以酒精與四氯化碳誘導肝損傷動物模式評估護肝功能之研究

Alcohol- and carbon tetrachloride-induced liver injury as animal models for hepatoprotection evaluation

中文摘要

本研究提出酒精性肝損傷動物模式，並且以四氯化碳誘導肝損傷之護肝功能評估方法探討其適用性。實驗動物爲雄性 Wistar 大白鼠，體重約 160~180g，依照血漿 AST 與 ALT 的活性分爲五組，包括：對照組 (C)、酒精組 (E)、酒精＋silymarin 組 (ES)、四氯化碳組 (CCL)、四氯化碳＋silymarin 組 (CCLS)，E 與 ES 組給予 Lieber-DeCarli 酒精液體飼料，C、CCL 與 CCLS 組則給予正常液體飼料；CCL 與 CCLS 組每週皮下注射四氯化碳一次 (0.75 mL 40％CCl4 in olive oil/kg BW)，C、E 與 ES 組則週皮下注射橄欖油一次 (0.75 mL olive oil/kg BW)；ES 與 CCLS 組另外在飼料中添加 silymarin，200 mg/kg BW。實驗期爲 12 週。結果顯示，實驗期間血漿 AST 與 ALT 活性方面，E、ES、CCL 與 CCLS 組皆較 C 組顯著增加，第 12 週時，CCLS 組較 CCL 組顯著減少。在相對肝重方面，E、ES、CCL 與 CCLS 組皆較 C 組顯著增加，而 silymarin 可以改善因酒精或四氯化碳造成相對肝重增加的情形。紅血球抗氧化酵素活性方面，CCLS 組之 superoxide dismutase (SOD) 與 glutathione peroxidese (GPX)的活性與 C 組比較顯著增加，E 與 ES 組之 catalase (CAT)活性與 C 組比較顯著增加，而 CCL 與 CCLS 組之 glutathione reductase (GRD) 活性則比 C 組顯著增加。肝臟抗氧化酵素活性方面，與 C 組比較之下，E、ES、CCL 與 CCLS 組之 SOD 與 GPX 活性顯著降低，CCL 組之 GRD 活性顯著降低，CCL 與 CCLS 組之 CAT 活性顯著降低。肝臟抗氧化物質 GSH/GSSG 比値方面，E、ES、CCL 與 CCLS 組皆顯著較 C 組減少，CCL 組又顯著較 E 組減少，而給予 silymarin 皆可以改善因酒精或四氯化碳造成肝臟 GSH/GSSG 比値減少的情形。肝臟維生素 E 含量方面，E、ES、CCL 與 CCLS 組皆顯著較 C 組減少，CCL 組又較 E 組減少。肝臟脂質過氧化物 thiobarbituric acid reactive substances (TBARS) 濃度方面，E、ES、CCL 與 CCLS 組與 C 組比較顯著增加。血漿脂質濃度的結果，在 total cholesterol (TC)與 high density lipoprotein-cholesterol (HDL-C)濃度方面，E 與 ES 組顯著較 C 組增加，CCL 與 CCLS 組則顯著較 C 組減少，而給予 silymarin 可以改善因爲四氯化碳造成血漿 TC 減少的情形；在 triglyceride (TG) 濃度方面，E、ES、CCL 與 CCLS 組皆顯著較 C 組增加；各組之血漿 low density lipoprotein-cholesterol (LDL-C)濃度皆無顯著差異。肝臟 TC、TG 含量方面，E、ES、CCL 與 CCLS 組較 C 組顯著增加，CCL 組又較 E 組顯著增加。發炎指標 myeloperoxidas (MPO)活性方面，E、ES、CCL 與 CCLS 組與 C 組比較皆顯著增加，而四氯化碳較酒精的影響更爲顯著。肝臟病理切片分析方面，E、ES 組肝臟有脂肪堆積的情形，CCL、CCLS 組除了脂肪堆積，另有細胞壞死、發炎與纖維化的情形，而給予 silymarin 皆可以減少脂肪堆積的情形。研究顯示，相較於四

氯化碳，長期攝取酒精所誘導之大白鼠肝損傷程度較輕微，給予 silymarin 可以增加肝臟抗氧化物質進而降低肝臟傷害。本研究所使用之酒精性肝損傷動物模式，可誘導大白鼠形成脂肪肝，將來可作爲預防或降低酒精性肝損傷的健康食品檢測模式。

英文摘要

In this study, we examined the applicability of an animal model for the alcohol-induced liver injury in hepatoprotective evaluation. According to both the plasma AST and ALT activities, 50 male Wistar rats were assigned to five groups: C (control feeding), E (ethanol feeding), ES (ethanol feeding combined with the supplementation of silymarin, 200 mg/kg BW/day), CCL (CCl4 injection and control feeding) and CCLS (CCl4 injection and control feeding combined with the supplementation of silymarin, 200 mg/kg BW/day). Rats were fed for 12 weeks on a control or an ethanol Lieber-DeCarli liquid diet. Rats in groups CCL and CCLS were intraperitoneally injected with 0.75 mL/kg BW of 40% CCl4 dissolved in olive oil once a week, while rats in groups C, E and ES were intraperitoneally injected with 0.75 mL/kg BW of olive oil only. Our dada indicated significant main effects of both ethanol feeding and carbon tetrachloride injection on increased relative liver weight (%); elevated plasma AST and ALT activities at weeks 2, 4, 6, 8, 10 and 12; lowered plasma TG concentrations at week 12; increased hepatic TG and TC contents; elevated hepatic MPO activity; increased plasma TBARS concentrations at week 12; increased hepatic TBARS level; reduced hepatic GSH/GSSG ratio; decreased hepatic vitamin E level; decreased hepatic antioxidant enzymes, GPX and SOD activities; and pathologically changed liver; when compared to control feeding. Moreover, after 12 weeks, the results also showed significant main effects of carbon tetrachloride injection on increased relative liver weight (%); elevated plasma AST and ALT activities; reduced all the plasma TG, TC and HDL-C concentrations; augmented hepatic TG and TC contents; elevated hepatic MPO activities; increased plasma TBARS concentration; reduced hepatic GSH/GSSG ratio; decreased vitamin E level; decreased hepatic antioxidant enzymes, GRD and CAT activities; and severe fatty change in livers; when compared to ethanol feeding. In rats fed an ethanol liquid diet, silymarin could improve relative liver weight, hepatic GSH/GSSG ratio, plasma TBARS concentration and liver fatty change; and in rats injected carbon tetrachloride, silymarin could improve plasma AST and ALT activities at week 12, plasma TC concentration, hepatic GSH/GSSG ratio, and liver fatty change. In conclusion, our results suggest that both long-term ethanol feeding and carbon tetrachloride injection significantly increased oxidative stress, lipid peroxidation, and decreases the ratio of GSH/GSSG in rats, and ethanol feeding induced a slight susceptibility to liver damage

than carbon tetrachloride injection. Silymarin showed the hepatoprotective effects by means of improving the antioxidative capacity treatment protected against ethanol or carbon tetrachloride induced liver damage. The animal model for the alcohol-induced liver injury in this study provided a technically simple way to reproduce early stages of alcohol liver disease, and could be a hepatoprotective evaluation in preventing alcohol liver disease.