生藥川芎口秦及天然物蜂膠之藥理作用的研究

Pharmacological Research of Tetramethylprazine and Propolis

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[摘要]

為了探討瞭解中國大陸常用民間生藥，川芎口秦（Tetramethylpyrazine）的保肝作用，本實驗室以動物實驗模式來研究此生藥的保護功能。急性肝細胞傷害的誘導是以具有肝毒性的抗黴菌藥物－Econazole 來進行：以 300 mg/kg 的劑量口服投與至大白鼠體內，而後再以生藥川芎口秦於肝毒性藥物投與後的第二、第四和第八小時後各自口服投與生藥 10mg/kg 和 50 mg/kg 的劑量，以觀察川芎口秦的保肝作用。在保肝效果的評估中，除了藉由檢測實驗動物的血清轉胺酵素值（SGOT 和 SGPT）之外，實驗同時就病理學上的變化，以評估肝細胞組織學上的損傷，諸如：脂肪變性（fatty degeneration）、氣球樣變性（ballooning degeneration）、肝小葉中心壞死現象（centrilobular necrosis）、炎症細胞（kupffer cells）、淋巴球（lymphocytes）、嗜中性白血球（neutrophils）與嗜伊紅性白血球（eosinophils）之增殖性發炎浸潤（ inflammatory infiltration）等項目的觀察來綜合判定。並以確實被證實對肝炎有效的乳薊草成分 silymarin 作為對照藥物。由實驗結果顯示，在川芎口秦的保肝作用實驗中，雄性大白鼠經口投與 Econazole（ 300 mg/kg）後 24 小時，會造成血清轉胺酵素值急遽升高（SGOT：1955.6±63.4 IU/L ；SGPT：348.6±17.8 IU/L），而於 econazole 經口投與後的第二、第四和第八小時經口投與川芎口秦的生理食鹽水溶液，SGOT 與 SGPT 值明顯抑制為：(1)10 mg/kg：SGOT：766.3± 44.9 IU/L；SGPT：221.3±20.6 IU/L，(2)50 mg/kg：SGOT：635.0±24.0 IU/L；SGPT：122.8±9.5 IU/L。為了探討瞭解中國大陸常用民間生藥，川芎口秦（Tetramethylpyrazine）的保腎作用，本實驗室以動物實驗模式來研究此生藥的保護功能。急性腎傷害的誘導是以具有腎毒性的物質－酒精來進行：預先口服生藥川芎口秦（10、25 和 50 mg/kg）三十分鐘後，再以濃度 95%之酒精口服於小白鼠體內，以觀察川芎口秦的保腎作用。在保腎效果的評估中，除了藉由檢測實驗動物的尿素氮及肌酐酸（BUN 和 Creatinine）之外，實驗同時就川芎口秦對於酒精所引起之脂質過氧化病理學上的變化，以評估腎細胞的損傷。 以實驗的結果顯示，在川芎口秦的保腎作用實驗中，酒精（10 ml/kg）口服投與雄性 WISTAR 小白鼠後，造成 BUN 及 Creatinine 升高（BUN：27.9 ±1.7 gm/dl；Cr：0.21±0. 01 gm/dl），而於事先三十分鐘口服投予川芎口秦生理食鹽水溶液後，其 BUN 與 Creatinine 值明顯抑制為：(1)10 mg/kg ：BUN：18.1 ±1.3；Cr：0.2 ±0.01，(2)25 mg/kg：BUN：13.6 ±1.2；Cr：0.06 ±0.03，(3)50 mg/kg：BUN：13.4 ±1.3；Cr：0.06±0.03。此外，在川芎口秦的抗氧化活性研究方面，於脂質過氧化試驗及細胞色素 C 還原比色法測試中，川芎口秦均顯現有抗脂質過氧化及清除超氧自由基的能力。由於川芎口秦的保腎及抗氧化活性研究結果，我們推測生藥之抗氧化活性與其保腎活性確實有關，抗氧化活性越佳則保腎活性越好，但是使

用過量也可能造成體內之腎毒性。蜂膠（Propolis）為蜜蜂採蜜時，同時採集了某些樹木（例如：白楊木、七葉樹、柳樹及橡樹等）的樹皮及嫩芽內的樹脂，這些物質和蜜蜂的唾液結合成一種具黏性的棕綠色芳香性膠狀物，蜜蜂將此種膠狀物吐在蜂巢內，人類因而能由蜂巢內採集到此種自然界中最寶貴之生藥──蜂膠。至於蜂膠對於腸胃的保護功能及機轉，至今尚未清楚。本實驗主要是在探討蜂膠是否對於急性無水酒精（Absolute ethanol）造成胃潰瘍有保護作用，並深入了解其作用機轉。由急性無水酒精胃傷害模式中，蜂膠（1、5、10%，P.O.）的投與可以呈現劑量依賴性（Dose-dependent）地減少胃黏膜由無水酒精（1.0 ml/kg，P.O.）投與所造成胃黏膜損傷的程度，此一保護結果可由大體照相（Gross）中觀察得知。此外在體外（ in vitro）及體內（in vivo）實驗的 TBA 實驗中亦發現蜂膠可直接與無水酒精之毒性代謝物質或代謝所產生之自由基（Free radical）結合，減少這些毒性物質對細胞的傷害。實驗證明，投與蜂膠可以減少無水酒精胃傷害。其可能之保護機轉為透過抑制自由基的效應而產生。

關鍵字: 川芎口秦 Tetramethylprazine、蜂膠 Propolis、
脂質過氧化 lipid peroxidantion、細胞色素 c cytochrome c

[摘要]

Abstract: 2,3,5,6-tetramethylpyrazine（TMP）is well known as a truecalcium antagonist. The aim of this study was to investigate the hepatoprotectiveand therapeutic effects of TMP on acute econazole-induced liver injury. The hepatological effect of various concentrations of TMP was first assessedby the biochemical assay of SGOT and SGPT and then by hepatohistological microscopic examination. The dose-response relationship of liver injury induced by various doses of econazole was observed simultaneously fromserum biochemical assay of SGOP and SGPT, and also from hepatohistological microscopic examination, in the determination of the hepatoprotectiveeffects of various concentrations of TMP on SGOT and SGPT elevation induced by hepatotoxic dose of econazole (300 mg/kg). The inhibitory effect ofvarious concentration of TMP or vitamin E (as positive control, 0.5mM: in vitro,0.69 mM: in vivo) on FeCl2-induced (in vitro)or econazole-induced (in vivo)lipid peroxidation was also investigated. The superoxide scavenging activity of various concentrations of TMP in econazole-damaged rat liver homogenatewas assessed by cytochrome C reduction method. Results showed that the hepatoprotective effect of TMP might be, at least in part, due to its inhibitory ability on membrance lipid peroxidation and free radicalformation, or due to its free radical scavenging ability. The improvement of theserum transaminases and MDA levels in rat liver homogenate, thehepatohistological microscopic examination, and the assessment of free radical scavenging activity by cytochrome C reduction method can

be used to detect the hepatoprotective and therapeutic effects of TMP on acute econazole-induced liver injury. Acute administration of absolute ethanol (10 ml/kg) per os to fasted mice produced extensive renal failure as measured by a rise in blood ureanitrogen and creatinine. Pretreatment with oral administration oftetramethylpyrazine (TMP) prevented such failure. The maximal effect against absolute ethanol- induced renal failure could be observed 1 h after TMP administration .Inorder to further investigate the renal protective mechanism of TMP, experimentson lipid peroxidation and superoxide scavenging activity were conducted.Renal homogenates made from mice treated with ethanol showed that TMPpretreatment had an antioxidant effect. Mice in acute renal failure had higher malonic dialdehyde concentrations than those pretreated with TMP. The renalprotective mechanism of TMP was attributed, in part, to its prominent superoxide scavenging effect, which protects the kidney from superoxide-induced renal damage. Reactive oxygen species have been implicated in the pathogenesis of avariety of acute injury models, including ischemia-reperfusion injury and ethanol- induced renal failure. Acute ingestion of absolute ethanol (5 g/kg) hasbeen reported to lead to an accelerated increase in lipid peroxidation, anindex of oxidative stress. Protection against renal injury can be achieved by avariety of agents, including scavengers of hydroxylation and superoxide dismutase, which converts superocide to hydrogen peroxide. Previous studies haveshown that renal failure is often associated with ischemic injury andnephrectomy. Absolute ethanol-induced renal failure is a useful model in mice, but the detailed mechanism of its pathogenesis is still not fully clear. Oxygen is essential for life, but it may also be dangerous. Reduction of oxygen in tissue produces an number of oxygen free radicals which mayinduce cellular damage and even cell death. Oxygen-handling cells have different systems, e.g. superoxide dismutase, peroxidases and catalases, whichprotect them against the toxic effects of oxygen free radicals. Tetramethylpyrazine (TMP) is a constituent of Ligusticum wallichii French.It not only blocks the entry of extracellular calcium through calciumchannels but also inhibits the release of intracellular stored calcium in vascular smooth muscle cells. In 1997, Feng et al. reported that pretreatment withYMP in hypoxic isolated rat heart enhances prostaglandin I2 outflow andattenuates the release of thromboxane A2 in rat heart during normoxia, hypoxia and reoxygenation, and hence could significantly protect the myocardium from hypoxic injury. Actually, TMP could be useful as a therapeutic agent in ischemic heart disease with coronary artery disease by suppressingcoronary vasoconstriction and ischemic changes in the tissues produced byendothelin-1. Since TMP has been confirmed to be a true calcium antagonist, it may playvery important roles in the area of tissue protection and preservation. In addition, in its use as a highly potent

antihypertensive drug, it mayexert favorable effects on renal hemodynamics related to the reversal of renal vasoconstrictors. Although the mechanisms of action of TMP in the settingof chronic renal failure are not yet fully established, its beneficialeffects may be related to protective actions such as the reduction of renalhypertrophy , modulation of mesangial cell uptake of macromolecules, changes in the permselectivity of the glomerulus and decreased free radical formation. Acute administration of absolute ethanol often leads to tissue damage, especially in the renal system. The aims of the present study were to investigate whether TMP administration per os in mice could protect thekidney from absolute ethanol-induced lesions, and if TMP did offer protection,what its mechanism of action might be. Acute p.o. administration of absolute ethanol (1.0 ml/kg) to fasted rats produced extensive necrosis of gastric mucosa. Pretreatment with p.o. administration of propolis ethanol extract (PEE) could effectively anddose- dependently prevent such necrosis. This protective effect is called " cytoprotection". The maximal cytoprotective effect against absoluteethanol (AE)-induced gastric mucosal lesion was observed at 1 hour after PEE administration. A gross examination of the gastric mucosa showed a marked improvement in groups receiving PEE. In order to further investigate the gastric protective mechanism of PEE, lipid peroxidation (LPO) levels invivo and in vitro were estimated. PEE exhibited dose-dependent superoxide scavenging activity and antioxidant effect in absolute ethanol inducedlipid peroxidation in rat gastric mucosal homogenate. It was concluded that the gastric protective mechanism of PEE contributed, at least in part, to its ability to inhibit lipid peroxidation, and hence indirectly protect the gastric mucosa from oxidative stress.