14 Shechter H, Gruener N, Shuval HI. A micromethod for the determination of nitrite in blood. Anal Chim Acta 1972; 60: 93-9.

173~176

激活与封闭库普弗细胞对小鼠免疫性肝损伤的影响

R575.02

王根生，刘耕陶（中国医学科学院，中国协和医科大学药物研究所药理室，北京 100050，中国）

关键词 库普弗细胞；肝；脂多糖；卡介苗；一氧化氮 肝损伤 维生素A

目的：研究库普弗细胞(KC)是否参与小鼠免疫性肝损伤．方法：给小鼠静脉注射卡介苗(BCG) 5 ×10^7 活菌后再静脉注射脂多糖(LPS) 7.5 μg 以诱导免疫性肝损伤．以维生素 A 激活 KC 和以印度墨汁或硅砂封闭 KC 后，测定血浆一氧化氮(NO)，谷丙转氨酶(AlaAT)，谷草转氨酶(AspAT)的变化并检查肝组织的病理改变．结果：注射 BCG 后，再注射 LPS 7.5 μg，可导致小鼠血浆 NO，AlaAT，AspAT 剧烈升高及严重的肝损伤．以维生素 A 激活 KC 后，肝损伤更为严重，而以印度墨汁或硅砂封闭 KC 后，肝损伤则显著减轻．结论：BCG+LPS 诱导的小鼠肝损伤与 KC 的功能关系密切，来源于 KC 的 NO 在 BCG+LPS诱导的肝损伤中起重要作用．

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Mar; 18 (2): 176-179

Effect of epidermal growth factor on cultured rat hepatocytes poisoned by CCl_4

WU Bin, WANG Chong-Wen, XU Jia-Rui, ZHU Jin-Quan (*Department of Gastroenterology, First Affiliated Hospital, Jiangxi Medical College, Nanchang 330006, China*)

KEY WORDS epidermal growth factor-urogastrone; alanine aminotransferase; aspartate aminotransferase; liver; carbon tetrachloride poisoning; cultured cells

AIM: To study the effects of epidermal growth factor (EGF) on CCl_4-induced primary cultured hepatocytes injury. **METHODS**: Alanine aminotransferase (AlaAT) and aspartate aminotransferase (AspAT) activities and K^+ concentractions were determined by the Auto-biochemistry Assay System. Malondialdehyde (MDA) was determined by thiobarbituric acid method. Radioactivity was determined by liquid scintillometry. Light microscopy and electron microscopy were used. **RESULTS**: EGF 40 $\mu g \cdot L^{-1}$ decreased CCl_4 (10 $mmol \cdot L^{-1}$)-induced damages of rat primary cultured hepatocytes by decreasing AlaAT and AspAT leakage and MDA production, and promoted RNA and DNA synthesis, with a high positive correlation between intracellular K^+ leakage and DNA syntheses ($r = 0.99$, $P < 0.01$). Cytopathological study showed that EGF decreased damage of liver cells. **CONCLUSION**: EGF maintains the stability of cellular lipid membrane and promotes syntheses of RNA and DNA of hepatocytes, and intracellular K^+ transference is a promotor of the message transmission of DNA synthesis.

Received 1995-08-15 Accepted 1996-10-04

CCl_4 induced the liver cell damage and necrosis by way of attacking the phospholipid fatty acid to lead to lipid peroxidation in the biomembrane[1,2]. This is a model of CCl_4-induced primary cultured hepatocytes injury[3,4].

Epidermal growth factor (EGF), found in mouse submaxillary gland in purifying nerve growth factor[5], promoted DNA synthesis of intoxicated hepatocytes[6-8]. The aim of this study was to examine the protective effects of EGF against CCl_4-induced primary cultured hepatocytes injury.

MATERIALS AND METHODS

Isolation and primary cuture of rat hepatocytes

Sprague-Dawley rats of either sex bred in Jiangxi Experimental Animal Centre weighing 184 ± s 25 g were used to isolate hepatocytes by the two-step liver cell perfusion method[9] with some modifications. The viable cells in the trypan blue exclusion test was >90 %. The cells were suspended in RPMI 1640 medium (Gibco, USA) with HEPES 10 mmol (Sigma, USA), 10 % fetal calf serum, and antibiotics. Its density was $2.5 \times 10^8 \cdot L^{-1}$. the cells were placed in a 5 % CO_2 + 95 % air incubator at 37 ℃.

Hepatotoxicity The suspension received either CCl_4 (10 $mmol \cdot L^{-1}$, Yixing Chem Co. Jiangsu) and EGF in saline (40 $\mu g \cdot L^{-1}$. Gibco, USA) or normal saline (control group) in primary hepatocytes cultured for 24 h separately. Malondialdehyde (MDA) was determined by the thiobarbituric acid (TBA) method[10]. The supernatant activities of alanine aminotransferase (AlaAT) and aspartate aminotransferase (AspAT) were measured by the Auto-biochemistry Analysis System (Beckman-7000, Encore-2, USA). The K^+ concentration was determined by the Auto-biochemistry Analysis System. Intracellular K^+ leakage = K^+ in supernatant-K^+ in medium.

Measurement of RNA and DNA synthesis The suspension of hepatocyte 200 μL was cultured with [^{3}H]uridine (37 kBq, 1036 $PBq \cdot mol^{-1}$) or [^{3}H]thymidine (37 kBq, 1036 $PBq \cdot mol^{-1}$, Shanghai Nuclear Tech Co) for 4 h, separately. All experiments were repeated several times. Hepatocytes were washed twice with cold PBS (0.05 mmol, pH 7.4) and collected by the cell collector on type 49 filter membrane. The filter membrane was counted by liquid scintillation. The radioactivity (Bq) was measured by the liquid scintillometer (Beckman LS 5801, USA).

Cytology The cultured hepatocytes were fixed with 3 % formalin and embedded in paraffin. Sections wre stained with H-E and analyzed by the light microscope (Olympus, Japan). The cultured hepatocytes were fixed with 3 % grutaraldehyde in cacodylate buffer (0.1 mmol, pH 7.4), refixed in 1 % osmium tetroxide solution in cacodylate, dehydrated in alcohols and embedded in E-pon 812. The sections were examined by the electron microscope (JEM-100CX, Japan).

Results were expressed as $\bar{x} \pm s$, Significances were analyzed using F and t tests.

RESULTS

Protection of cell membrane The supernatant AlaAT and AspAT levels increased with the time and reached the peak at 16 h after CCl_4 addition in the cell culture. When EGF was administered with CCl_4, the supernatant AlaAT and AspAT levels reached the peak at 12 h and 4 h, respectively, then decreased gradually (Tab 1).

Tab 1. Effect of epidermal growth factor (EGF) on leakage of AlaAT and AspAT and product of malandioldehyde (MDA) from CCl_4-intoxicated primary cultured rat hepatocytes. n = 15 rats, $\bar{x} \pm s$. $^cP<0.01$ vs CCl_4 control.

Time/h	Control	EGF
	AlaAT/$\mu mol \cdot min^{-1} \cdot L^{-1}$	
4	26 ± 3	12.7 ± 1.4^c
8	27 ± 4	15.0 ± 2.4^c
12	27 ± 5	16.3 ± 2.1^c
16	30 ± 5	15.9 ± 2.3^c
	AspAT/$\mu mol \cdot min^{-1} \cdot L^{-1}$	
4	25 ± 3	18.0 ± 2.2^c
8	31 ± 5	17.0 ± 2.3^c
12	32 ± 5	13.2 ± 2.2^c
16	35 ± 5	12.7 ± 3.5^c
	MDA/$nmol \cdot L^{-1}$	
4	9.5 ± 0.8	6.2 ± 1.0^c
8	10.9 ± 0.8	6.9 ± 0.9^c
12	11.1 ± 0.7	6.8 ± 1.0^c
16	11.6 ± 0.8	6.9 ± 0.7^c

Inhibition of lipid peroxidation MDA showed a moderate increase with time in the control group, but a moderate decrease with time in EGF-treated group, indicating that EGF inhibited lipid peroxidation of rat hepatocytes induced by CCl_4 (Tab 1).

Effect on RNA and DNA syntheses The time course of RNA and DNA syntheses of primary cultured rat hepatocytes in the presence of EGF was enhanced. [^{3}H]uridine and [^{3}H]thymidine incorporation showed a moderate increase with time, by 120 % and 20 % at 4 h, by 183 % and 104 % at 16 h, respectively, compared with control (Fig 1). The results strongly suggest that EGF promote syntheses of RNA and DNA of hepatocytes.

Effect of intracellular K^+ leakage on DNA synthesis The intracellular K^+ leakage did not affect [^{3}H]thymidine incorporation into DNA of CCl_4-intoxicated primary cultured rat hepatocytes in control group ($\hat{Y} = 38 + 21X$, $r = 0.33$, $P > 0.05$), but it affected [^{3}H]thymidine incorporation into DNA in a high positive correlation manner in EGF-treated group. The increase of intracellular K^+ leakage was associated with DNA synthesis (Fig 2).

Effect on cytology of cultured rat liver cells The necrotic liver cells were many in control group,

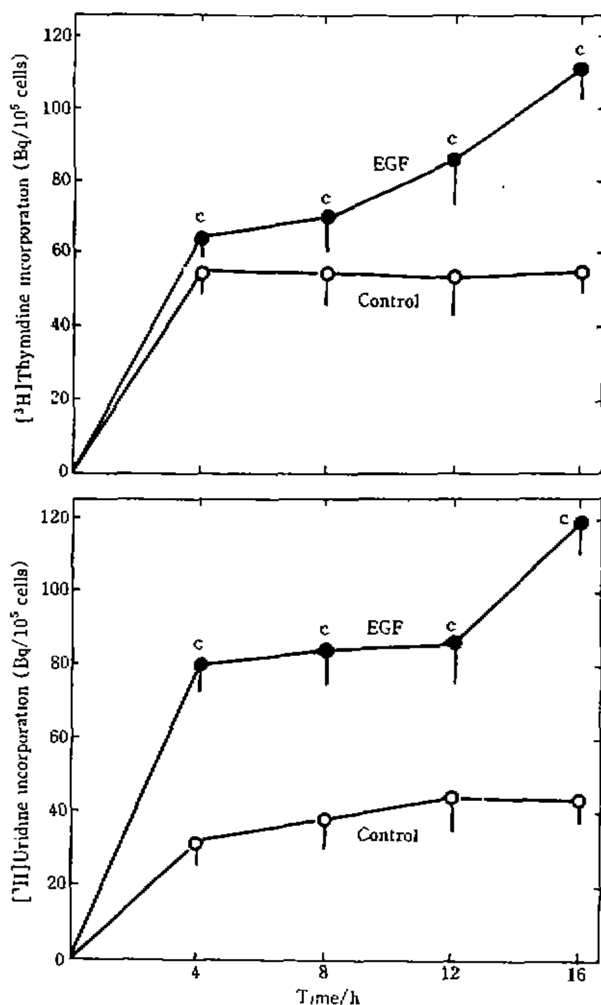

Fig 1. **Effect of EGF on [³H] uridine incorporation RNA and [³H] thymidine incorporation DNA in CCl_4-intoxicated primary cultured rat hepatocytes.**
$n = 15$ rats, $\bar{x} \pm s$. ᶜ$P < 0.01$ vs control.

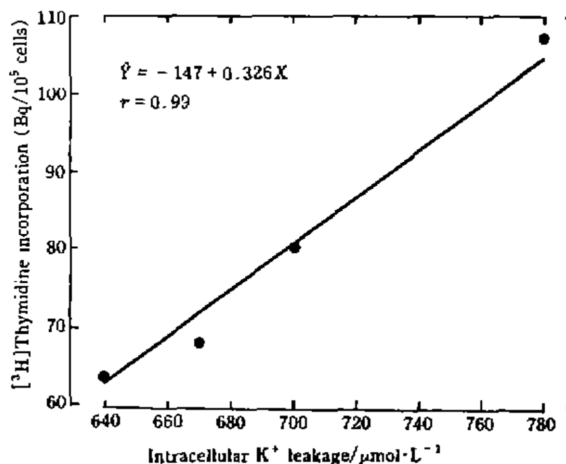

Fig 2. Relationship between intracellular K^+ leakage and [³H] thymidine incorporation.

but few in EGF-treated group. Ultrastructural examination demonstrated that in control group the heterochromatins converged, the nuclear envelopes were irregular, the mitochondria were swollen and its crest disappeared, the ribosomes became less, in EGF group the heterochromatins were regular, the cell membranes and nuclear envelopes were intact, the mitochondria and ribosomes were richer than those in CCl_4 control group (Fig 3, Plate 1).

DISCUSSION

The hepatotoxicity of CCl_4 results from its metabolic conversion to free radical products, the free radicals will attack directly cell membrane protein, DNA and so on, resulting in liver cell damage even necrosis[1,11].

Our present study showed that EGF reduced AlaAT and AspAT leakage and inhibited lipid peroxidation of rat hepatocytes induced by CCl_4 *in vitro*. Cytology study demonstrated that EGF reduced liver cell necrosis and many cell organelle injury, stabilized cell membrane and mitochodria membrane. The mechanism is that EGF reduced free radical hepatotoxicity by inhibiting lipid peroxidation.

Ca^{2+} affected DNA synthesis[12-14]. Our experiment showed that EGF promoted intracellular K^+ leakage as well as RNA and DNA synthesis, and the intracellular K^+ leakage and DNA synthesis had a high postive correlation. This means that intracellular K^+ leakage affects DNA synthesis on CCl_4-intoxicated hepatocytes. EGF, a polypeptide hormone, when combined with special receptor in hepatocyte, activates ion-pump and promotes K^+ and Ca^{2+} transference, and then stimulates the second message transmission and promotes RNA synthesis of liver cells, and further promotes DNA synthesis by the RNA. Furthermore, intracellular K^+ transference is a promotor of the message transmission of DNA synthesis of liver cells.

In conclusion, EGF protects primary cultured hepatocytes against CCl_4 hepatotoxicity, and intracellular K^+ transference is a promotor of the

message transmission of DNA synthesis of hepatocytes.

REFERENCES

1. Tribble DL, Aw TY, Jones DP. The pathophysiological significance of lipid peroxidation in oxidative cell injury. Hepatology 1987; 7: 377 - 86.
2. Nastainczyk W, Ahr HJ, Ullrich V. The reductive metabolism of halogenated alkanes by liver microsomal cytochrome P450. Biochem Pharmacol 1982; 31: 391 - 6.
3. Berger ML, Bhatt H, Combes B, Estabrook RW. CCl_4-induced toxicity in isolated hepatocytes: the importance of direct solvent injury. Hepatology 1986; 6: 36 - 45.
4. Slater TF, Cheeseman KH, Ingold KU. Carbon tetrachloride toxicity as a model for studying free-radical mediated liver injury. Philos Trans R Soc Lond (Biol) 1985; 311: 633 - 45
5. Cohen S. Purification and metabolic effects of a never growth-promoting protein from snake venom. J Biol Chem 1959; 234: 1129 - 37.
6. Jones DE Jr, Tran-Patterson R, Cui DM, Davin D, Estell KP, Miller DM. Epidermal growth factor secreted from the salivary gland is necessary for liver regeneration. Am J Physiol 1995; 268 (5 Pt 1): G872 - G878
7. Tomomura A, Sawada N, Sattler GL, Kleinman HK, Pitot HC. The control of DNA synthesis in primary cultures of hepatocytes from adult and young rats: interactions of extracellular matrix components, epidermal growth factor, and the cell cycle. J Cell Physiol 1987; 130: 221 - 7.
8. Marti U, Burwen SJ, Jones AL. Biological effects of epidermal growth factor, with emphasis on the gastrointestinal tract and liver: an update. Hepatology 1989; 9: 126 - 38.
9. Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol Chem 1989; 264: 4747 - 50
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351 - 8.
11. Burk RF, Lane JM, Patel K. Relationship of oxygen and glutathione in protection against carbon tetrachloride-induced hepatic microsomal lipid peroxidation and covalent binding in the rat. Rationale for the use of hyperbaric oxygen to treat carbon tetrachloride ingestion. J Clin Invest 1984; 74: 1996 - 2001.
12. Eckl PM, Whitcomb WR, Michalopoulos G, Jirtle RL. Effects of EGF and calcium on adult parenchymal hepatocyte proliferation. J Cell Physiol 1987; 132: 363 - 6.
13. Petronijevic T, Edwards AM. Role of Ca^{2+} in stimulation of DNA synthesis by epidermal growth factor and tumor promoters in cultured rat hepatocytes. J Cell Physiol 1993; 155: 44 - 53.
14. Bilodeau M, Provencher SJ, Neron S, Haddad P, Vallieres S, Gascon-Barre M. Hypocalcemia decreases the early and late responses to epidermal growth factor in rat hepatocytes. Hepatology 1995; 21: 1576 - 84

176~179 R977.6

表皮生长因子对四氯化碳中毒的大鼠培养肝细胞的作用

吴斌, 王崇文, 徐家瑞, 祝金泉
(江西医学院第一附属医院消化科, 南昌 330006, 中国)

关键词 表皮生长因子-尿抑胃激素; 丙氨酸转氨酶; 天门冬氨酸转氨酶; 肝; 四氯化碳中毒; 培养的细胞

肝细胞 培养

目的: 研究表皮生长因子(EGF)对四氯化碳(CCl_4)所致大鼠原代培养肝细胞损伤的作用. 方法: 丙氨酸转氨酶(AlaAT)和天门冬氨酸转氨酶(AspAT)活力及 K^+ 浓度用自动生化分析仪测定. 丙二醛(MDA)用苯巴比土酸比色法测定. 放射活力用液体闪烁测量仪测定. 细胞病理用光学显微镜和电子显微镜检查. 结果: EGF 显著降低 AlaAT, AspAT 及 MDA 水平, 增加中毒肝细胞 RNA 和 DNA 的合成, 且 K^+ 漏出与 DNA 的合成呈正相关. 细胞病理显示 EGF 减轻 CCl_4 对肝细胞的毒性作用. 结论: EGF 对 CCl_4 所致原代培养肝细胞损伤有拮抗作用, 肝细胞内 K^+ 转运是 DNA 合成起信息传递的启动因子.

13th International Congress of Pharmacology

1998 Jul 26 - 31 München

Please contact INTERPLAN, Convention and Visitor Service
Anton Kössl, Sophienstr. 1
D-80333 München, GERMANY
Fax: 49-89-591610. Phone: 49-89-594492.