# 行政院國家科學委員會專題研究計畫 成果報告

## Luteolin 對抗大鼠缺血以及再灌注傷害的保護作用及其作用機轉研究

## 研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2320-B-040-027-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：中山醫學大學醫學系藥理學科

計 畫 主 持 人：黃相碩

計畫參與人員：碩士班研究生-兼任助理：李君珮、盧怡珠
大學生-兼任助理：黃薇芳、謝欣穎
工讀生：劉幸怡

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 96 年 08 月 07 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告
□期中進度報告

# Luteolin 對抗大鼠缺血以及再灌注傷害的保護作用及其作用機轉研究

計畫類別：■ 個別型計畫　□ 整合型計畫
計畫編號：NSC 95 － 2320 － B － 040 － 027 －
執行期間： 2006 年 08 月 01 日至 2007 年 07 月 31 日

計畫主持人：黃相碩
共同主持人：
計畫參與人員：劉幸怡；李君珮；盧怡珠；黃薇芳；謝欣穎

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：中山醫學大學醫學系藥理科

中 華 民 國 96 年 08 月 07 日

中文摘要及關鍵詞：

Luteolin 是廣泛的存在許多種類的水果和蔬菜中的一種黃酮類物質，本計劃引起大白鼠心臟缺血或是缺血再灌注傷害來研究 Luteolin 的心臟保護作用，實驗將麻醉大白鼠左冠狀動脈結紮三十分鐘研究心臟缺血傷害，此外將麻醉大白鼠左冠狀動脈結紮五分鐘而後再灌注三十分鐘研究心臟缺血再灌注傷害，動物在冠狀動脈結紮前投與含有或是不含有 Luteolin 之溶劑，以此兩種動物模式評估 Luteolin 在大白鼠心臟缺血或是缺血再灌注傷害的作用，實驗比較大白鼠在心臟缺血或是缺血再灌注傷害期間所引發心律不整的嚴重程度以及死亡率，此外我們還比較投與 Luteolin 與否對於大白鼠心臟缺血或是缺血再灌注傷害期間血漿中 LDH 的活性的影響，藉以評估大白鼠用 Luteolin 治療後遭受心臟缺血或是缺血再灌注傷害時細胞傷害的情形，研究發現預先投與濃度 1 μg/kg 的 Luteolin 具心臟保護作用，可以對抗心臟缺血或是缺血再灌注所造成的傷害。預先投與濃度 1 μg/kg 的 Luteolin，在心臟遭受缺血傷害期間不僅可以減少心室心搏過速的發生率以及發生時間長短，也可以減少心室纖維顫動的發生時間長短，還可以減少動物死亡率。此外預先投與濃度 1 μg/kg 的 Luteolin，在心臟遭受缺血再灌注傷害期間不僅可以減少心室心搏過速和心室纖維顫動的發生率以及發生時間長短，還可以減少動物死亡率。收集頸動脈血，發現在於大白鼠心臟缺血或是缺血再灌注傷害期間，投與 Luteolin 也減少血漿中 LDH 的活性。綜合研究成果，我們發現在於大白鼠遭受心臟缺血或是缺血再灌注傷害期間，Luteolin 具有抗心律不整的作用，是一個很有潛力的心臟保護劑。

關鍵字： 心臟保護劑；Luteolin；心肌；大白鼠；缺血；再灌注

英文摘要及關鍵詞：

The cardioprotective effects of luteolin, a flavonoid wildly presents in many kinds of fruits and vegetables, which exhibit antioxidant properties, were investigated in the rats after myocardial ischemia and ischemia-reperfusion (I/R) injury. Anesthetized rats were subjected to the left main coronary artery occlusion 30 min or 5 min followed by a 30-min period of reperfusion for evaluation the effect of luteolin on the myocardial ischemia or I/R injury. Animals were preinfused with or without luteolin before occlusion of coronary artery and the severity of myocardial ischemia and I/R induced arrhythmias and mortality were compared. In addition, we test the lactate dehydrogenase (LDH) activity in plasma to correlate cellular damage with the myocardial ischemia and I/R lesions treated by luteolin. Cardioprotective effects of luteolin (1 μg/kg) were observed against myocardial ischemia and I/R injury. During myocardial ischemia injury period, pretreatment luteolin (1 μg/kg) not only reduced both the incidence and duration of ventricular tachycardia (VT) and the duration of ventricular fibrillation (VF) but also decreased the mortality. While myocardial I/R injury period, pretreatment luteolin (1 μg/kg) reduced both the incidence and duration of VT and VF and mortality. During the same period, pretreatment luteolin also decreased LDH levels in the carotid blood. It is concluded that luteolin is a potent cardioprotective agent with antiarrhythmic effect in myocardial ischemia and I/R injury rats.

*Keywords:* Cardioprotective agent; Luteolin; Myocardium; Rat; Ischemia; Reperfusion

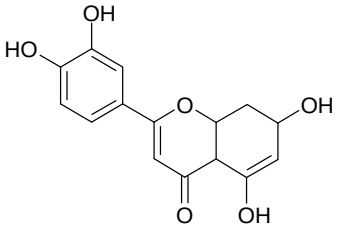
# 目　　錄

## 報告內容：

### 前言：

Evidences from studies on the myocardium suggest that reactive oxygen species, including superoxide radical, hydrogen peroxide, hydroxyl radical, singlet oxygen, contribute to the pathophysiology of myocardial ischemia and I/R injury (Kloner, 1988; Park and Lucchesi, 1999). The interaction of oxygen-derived free radicals with cell membrane lipids and essential proteins induce myocardial cell damage, leading to depressed cardiac function and irreversible tissue injury. Myocardial ischemia and I/R injury will induce potentially lethal ventricular arrhythmia resulting in circulation collapse and end up in sudden death (Manning and Coltart, 1984). Therefore, effective inhibition of reactive oxygen species production or elimination of oxygen-derived free radicals become important strategy for the treatment of the ventricular arrhythmia and sudden death caused by myocardial ischemia or I/R injury (Garlick et al., 1987; Huang et al., 2001).

Luteolin (Fig.1) is one of the most wildly presented flavonoids in many kinds of fruits and vegetables (Williams et al., 1996; Lin et al., 1997). There are several literatures had been reported that luteolin possess antineoplastic (Pettit et al., 1996; Chang et al., 2005), antihepatotoxic, antiallergic, antiosteoporotic (Di Carlo et al., 1999), antidiabetic activity (Zarzuelo et al., 1996), and anti-inflammatory activities (Wu et al., 2005). Recently, luteolin also has been shown to protect DNA against free radicals injury in human melanoma HMB-2 cells (Horvathova et al., 2005). The antioxidant activity of luteolin may have beneficial effect on the model of myocardial ischemia and I/R injury. Luteolin also has been shown to significantly enhance left ventricular pressure and the global and relative coronary flow in Langendorff rabbit hearts subjected to repetitive myocardial ischemia (Rump et al., 1994). However, there is no literature to consider luteolin for the possible use as a therapeutic drug in treating the acute scenarios on myocardial ischemia and I/R injury induced arrhythmia.




### 研究目的：

The purpose of this experimental study was to evaluate the antiarrhythmic effect of luteolin on myocardial ischemia or I/R injury in anesthetized rats subjected to transient coronary artery occlusion and reperfusion. Animals were pretreated with or without luteolin before coronary artery ligation and the severity of myocardial ischemia and I/R induced arrhythmias, including the incidence and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) and mortality were compared. In addition, we test the lactate dehydrogenase (LDH) activity in plasma to correlate cellular damage with the myocardial ischemia and I/R lesions treated by luteolin.

文獻探討：

Chang, J., Hsu, Y., Kuo, P., Kuo, Y., Chiang, L., Lin, C., 2005. Increase of Bax/ Bcl-XL ratio and arrest of cell cycle by luteolin in immortalized human hepatoma cell line. Life Sciences 76(16), 1883–1893.

Chen, Y.T., Zheng, R.L., Jia, Z.J., Ju, Y., 1990. Flavonoids as superoxide scavengers and antioxidants. Free Radical Biology and Medicine 9(1), 19-21.

Di Carlo, G., Mascolo, N., Izzo, A.A., Capasso, F., 1999. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sciences 65(4), 337-353.

Garlick, P.B., Davies, M.J., Hearse, D.J., Slater, T.F., 1987. Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. Circulation Research 61(5), 757-760.

Horvathova, K., Chalupa, I., Sebova, L., Tothova, D., Vachalkova, A., 2005. Protective effect of quercetin and luteolin in human melanoma HMB-2 cells. Mutation Research 565(2), 105-112.

Huang, S.S., Liu, S.M., Lin, S.M., Liao, P.H., Lin, R.H., Chen, Y.C., Chih, C.L., Tsai, S.K., 2005. Antiarrhythmic effect of caffeic acid phenethyl ester (CAPE) on myocardial ischemia/reperfusion injury in rats. Clinical Biochemistry 38(10), 943-947.

Huang, S.S., Tsai, S.K., Chiang, L.Y., Chih, L.H., Tsai, M.C., 2001. Cardioprotective Effects of Hexasulfobutylated C60 (FC4S) in Anesthetized Rats During Coronary Occlusion/Reperfusion Injury. Drug Development Research 53, 244–253.

Jolly, S.R., Kane, W.J., Bailie, M.B., Abrams, G.D., Lucchesi, B.R., 1984. Canine myocardial reperfusion injury: Its reduction by the combined administration of superoxide dimutase and catalase. Circulation Research 54(3), 277-285.

Kilgore, K.S., Lucchesi, B.R., 1993. Reperfusion injury after myocardial infarction: the role of free radicals and the inflammatory response. Clinical Biochemistry 26(5), 359-370.

Kloner, R.A., 1988. Introduction to the role of oxygen radicals in myocardial ischemia and infarction. Free Radical Biology and Medicine 4(1), 5-7.

Kukreja, R.C., Hess, M.L., 1992. The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. Cardiovascular Research 26(7), 641-655.

Kusama, Y., Bernier, M., Hearse, D.J., 1990. Exacerbation of reperfusion arrhythmias by sudden oxidant stress. Circulation Research 67(2), 481-489.

Li, Y.C., Hung, C.F., Yeh, F.T., Lin, J.P., Chung, J.G., 2001. Luteolin-inhibited arylamine N-acetyltransferase activity and DNA-2-aminofluorene adduct in human and mouse leukemia cells. Food and Chemical Toxicology 39(7), 641-647.

Lin, C.N., Kuo, S.H., Chung, M.I., Ko, F.N., Teng, C.M., 1997. A new flavone C-glycoside and antiplatelet and vasorelaxing flavones from Gentiana arisanensis. Journal of Natural Products 60(8), 851–853.

Manning, A.S., Coltart, D.J., 1984. Ischemia and reperfusion-induced arrhythmias in the rat:

effects of xanthine oxidase inhibition with allopurinol. Circulation Research 55(4), 545-548.

Mertz, T.E., Kaplan, H.R., 1982. Pirmenol hydrochloride (CI-845) and reference antiarrhythmic agents: effects on early ventricular arrhythmias after acute coronary artery ligation in anesthetized rats. Journal of Pharmacology and Experimental Therapeutics 223(2), 580-586.

Nagao, A., Seki, M., Kobayashi, H., 1999. Inhibition of xanthine oxidase by flavonoids. Bioscience, Biotechnology and Biochemistry 63(10), 1787-1790.

Park, J.L., Lucchesi, B.R., 1999. Mechanisms of myocardial reperfusion injury. Annals of Thoracic Surgery 68(5), 1905-1912.

Pettit, G.R., Hoard, M.S., Doubek, D.L., Schmidt, J.M., Pettit, R.K., Tackett, L., Chapuis, J.C., 1996. Antineoplastic agents 338. The cancer cell growth inhibitory constituents of Terminalia arjuna (Combretaceae). Journal of Ethnopharmacology 53(2), 57–63.

Rump, A.F., Schussler, M., Acar, D., Cordes, A., Theisohn, M., Rosen, R., Klaus, W., Fricke, U., 1994. Functional and antiischemic effects of luteolin-7-glucoside in isolated rabbit hearts. General Pharmacology 25(6), 1137-1142.

Smith, E.F. 3rd, Griswold, D.E., Egan, J.W., Hillegass, L.M., Dimartino, M.J., 1989. Reduction of myocardial damage and polymorphonuclear leukocyte accumulation following coronary artery occlusion and reperfusion by the thromboxane receptor antagonist BM 13.505. Journal of Cardiovascular Pharmacology 13(5), 715-722.

Tsai, S.K., Lin, S.M., Huang, C.H., Hung, W.C., Chih, C.L., Huang, S.S., 2004. Effect of desflurane-induced preconditioning following ischemia-reperfusion on nitric oxide release in rabbits. Life Sciences 76(6), 651-660.

van Meeteren, M.E., Hendriks, J.J., Dijkstra, C.D., van Tol, E.A., 2004. Dietary compounds prevent oxidative damage and nitric oxide production by cells involved in demyelinating disease. Biochemical Pharmacology 67(5), 967-75.

Walker, M.J., Curtis, M.J., Hearse, D.J., Campbell, R.W., Janse, M.J., Yellon, D.M., Cobbe, S..M., Coker, S.J., Harness, J.B., Harron, D.W., Higgins, A.J., Julian, D.G., Lab, M.J., Manning, A.S., Northover, B.J., Parratt, J.R., Reimersma, R.A., Riva, E., Russell, D.C., Sheridan, D.J., Winslow, E., Woodward, B., 1988, The Lambeth Conventions: guidelines for the study of arrhythmias in ischemia infarction, and reperfusion. Cardiovascular Research 22(7), 447-455.

Wang, P., Zweier, J.L., 1996. Measurement of nitric oxide and peroxynitrite generation in the postischemic heart: evidence for peroxynitrite-mediated reperfusion injury. Journal of Biological Chemistry 271(46), 29223-29230.

Werns, S.W., Lucchesi, B.R., 1990. Free radicals and ischemic tissue injury. Trends in Pharmacological Sciences 11(4):161-166.

Williams, C.A., Goldstone, F., Greenham, J., 1996. Flavonoids, cinnamic acids and coumarins from the different tissues and medicinal preparations of Taraxacum officinale. Phytochemistry 42(1), 121–127.

Wu, M.J., Weng, C.Y., Ding, H.Y., Wu, P.J., 2005. Anti-inflammatory and antiviral effects of Glossogyne tenuifolia. Life Sciences 76(10), 1135-1146.

Yee, S.B., Lee, J.H., Chung, H.Y., Im, K.S., Bae, S.J., Choi, J.S., Kim, N.D., 2003. Inhibitory

effects of luteolin isolated from Ixeris sonchifolia Hance on the proliferation of HepG2 human hepatocellular carcinoma cells. Archives of Pharmacal Research 26(2), 151-156.

Zarzuelo, A.. Jimenez, I., Gamez, M.J., Utrilla, P., Fernadez, I., Torres, M.I., Osuna, I., 1996. Effects of luteolin 5-O-beta-rutinoside in streptozotocin-induced diabetic rats. Life Sciences 58(25), 2311-2316.

研究方法：

*Animals*

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male Sprague-Dawley rats (National Lab. Animal Breeding and Research Center, Taipei, Taiwan) weighting 250~300 g were used. These animals were housed in a room with controlled temperature (24±1°C) and humidity (55±5%) under a 12:12 h light-dark cycle. They were allowed free access to food and water.

*Surgical procedure*

Rats undergoing myocardial ischemia and I/R injury were induced by a temporary occlusion of the left main coronary artery in procedures as described previously (Huang et al., 2005). Briefly, Male sprangue-Dawley rats were anesthetized with intraperitoneal urethane (1.25 g/kg i.p.) and placed on an operating table. The trachea was cannulated for artificial respiration and the jugular vein was cannulated for drug administration. Polyethylene catheters (PE-50) were inserted into the common carotid artery for continuous monitoring of heart rate and arterial blood pressure by a Statham P23 XL transducer and displayed on a Gould RS-3400 physiological recorder (Gould, Cleveland, OH, USA). A standard lead-1 electrocardiogram (ECG) was also recorded by attaching silver electrodes to extremities of animals.

After tracheotomy, the animals were ventilated with room air by a respirator for small rodents (Model 131, NEMI, U.S.A.) with a stroke volume of 15 ml/kg body weight and at a rate of 60 strokes/min to maintain normal $P_{O_2}$, $Pc_{O_2}$ and pH parameters (blood gas analyzer, GEM-5300 I.L. CO, USA). The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs, approximately 2 mm to the left of the sternum. The heart was quickly expressed out of the thoracic cavity, inverted and a 6/0 silk ligature was placed around the left main coronary artery. The heart was repositioned in the chest and the animal was allowed to recover for 15 min. Animals in which the procedure produced arrhythmia or a sustained decrease in BP to less than 70 mmHg were not included in the study.

A small plastic snare formed from a Portex P-270 cannula was threaded through the ligature and placed in contact with the heart. The coronary artery then was occluded by tightening the ligature and reperfusion was achieved by releasing the tension applying to the ligature (operated groups). Successful ligation of the coronary artery was validated by observation of a decrease in arterial pressure and ECG changes (increase in R wave and ST segment elevation) indicative of ischemia. Sham operated animals underwent all surgical procedures, except the silk passing around the left coronary artery was not tied (sham groups) (Smith et al., 1989).

*Evaluation of arrhythmia*

For evaluation the effect of luteolin on the myocardial ischemia or I/R injury, the coronary artery was occluded for 30 min or 5 min followed by 30 min reperfusion. Before and during the ischemia or I/R period, heart rate, blood pressure, and ECG changes were recorded simultaneously on a personal computer with a wave form data analysis software (Acqknowledge, Biopac System, Goleta, California, USA). Ventricular ectopic activity was evaluated according to the diagnostic criteria advocated by the Lambeth Convention (Walker et al., 1988). The incidence and duration of ventricular tachyarrhythmias, including ventricular tachycardia (VT) and ventricular fibrillation (VF) were determined, in surviving as well as the nonsurviving animals. In rats with irreversible VF, the duration of VF was recorded up the time when BP fell to <15 mmHg.

*Drug administration*

Luteolin was purchased from Sigma Chemical Company (St. Louis, Mo. USA) and luteolin solution was fresh prepared before administration. Luteolin (0.01, 0.1 or 1 μg/kg) or vehicle (dimethyl sulfoxide-0.9% NaCl, $1:10^4$; v/v) were infused via a jugular vein 15 min before coronary artery occlusion. Rats injected with vehicle were used as control. No effect of vehicle on ischemia and I/R induced arrhythmia at such concentration. Animals were randomly allocated to each drug treatment and vehicle group.

*Plasma LDH analysis*

Myocardial cellular damage was evaluated by measuring the lactate dehydrogenase (LDH) activity in plasma. Samples of arterial blood were drawn from the carotid catheter at the end of ischemia and reperfusion, then collected in polyethylene tubes containing 50 μl heparin (250 IU). The blood was kept at 4°C until it was centrifuged at 2000 × g for 15 min. The plasma was recovered and aliquots were used for determination of LDH activity. The LDH activity was measured according to the method of Tsai et al (Tsai et al., 2004) by spectrophotometrically following the rate of conversion of NADH to $NAD^+$, at 340 nm, with a commercially available assay kit (Sigma, St Louis, MO).

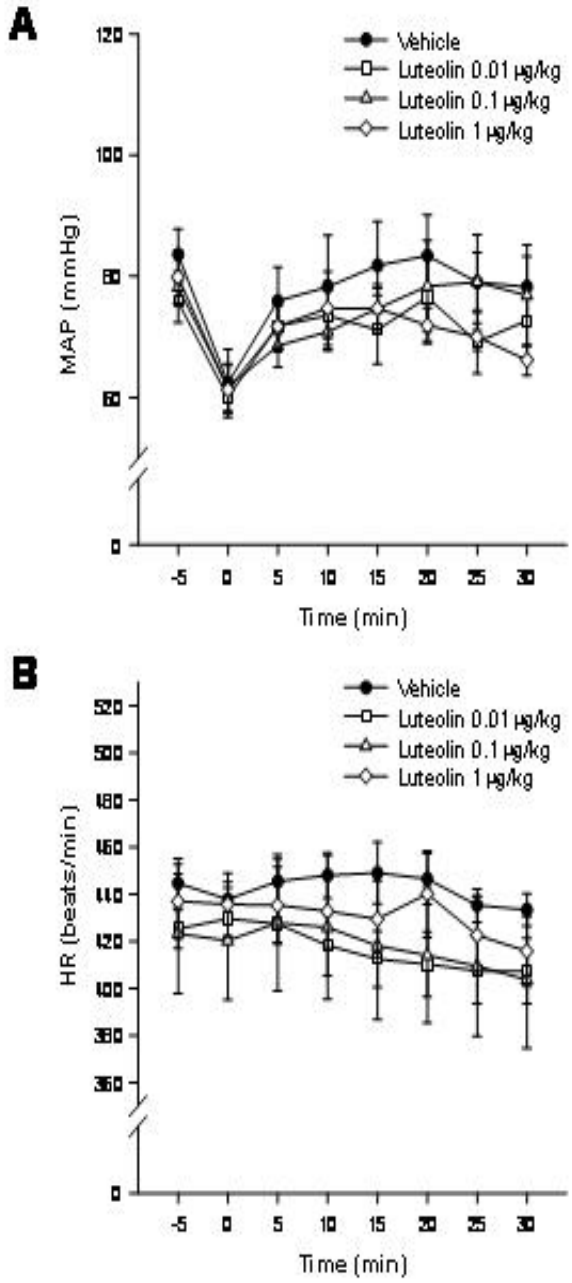
*Statistics*

Data were expressed as mean ± standard error of mean (SEM). The difference of blood pressure, heart rate, duration of VT and VF between vehicle and drug treatment groups in arrhythmia study was carried out by using analysis of variance (ANOVA) followed by Bonferroni's test. The difference in the percentage incidence of VT, VF and mortality were analyzed with a $\chi^2$ test. While the difference in plasma LDH levels were statistically evaluated by unpaired Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

結果：

*Hemodynamic changes during coronary artery occlusion*

Jugular vein injection of luteolin did not change the mean arterial pressure and the heart rate in rats subjected to myocardial ischemia (Fig. 2). No significant difference was seen among control and luteolin treated groups (1 μg/kg, n=7).




*Myocardial ischemia induced rhythm disturbances*

Effects of luteolin on coronary ligation elicited arrhythmias in anesthetized rats were shown in Table 1. In the vehicle-treated group, severe ventricular arrhythmias occurred at 6-7 min and peaked at 8-12 min, and normally subsided by approximately 15 min after coronary occlusion. Among 14 rats in the vehicle-treated group, 12 animals (86%) exhibited VT (39.9 ± 13.4 sec in duration), and 9 animals (64%) exhibited VF (81.0 ± 25.5 sec in duration). However, administration of luteolin at the doses of 1 μg/kg 15 min prior to coronary occlusion, significantly reduced the incidence of VT (29%) and the duration of VT (6.1 ± 4.5 sec) and VF (3.6 ± 2.7 sec), respectively. The duration of VT was significantly reduced to 7.7 ± 4.1 sec by 0.1 μg/kg luteolin. The mortality rate was significantly decreased from 50% to 0% in rats treated with 1 μg/kg luteolin.

Table 1. Effect of luteolin on coronary ligation (30 min) induced arrhythmias in anesthetized rats.

| | | Ventricular Tachycardia | | Ventricular Fibrillation | | Mortality |
|---|---|---|---|---|---|---|
| | n | Incidence (%) | Duration (s) | Incidence (%) | Duration (s) | (%) |
| Sham | | | | | | |
| Vehicle | 4 | — | — | — | — | — |
| Luteolin 1 μg/kg | 4 | — | — | — | — | — |
| Operated (Coronary ligation) | | | | | | |
| Vehicle | 14 | 86 | 39.9±13.4 | 64 | 81.0±25.5 | 50 |
| Luteolin 0.01 μg/kg | 9 | 44 | 20.5±12.1 | 33 | 29.9±18.1 | 22 |
| 0.1 μg/kg | 8 | 50 | 7.7±4.1* | 25 | 12.7±12.3 | 13 |
| 1 μg/kg | 7 | 29* | 6.1±4.5* | 29 | 3.6±2.7* | 0* |
| | | | | | | |

Vehicle is 0.01% DMSO in normal saline; n = number of experiments; values for duration of VT and VF are shown as the mean ± S.E.M.
* Statistical difference at the level of $p < 0.05$ as compared with vehicle.

*Myocardial I/R induced rhythm disturbances*

Effects of luteolin on myocardial I/R-elicited arrhythmias in anesthetized rats were shown in Table 2. The severity of I/R-induced arrhythmias is critically dependent on the duration of the proceeding period of ischemia. In this study, we selected a 5-min period of ischemia followed by a 30-min period of reperfusion in order to produce the maximal effect of the rhythm disturbance (Werns and Lucchesi, 1990). During myocardial I/R injury, rats treated with 0.1 μg/kg and 1 μg/kg of luteolin significantly reduced the duration of VT (1.1 ± 0.7 sec, 0.9 ± 0.9 sec vs. 15.2 ± 4.9 sec, $p<0.05$) and VF (3.3 ± 3.0 sec, 0.7 ± 0.7 sec vs. 77.8 ± 18.5 sec, $p<0.05$), but the incidence of VT (14% vs. 69%, $p<0.05$) and VF (14% vs. 69%, $p<0.05$) were significantly reduced only at the doses of 1 μg/kg luteolin treatment compared to that of vehicle-treated rats. The mortality rate was declineded from 56% to 0% by 1 μg/kg luteolin.

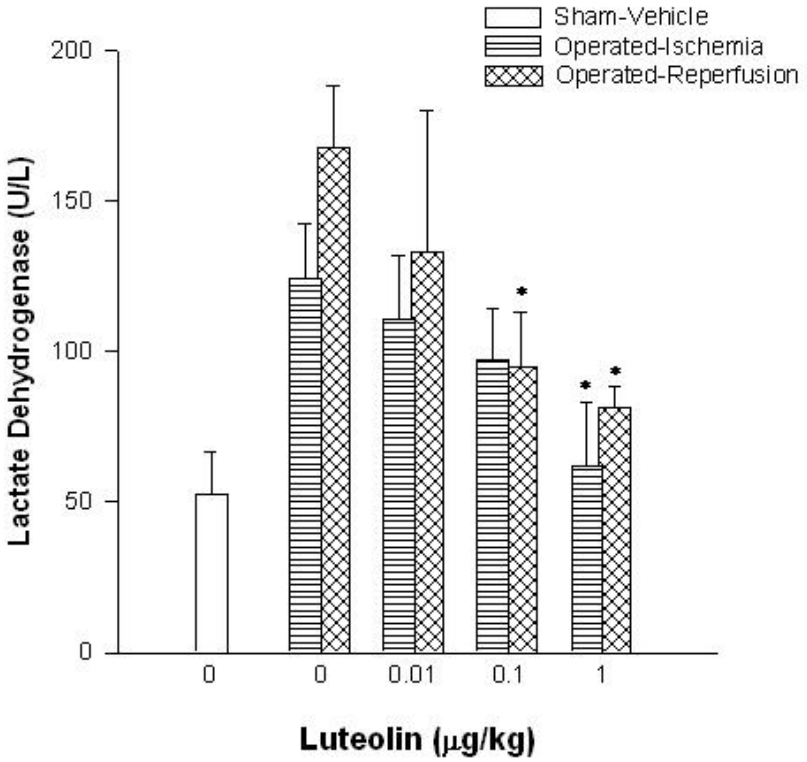
Table 2. Effect of luteolin on reperfusion (30 min) induced arrhythmias in anesthetized rats.

| | | Ventricular Tachycardia | | Ventricular Fibrillation | | Mortality |
|---|---|---|---|---|---|---|
| | n | Incidence (%) | Duration (s) | Incidence (%) | Duration (s) | (%) |
| Sham | | | | | | |
| Vehicle | 4 | — | — | — | — | — |
| Luteolin 1 μg/kg | 4 | — | — | — | — | — |
| Operated | | | | | | |
| Vehicle | 16 | 69 | 15.2±4.9 | 69 | 77.8±18.5 | 56 |
| Luteolin 0.01 μg/kg | 10 | 60 | 3.4±1.9 | 50 | 31.2±27.4 | 30 |
| 0.1 μg/kg | 8 | 25 | 1.1±0.7* | 25 | 3.3±3.0* | 13 |
| 1 μg/kg | 7 | 14* | 0.9±0.9* | 14* | 0.7±0.7* | 0* |
| | | | | | | |

Vehicle is 0.01% DMSO in normal saline; n = number of experiments; values for duration of VT and VF are shown as the mean ± S.E.M.
* Statistical difference at the level of $p < 0.05$ as compared with vehicle.

*Plasma LDH level*

The biochemical indicator of cellular damage was examined by measuring the LDH leakage to the plasma in the end of myocardial ischemia and I/R injury period. The effects luteolin on the changes in LDH activity in plasma during ischemia and I/R injury of rats were shown in Fig. 3. Low LDH activity in the plasma was recorded in the sham-operated animals (52.8 ± 13.9 U/L (n=7)). Instead, after myocardial ischemia and I/R injury, a large increase of the enzyme was found in the plasma of rats given vehicle (124.5 ± 18.1 and 167.8 ± 20.4 U/L (n=7), respectively). In contrast, the administration of luteolin dose-dependently reduced the LDH release. Luteolin at the dose of 1 μg/kg, the LDH activities in the plasma were reduced to 62.2 ± 20.7 and 81.2 ± 7.3 U/L (n=7), respectively, in rats after myocardial ischemia and I/R injury.




討論：

Ischemia is characterized in part by low tissue oxygen tension. It is well documented that salvage of the ischemic myocardium is dependent upon timely reperfusion**,** it is likely that the very events critical for survival may, in fact, lead to further tissue injury. There are several evidences from studies on the myocardium suggest that reactive oxygen species (superoxide radical, hydrogen peroxide, hydroxyl radical, singlet oxygen) contribute to the pathophysiology of myocardial ischemia and I/R injury. These reactive oxygen species, which are formed within the myocardial ischemia and first moments of reperfusion period, are known to be cytotoxic to surrounding cells (Werns and Lucchesi, 1990; Kukreja and Hess, 1992; Kilgore and Lucchesi, 1993). Therefore, Myocardial ischemia and I/R injury will induce ventricular arrhythmia resulting in circulation collapse and end up in sudden death (Kusama et al., 1990; Mertz and Kaplan, 1982). The strong evidence lies in the ability of free radicals scavengers to reduce the ventricular arrhythmia and to limit myocardium damage in experimental models caused by myocardial ischemia and I/R injury (Garlick et al., 1987; Jolly et al., 1984).

In the present study, we showed that in anesthetized rats the administration of luteolin (1 μg/kg) prior to coronary artery occlusion significantly reduced the mortality and suppressed the myocardial arrhythmias during myocardial ischemia and I/R injury. During the same period, luteolin pretreatment also decreased LDH levels in the carotid blood. These results indicated that luteolin possesses the robust cardioprotective effect against myocardial ischemia and I/R injury.

Luteolin is one of the most wildly distributed flavonoids, a group of naturally occurring polyphenolic compound, which present in many kinds of fruits and vegetables (Williams et al., 1996). Luteolin has a wide range of biological and pharmacological properties including antineoplastic activities (Li et al., 2001; Yee et al., 2003), anti-inflammatory effect (Wu et al., 2005), antiplatelet and vasodilatory activity (Lin et al., 1997) and antioxidant effect (Chen et al., 1990). The cardioprotective effect of luteolin may be due to the antioxidant activity in biological systems. Luteolin had been reported to inhibit xanthine oxidase activity, which has been implicated in oxidative injury to tissue by ischemia-reperfusion, at low concentrations ($IC_{50}$ value is 0.96 μM) (Nagao et al., 1999). In human melanoma HMB-2 cells, luteolin showed a concentration-dependent inhibitory activity toward DNA damage induced by $H_2O_2$ (Horvathova et al., 2005). The mass production of oxygen-derived free radicals during myocardial ischemia and I/R period may be arrested by antioxidant activity of luteolin. In addition, in isolated rabbit heart, luteolin also possess cardioprotective properties against repetitive myocardial ischemia injury by improvement of left ventricular pressure. Luteolin also enhanced the global and relative coronary flow and therefore might improve myocardial perfusion in the ischemic border area (Rump et al., 1994). Recently, Marieke et al., showed that luteolin inhibited nitric oxide (NO) production and reduced the expression of inducible NO synthase (iNOS) in lipopolysaccharide stimulated NR8383 macrophages (van Meeteren et al., 2004). NO is an agent that can induce cell damage. NO can inhibit mitochondria function and break DNA single-strand. In addition, NO and superoxide radicals can rapidly combine to form a strong reactive metabolite, peroxynitrite, which is a potent oxidant that can potentially cause membrane lipid peroxidation leading to myocardial dysfunction (Wang and Zweier, 1996). Luteolin decrease NO production may be direct scavenge NO radicals or inhibit iNOS protein synthesis. During myocardial ischemia and I/R period, luteolin may suppress NO production and scavenge reactive oxygen species to prevent NO interaction with superoxide radicals, which then to avoid the injury of free radicals.

In conclusion, our study presents the first evidence that pretreatment with luteolin could effectively protect myocardium against myocardial ischemia and I/R induced cardiac injury. We wound speculate that the beneficial cardioprotective effect may be related to the antioxidant activity, vasodilating effect, decrease the production of NO or all these mechanisms together.

計畫成果自評：

This study had finished and prepared for publish.