEY WORDS**] interleukin-6; tissue factor pathway inhibitor; umbilical vein; endothelial cells; reverse transcript polymerase chain reaction

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组织因子途径抑制物(tissue factor pathway inhibitor, TFPI)是TF/FⅦa惟一的生理性抑制物，是外源性凝血途径的重要阻断剂[1]，主要由血管内皮细胞合成。IL-6不仅是体内最为重要的调节天然免疫应答、体液和细胞免疫应答的细胞因子之一，而且作为前炎性反应因子IL-6与IL-1均在全身性炎性反应中扮演重要角色，许多细菌和病毒感染性疾病均能诱导炎性反应、IL-6升高并诱发凝血而继发弥散性血管内凝血(disseminated intravascular coagulation, DIC)，其中高水平IL-6与急性炎性反应密切相关[2]。IL-6除在启动炎性反应的发生中能激活内皮细胞表达ICAM-1等黏附分子诱发局部

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炎性反应之外，能否诱导内皮细胞凝血功能变化目前尚不清楚。本研究应用 IL-6 作为刺激因子作用于体外培养的人脐静脉血管内皮细胞（HUVECs），观察 TFPI mRNA 表达的时效变化，以探索 IL-6 对内皮细胞凝血调控功能的影响。

1　材料和方法

1.1　*仪器和试剂*　二氧化碳培养箱（Napco 5400）、低温离心机（Sigma 3-18k）、PCR 仪（Astec PC808）、凝胶成像分析系统（Syngene 公司）。新生小牛血清（杭州四季青生物公司）。白细胞介素 6（interleukin-6，IL-6，R&D System 公司）。RPMI 1640 培养基、胰蛋白酶和 Hepesf 均为 Gibco 公司产品。CCK-8 为日本同仁化学研究所产品，TRIzol 购自上海申能博彩公司，RT-PCR 试剂盒购自 Gibco 公司，TFPI 和 GAPDH 引物由北京赛百盛公司合成。

1.2　*HUVEC 的培养*　取新生儿脐带（暨南大学第一附属医院妇产科提供），应用 0.125%胰酶：0.01%EDTA 消化液灌注消化法，从脐静脉中分离出血管内皮细胞。离心沉淀后，加入 20%新生牛血清（FCS）培养液 20% FCS、RPMI 1640 培养液、Hepes 5 mg/ml、*L*-谷氨酰胺 30 μg/ml、青霉素与链霉素各 100 U/ml），接种于表面积为 25 cm^2 培养瓶中，在 5%CO_2 的 37℃培养箱内培养。3 d 后细胞贴壁生长至融合状态，用 0.125%胰酶：0.01%EDTA 液消化传代。选择生长状态良好第 2、3 代细胞进行实验。ABC 酶标法（武汉博士德生物公司产品）检测胞质中第Ⅷ因子相关抗原。

1.3　*分组和处理*　细胞分为 6 组：（1）对照组：给予培养液作对照；（2）0.125 ng/ml IL-6 组；（3）0.25 ng/ml IL-6 组；（4）0.5 ng/ml IL-6 组；（5）1.0 ng/ml IL-6 组；（6）2.0 ng/ml IL-6 组，分别加入相应的 IL-6（终质量浓度）。

1.4　*细胞活力的检测*　将 HUVECs 混悬的培养液（1×10^5/ml）分别加入 5 块 96 孔板上，每块板上均分 4 组（即对照组和 3 个不同浓度的 IL-6 处理组），每组接种 6 个孔，24 h 后吸出培养液，用 4% FCS 的 RPMI 1640 培养液冲洗 3 遍，各孔中留 100 μl，分别在 6、12、24、48 和 72 h 取出其中一块 96 孔板，于实验各孔分别加入 CCK-8 试剂 10 μl，37℃继续孵育 2 h，选择 450 nm 波长，在酶联免疫检测仪上测定光密度值（*D* 值）。

1.5　RT-PCR　对照组和实验组（0.5 ng/ml IL-6）孵育完成后，用 0.125%胰酶：0.01%EDTA 液消化，室温 1 200×*g* 离心 5 min 收集细胞，加入 1 ml TRIzol 试剂，移入无 RNA 酶的 1.5 ml Ependorff 管中。RNA 提取方法按照 Gibco 试剂盒说明书中的操作步骤进行。提取的 RNA 沉淀用 30 μl 的 DEPC-H_2O 稀释，并用紫外分光光度计测定 D_{260}/D_{280} 值进行 RNA 纯度及含量计算。实验重复 3 次，每次做复管。逆转录步骤参照试剂盒说明书进行。具体取总 RNA 1 μl（2 g/L）、Oligo（dT）$_{15}$ 1 μl 和 DEPC-H_2O 至 10 μl 于 0.2 ml 微量离心管中。恒温水浴 70℃ 5 min 后置于冰上 5 min，分别加入 5× buffer 5 μl、10 mmol/L dNTP 2.5 μl、逆转录酶 MMnLV（10 U/μl）3 μl、加 DEPC-H_2O 补足体积至 25 μl。恒温水浴 42℃×1 h、65℃×10 min。PCR 操作步骤按说明书进行。TFPI 上游引物为 5′-CAG TGC GAA GAA TTT ATA TAT GG-3′，下游为 5′-TTG CAT TCT TCC AGT GTC TC-3′，扩增片段长度为 284 bp。同时设置 GAPDH 作为内参照，其引物为上游 5′-GTC AGT GGT GGA CCT GAC CT-3′和下游 5′-TGA GGA GGG GAG ATT CAG TG-3′，扩增片段长度为 400 bp。TFPI 与 GAPDH 的 PCR 反应条件均为：95℃预变性 5 min；94℃×40 s，55℃×40 s，72℃×60 s，30 个循环；72℃×10 min。PCR 产物进行 2%琼脂糖凝胶电泳，并与标记物（MG0911，华美生物工程公司）比较，将 TFPI 不同时相的条带与相应内参照带的光密度作比较。

1.6　*统计学处理*　用 SPSS 10.0 进行统计分析，实验数据以 $\bar{x}\pm s$ 表示。用多因素多水平析因设计检验各因素的影响水平，用 *t* 检验分析不同组别相同时相基因表达的差异性，One-way ANOVA 检验 IL-6 刺激的时效变化。

2　结　果

2.1　*HUVEC 细胞的分离和鉴定*　所分离的 HUVECs 在相差倒置显微镜下细胞为单层鹅卵石状镶嵌排列；ABC 酶标法显示细胞胞质中第Ⅷ因子相关抗原阳性，棕黄色的颗粒均匀地分布在整个细胞质中，苏木精复染，细胞核呈蓝色（图 1），证明所分离的细胞为血管内皮细胞。

2.2　*IL-6 对 HUVECs 活性的影响*　实验组和对照组细胞在 6～72 h 各个不同时段应用 CCK-8 所测得的活性结果如图 2，当 IL-6 在 0.125～0.5 ng/ml 时，随着 IL-6 浓度升高，处理组的 *D* 值有升高趋势，但与对照组相比其差异无统计学意义（$P>0.05$）；而当 IL-6 的浓度高于 0.5 ng/ml 时，处理组

作用 24 h 对内皮细胞没有明显的损伤作用，细胞活性没有变化；当 IL-6 高于 1.0 ng/ml 时，细胞活性与对照组相比开始下降（$P<0.05$），提示高浓度的 IL-6 作用可以造成内皮细胞的损伤，因此我们选择对内皮细胞无损伤的最高浓度 0.5 ng/ml 进行实验，观察 IL-6 对血管内皮细胞作用后，对 HUVECs 表达组织因子途径抑制物（TFPI）的影响。

TFPI 是相对分子质量为 43 000 的糖蛋白，由 276 个氨基酸残基构成，包含 3 个 Kunitz 功能区的 Kunitz 型蛋白酶抑制剂；正常状态下血管内皮细胞 TFPI 基因保持低水平表达。TFPI 生理功能是通过灭活 FⅩa 和抑制 TF/FⅦa 的蛋白水解酶活性来抑制 TF 的作用，两者比例是否协调是决定凝血途径是否活化的关键因素[5,6]。本研究发现，正常状态下 HUVECs 低水平表达 TFPI（TFPI mRNA/GAPDH mRNA 平均为 0.399），虽然不同时相的 mRNA 水平有所波动，但无统计学差别（$P>0.05$），提示正常状态血管内皮细胞维持 TFPI mRNA 的低水平稳定表达，这有利于与循环系统中新生的 TF 迅速结合，从而可有效防止凝血系统活化而导致凝血。有研究证实 IL-6 能上调组织因子（TF）表达[7]，而本实验发现浓度为 0.5 ng/ml 的 IL-6 作用 HUVECs 后，内皮细胞表达的 TFPI mRNA/GAPDH mRNA 与对照组相比在 IL-6 处理 6～24 h 时显著下降（$P<0.05$），以后其比值逐渐恢复到正常水平。IL-6 抑制内皮细胞 TFPI mRNA 的表达，使生成的 TFPI 分子减少，进一步可诱导促凝活性增加，提示 IL-6 增高与血液的高凝状态有关。

进一步应用 One-way ANOVA 对 IL-6（0.5 ng/ml）组不同时相间进行比较，发现不同时相间有显著性差异（$P<0.05$），6～24 h 的 TFPI mRNA 水平显著低于 48～72 h，提示 IL-6 作用可迅速而短暂地抑制 TFPI mRNA 的表达且具有明显的时效性，在 IL-6 作用的 6～24 h，可显著抑制 TFPI mRNA 的表达（6～48 h），但随时间延长而 TFPI mRNA 的转录水平亦逐渐恢复到正常水平。IL-6 作为一种前炎症因子，具有诱发急性炎症反应等多种生物学活性，它可通过与多种细胞因子共受体 gp130 结合，激活细胞内 Vav、Rac、MEKK 和 SEK/MKK-4 信号途径，诱导转录因子 STAT3 的 Ser^{727} 磷酸化，导致 STAT3 形成二聚体进入细胞核，结合到相应基因的特定一致系列（class Ⅱ IL-6 responsive elements，IL-6RE Ⅱ）[8]，从而影响 TFPI 等靶基因转录与蛋白合成，因此推测 IL-6 有可能是通过 IL-6R → Jak1、Jak2 和 Tyk2 → Vav、Rac、MEKK 和 SEK/MKK-4 → STAT3 → IL-6RE Ⅱ 胞内信号通路[8]，影响 TFPI mRNA 转录与表达。在许多感染性疾病患者血液中 IL-6 常维持在较高水平（0.25～1.0 ng/ml），高水平的 IL-6 一方面可诱导血管内皮细胞表达 TF，另一方面抑制 TFPI 表达，这可造成循环系统中血液状态失衡，诱发凝血功能亢进，血液进入高凝态，进一步可诱发 DIC。

IL-6 除参与局部炎性反应、免疫病理损伤和抗体依赖性增强效应外，在许多感染性疾病中，高水平的 IL-6 还可诱导内皮细胞 TF/TFPI 失调，进一步可诱导机体凝血和抗凝系统失衡而致局部血栓形成，从而参与急性期凝血系统活化与 DIC 发生，因此 IL-6 在诱导患者凝血系统失常并继发出血、病情进一步恶化等方面可能发挥重要的作用。

[参 考 文 献]

[1] Gando S, Kameue T, Matsuda N, *et al*. Imbalances between the levels of tissue factor and tissue factor pathway inhibitor in ARDS patients[J]. *Thrombosis Res*, 2003, 109(2-3): 119-124.

[2] Soda K, Kano Y, Kawakami M, *et al*. Excessive increase of serum interleukin 6 jeopardizes host defense against multi-bacterial infection[J]. *Cytokine*, 2003, 21(6): 295-302.

[3] Edwards LC, Freeman HS, Claxton LD. Developing azo and formazan dyes based on environmental considerations: Salmonella mutagenicity[J]. *Mutation Res*, 2004, 546(1-2): 17-28.

[4] Tipton DA, Lyle B, Babich H, *et al*. *In vitro* cytotoxic and anti-inflammatory effects of myrrh oil on human gingival fibroblasts and epithelial cells[J]. *Toxicology in Vitro*, 2003, 17(3): 301-310.

[5] Ushigome H, Sano H, Okamoto M, *et al*. The role of tissue factor in renal ischemic reperfusion injury of the rat[J]. *J Surg Res*, 2002, 102(2): 102-109.

[6] Marutsuka K, Hatakeyama K, Yamashita A, *et al*. Adrenomedullin augments the release and production of tissue factor pathway inhibitor in human aortic endothelial cells[J]. *Cardiovascular Res*, 2003, 57(1): 232-237.

[7] Chen YC, Wang SY. Activation of terminally differentiated human monocytes/macrophages by dengue virus: productive infection, hierarchical production of innate cytokines and chemokines, and the synergistic effect of lipopolysaccharide[J]. *J Virol*, 2002, 76(19): 9877-9887.

[8] Mulligan-Kehoe MJ, Kleimman HK, Drinane M, *et al*. A truncated plasminogen activator inhibitor-1 protein blocks the availability of heparin-binding vascular endothelial growth factor A isoforms[J]. *J Biol Chem*, 2002, 277(50): 49077-49089.

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· Original article ·

Effects of soybean isoflavone on liver oxidative stress resulting from ^{60}Co-gamma rays

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[**ABSTRACT**] **Objective**: To study the effect of soybean isoflavone on liver oxidative stress resulting from ^{60}Co-gamma rays. **Methods**: Totally 80 normal female Kunming mice were evenly randomized into 5 groups according to body weight: 3 intervention groups, single irradiation group and normal control group. The normal group and single irradiation groups were given 0.5% CMC-Na, and the 3 intervention groups were given different doses of soybean isoflavone (50 mg/kg, 100 mg/kg, 400 mg/kg) respectively for 14 d. The whole body of single irradiation group and intervention groups were subjected to 4.56 Gy ^{60}Co-γ radiation once on the 7th day, and then the mice were killed on the 2nd day and the 7th day after radiation. **Results**: The CAT activity of liver tissue of 100,400 mg/kg intervention groups and 3 SI groups were significantly increased on the 2nd day and 7th day after irradiation($P<0.05$), respectively; the GSH-Px activity of 100 mg/kg SI group was significantly increased ($P<0.05$) on the 7th day after irradiation; the T-SOD activity of 50 mg/kg SI group was significantly decreased ($P<0.05$) on the 2nd day after irradiation, while no difference was observed among remaining groups. The MDA content of 100 mg/kg group was significantly decreased on the 7th day after radiation compared with control group, and MDA content of each group subjected to irradiation were increased on the 2nd day after irradiation, but 3 SI groups nearly decreased to normal level on the 7th day after irradiation. **Conclusion**: The soybean isoflavone can enhance the antioxidant capability of mice, but it does not show a dose-effect relationship.

[**KEY WORDS**] liver injury; γ ray; free radicals; antioxidant-enzyme; soybean isoflavone

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The mechanisms of radiation hepatitis and fibrosis, which are the major complication of liver lesion resulting from radiation, remain unclear [1]. Weiss JF *et al* [2] considered that irradiation could indirectly activate H_2O and yield a large amount of free radicals, which destroyed the balance between oxidation and anti-oxidation and led to the oxidative stress that responsible for carcinomatous change or necrosis of hepatocyte. Since the radiation hepatitis is unrecoverable, its prevention becomes important.

Soybean isoflavones (SI) mainly contents genistin and daidzin. After ingestion, soybean isoflavones are hydrolyzed by intestinal glucosidases and release the aglycones, glycitein, daidzein, and genistein. Among those daidzein and genistein play major biological role. Previous studies *in vitro* and *in vivo* showed that SI had antioxidant capability and could prevent biomacromolecule from damage induced by free radicals. Phenolic phydoxyl provided by genistein and daidzein can react with free radicals, thus scavenging them or cut out the chain reaction of free radicals. In addition, Cai QY *et al* [3] have reported that genistein significantly increased the activity of antioxidant-enzyme in intestine, skin, *etc.* of mice, which suggested that the enhancement to antioxidant-enzyme activity of dietary SI is possibly the mechanism of its action as chemical preventor. Wei H *et al* [4,5] found that the genistein could relieve the oxidative injury induced by ultraviolet, but both energy and penetrability of X ray or γ ray used in radiotherapy are stronger than that of ultraviolet [6,7], so whether the SI could relieve the oxidative injury of liver induced by radiation or not need further investigation.

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1 MATERIALS AND METHODS

1.1 Animals and reagents Female Kunming mice were purchased from Center of Laboratory Animal, Second Military Medical University. The animals were, on average, 60 to 80 days old at the time of irradiation and weighed between 23 and 29 g. They were fed with standard mice pellet food and water for one week's adaptation. SI powder (daidzin 16.42%, daidzein 1.03%, genistin 22.63%, genistein 1.18%) were provided by Dalian Green Peak Bio-Products Company LTD, and 0.58%, 1.15%, 4.62% SI suspensions used in intervention were prepared through dissolving in 0.5% CMC-Na.

1.2 Radiation For *in vivo* experiment, radiation was performed with ^{60}Co-γ rays in actinotheraphy department of Changhai Hospital. The mice were placed in Perspex-covered boxes and exposed to 4.56 Gy at a dose rate of 0.57 Gy/min.

1.3 Intervention methods Totally 80 normal female Kunming mice were evenly randomized into 5 groups according to body weight: 3 intervention groups, single irradiation group (D group in short), and normal group (N group in short). The N group and D group were given 0.5 ml 0.5% CMC-Na, and the 3 intervention groups were given different doses of soybean isoflavone (50 mg/kg, 100 mg/kg, 400 mg/kg, A, B, C group correspondingly in short) respectively for 14 d. For whole body irradiation experiments, the D group and A, B, C intervention groups were given 4.56 Gy ^{60}Co-γ radiation once on the 7th day, and then the mice were killed on the 2nd day and the 7th day after radiation.

1.4 Determination of MDA content and antioxidant enzyme activity The MDA content and antioxidant enzyme activity (CAT, SOD, GSH-Px) were tested according to the recommended procedures provided by the kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical analysis Statistical evaluation of the data was done with SPSS 10.0 software. Data were expressed as $\bar{x} \pm s$. The differences of MDA content and antioxidant enzyme activity inside the experimental groups were analyzed by paired t test, and that among each group were determined with LSD test.

2 RESULTS

2.1 Effect of SI on hepatic CAT activity after irradiation CAT activity of B, C group significantly increased compared with D group ($P<0.05$) after the 2nd day of irradiation; after the 7th day of irradiation, the CAT activity of 3 intervention group were significantly higher than that of D group ($P<0.05$), and D group was the lowest one in 5 groups. As to 3 intervention group, the CAT activity of B and C group were higher than that of A group after the 2nd day of irradiation, and the higher the intervention dose, the higher the CAT activity after the 7th day of irradiation. In C group CAT activity was significantly increased on the 7th day compared with that on the 2nd day after irradiation(Tab 1).

Tab 1 Effect of SI on hepatic CAT, GSH-Px, T-SOD activity and MDA content after irradiation

($n=8, \bar{x} \pm s$)

Group	CAT activity (NU/mg protein)		GSH-Px activity (NU/mg protein)		T-SOD activity (NU/mg protein)		MDA content(nmol/ml protein)	
	After 2 d	After 7 d	After 2 d	After 7 d	After 2 d	After 7 d	After 2 d	After 7 d
N	3.09±0.67	4.22±1.06	7.73±1.26	13.91±1.76	48.51±16.24	51.84±26.52	3.14±1.01	2.10±0.81
D	2.59±0.46	3.46±1.39	7.63±1.11	12.74±0.96	54.41±15.22	55.22±20.09	3.32±0.77	2.96±1.08
A	3.39±1.16	5.05±1.79*	8.35±1.53	14.41±3.55	36.76±8.12▲	49.01±17.56	4.24±1.29	2.28±0.66△
B	3.97±1.47*	5.15±1.19*	8.30±1.30	15.30±2.39*	46.11±13.67	44.67±14.75	3.71±0.62	1.84±0.86*△△
C	3.93±0.82*	5.68±1.59*△	7.59±0.58	13.91±1.68	53.65±21.41	43.69±12.25	4.37±1.58	2.19±1.01△

* $P<0.05$ *vs* group D; △ $P<0.05$, △△ $P<0.01$ *vs* after 2 d group; ▲ $P<0.05$ *vs* group C and D

2.2 Effect of SI on hepatic GSH-Px activity after irradiation As show in Tab 1, the GSH-Px activi-

ty of A and B group were higher than that of other groups, but there were no significant differences ($P>0.05$) among each group on the 2nd day after irradiation; on the 7th day after irradiation, the GSH-Px activity of B group was significantly higher than that of D group($P<0.05$) while no significant differences were observed between A, C, N and B group.

2.3 Effect of SI on hepatic T-SOD activity after irradiation Tab 1 shows that T-SOD activity of each intervention group and N group were lower than that of D group, but there were no significant difference between them except A group on the 2nd day after irradiation. T-SOD activity of D group was still the highest one but no significant difference was observed among each group on the 7th day after irradiation. As for the change trend of each SI group of the 2nd and the 7th day after irradiation, A group increased, B group remained balance while C group decreased, but no significant difference were observed among all groups.

2.4 Effect of SI on hepatic MDA content after irradiation Tab 1 shows that hepatic MDA content of both single irradiation group and each intervention group increased compared with that of normal group on the 2nd day after irradiation, though the increase extent of A (35%), B (18%), and C (39%) group were higher than that of D (5.7%) group, the difference was insignificant; on the 7th day after irradiation, although hepatic MDA content of each group decreased, the extent was different. A and C group decreased significantly ($P<0.05$), B group also decreased significantly ($P<0.01$), whereas the change of D group was insignificant, and the MDA content of B group was significantly lower than that of D group ($P<0.05$). Furthermore, compared with N group, D group increased by 41%, A and C group increased by 8.5% and 4.2%, respectively, while B group was lower. MDA content of B group was the lowest one both on the 2nd day and 7th day after irradiation compared with the other intervention groups.

3 DISCUSSION

Liver is one of the sensitive organs to irradiation [1]. The major complication of liver lesion resulting from irradiation is hepatitis and fibrosis. Previous study observed the change of antioxidant enzyme in liver homogenate of mice after received 3, 6, 9 and 12 Gy dose of irradiation, the results showed that with the increase of irradiation dose, the activity of T-SOD, CAT, and GSH-Px decreased, while MDA content increased obviously. In this process, the biomacromolecule was destructed, which lead to carcinomatous change or necrosis of hepatocyte, and the release of bioactive matter stimulated the hyperplasia of fibroblast.

In this report, we studied the radio-protective effect of different dose of SI at different time after irradiation against ^{60}Co-γ-induced oxidative injury on liver. The results showed that the effect of different dose of SI was different on hepatic antioxidant enzyme activity and MDA content at different time after irradiation. In conclusion, hepatic CAT activity of SI group can be significantly increased compared with single irradiation group at the early and later stage after irradiation, while the change of GSH-Px activity was different from that of CAT activity. GSH-Px activity of medium dose of SI group was significantly higher than that of single irradiation group at later stage after irradiation. In addition, MDA content of intervention group significantly decreased at later stage after irradiation, which indicate that SI enhanced the antioxidant ability of mice received irradiation. Combining with the change of GSH-Px activity in intervention group especially medium one, our results are in agreement with those findings[8] that increasing of GSH-Px activity can efficiently prevent hepatocyte from lipid peroxidation.

Recent study showed that SI could slightly increase the activity of SOD of high fat group in blood and liver [9], while our result showed that the level of SOD activity of SI group was lower