行政院國家科學委員會專題研究計畫　成果報告

高脂血症對心肌微循環血管屏障的影響及其在缺血—再灌流傷害中之角色

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【中、英文摘要及關鍵詞(keywords)】

血膽固醇過高症與全身性動脈粥樣硬化相關聯。血膽固醇過高症對微循環的衝擊擾亂正接受眾多的研究。在這一項研究中，我們使用了加量膽固醇飲食餵食兔子。動態的對比劑增益磁共振影像顯示血膽固醇過高症和微循環表面區通透性的增加相關但是沒有影響心肌血流。那些血膽固醇過高症的兔子病理結果描述 isolectin－或平滑肌－肌動蛋白的微血管增加。而心肌層的微血管週邊和間質組織中 VEGF 的表達也出現。血膽固醇過高症不需要造成顯著的灌流缺陷就增加冠狀動脈心肌微循環表面之通透性。其增加的微血管密度，或外皮細胞參與了微血管的再塑形。 VEGF 可被視為一個可能的機制。

Hypercholesterolemia (HC) is associated with atherosclerosis in systemic arteries. Its impacts on microcirculatory disturbances are receiving rigorous investigations. In this study, we used the cholesterol-enrich diet to treat the rabbit. The dynamic contrast-enhanced magnetic resonance imaging showed that the induced hypercholesterolemia was associated with increase in permeability surface area product but did not influence the myocardial blood flow. The pathological examination of those hypercholesterolemic rabbits delineated greater density of isolectin- and smooth muscle α-actin bound microvessels. Patchy expression of VEGF in perivascular and interstitial space of myocardium was also noted.

Hypercholesterolemia was associated with increased coronary leakage without evident perfusion defect. Increased microvessel density, either pericyte-bound or not, suggested microvascular remodeling. VEGF could be induced in the setting and served as a possible underlying mechanism.

【keywords】Dynamic contrast-enhanced magnetic resonance imaging, Hypercholesterolemia, Microcirculatory permeability, VEGF 。

【報告內容】

Hypercholesterolemia Is Associated with Disrupted Microvascular Barrier Integrity in Rabbit Hearts

【Introduction】

Hypercholesterolemia (HC) is associated with atherosclerosis in systemic arteries. Its impacts on microcirculatory disturbances, such as enhanced leukocyte endothelial interaction in rat cresmaster muscle flaps and impaired positron emission tomography (PET) defined coronary flow reserve in human subjects, are receiving rigorous investigations.

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is an excellent non-invasive imaging modality to evaluate tumor microvasculature. Recently its applications in coronary microcirculation expanded our understanding about collateral formation in ischemic heart diseases. We previously reported increased fluorescein extravasation in iridic microvasculature of hypercholesterolemic rabbits. In this study, we developed a platform to perform and analyze coronary DCE-MRI images. We also tested the hypothesis that in hypercholesterolemic rabbits coronary microvascular barrier integrity would be disrupted due to neovascularization and enhanced VEGF expression.

【Methods】

Animals

15 New Zealand white rabbits were enrolled at the age of 8 weeks. (body weight: 1.2-1.5 kg) Rabbits were randomly assigned into the hypercholesterolemic group (HC, n=8), which were fed atherogenic diet (enriched with 0.5% cholesterol and 10% coconut oil) for 12 weeks, and the control group (Ctrl, n=7). The others fed on regular rabbit chow served as the control group. The protocol follows the regulation of Republic of China Animal Protection Law (Scientific Application of animals, 1998).

Examination Schedule

All rabbits received examinations at the age of 8, 14, and 20 weeks. Examinations included body weight measurement, serum lipid level anlysis, and coronary DCE-MRI. Afterwards, rabbits were sacrificed with a bolus of pentobarbital (100 mg/kg) and exsanguination. Myocardial samples from middle segments of left ventricle were then snap frozen with liquid nitrogen or fixed in 4% formaldehyde solution for pathological studies.

DCE-MRI

Animal was sedated with an intramuscular injection of ketamine and xylazine. With a 1.5T scanner and a surface array coil, coronary DCE-MRI was performed using a True-FISP-IR sequence. (TR=170ms; TE=1.2ms; TI= 260ms; slice thickness=9mm; FOV=108x144 mm; matrix=96x128)160 ECG-gated consecutive measurements of the same mid-ventricular axial slice were acquired during a bolus of Gd-DTPA. (0.02 mmol/kg, injection flow rate=0.5 ml/sec) Semiautomated image registration and segmentation programs were created to readout signal to time curve from myocardium and the cavity of left ventricle. A constrained deconvolutional

approach was used to extract indices of permeability-surface area product (Ktrans) and regional tissue perfusion (f) under the assumption of a general pharmacokinetic model for Gd-DTPA:

$$Myo(t) = \int_{-\infty}^{\infty} LVC(t)MTF(t-x)dx$$

TF(t) = Ee-EFr / Ve(t-Tc), where E = 1-e-ktrans / Fr(1-rHct)

[Myo(t): myocardial enhancement function; LVC(t) left ventricle cavity enhanced function; TF(t): transfer function; Fr: blood flow, Ve: vascular fraction, Tc: mean transit time; Ktrans: permeability surface area product; rHct: capillary hematocrit]

Pathological Analysis

Snap-frozen samples were embedded for cryosection and processed for immunofluorescence labeling. Images were acquired with laser scanning confocal microscopy. To examine microvessel density, Bandeiraea simplicifolia isolectinB4 and anti-smooth muscle a-actin monoclonal antibody were used. Only cross-sectioned microvessels were counted. Anti-VEGF monoclonal antibody was used to reveal spatial pattern of VEGF expression.

【Results】

Atherogenic diet resulted in significantly increased body weight and serum level of lipids, as shown in figure 1.

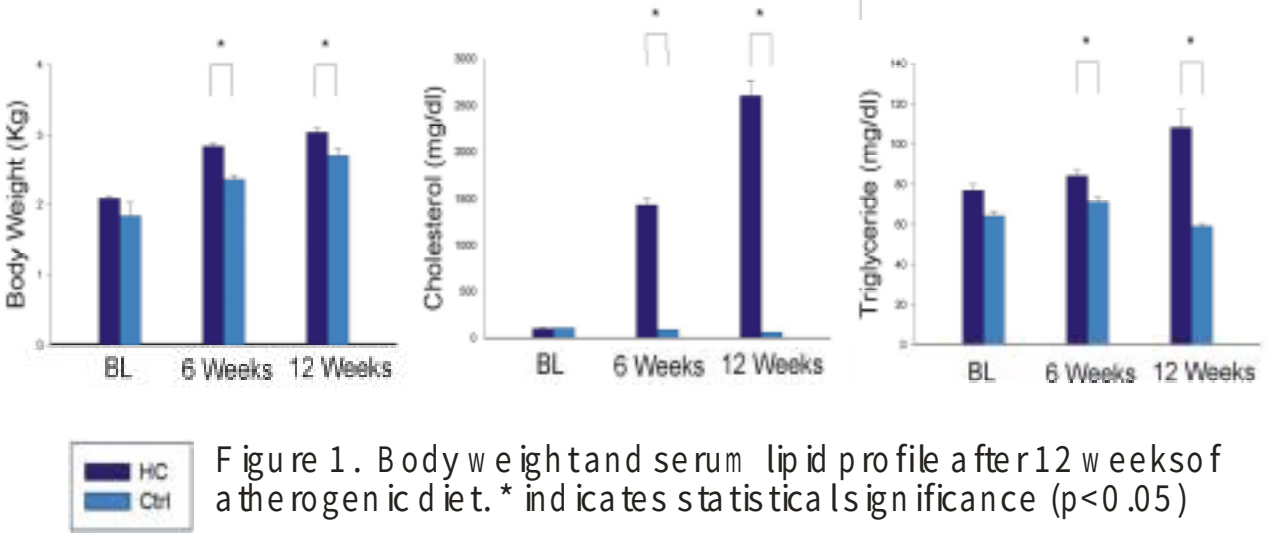

Figure 1. Body weight and serum lipid profile after 12 weeks of atherogenic diet. * indicates statistical significance ($p<0.05$)

From coronary DCE-MRI, diet-induced hypercholesterolemia for 6 weeks was associated with an increase in Ktrans but not in f compared with the controls. (Figure 2) Feeding with cholesterol enriched diet up to 12 weeks would not further influence both Ktrans and f despite a continual rise of serum cholesterol concentration. In addition, in control group we noted a decrease in Ktrans with time from the age of 8 weeks old to the age of 14 weeks old, which was less pronounced in the HC group.

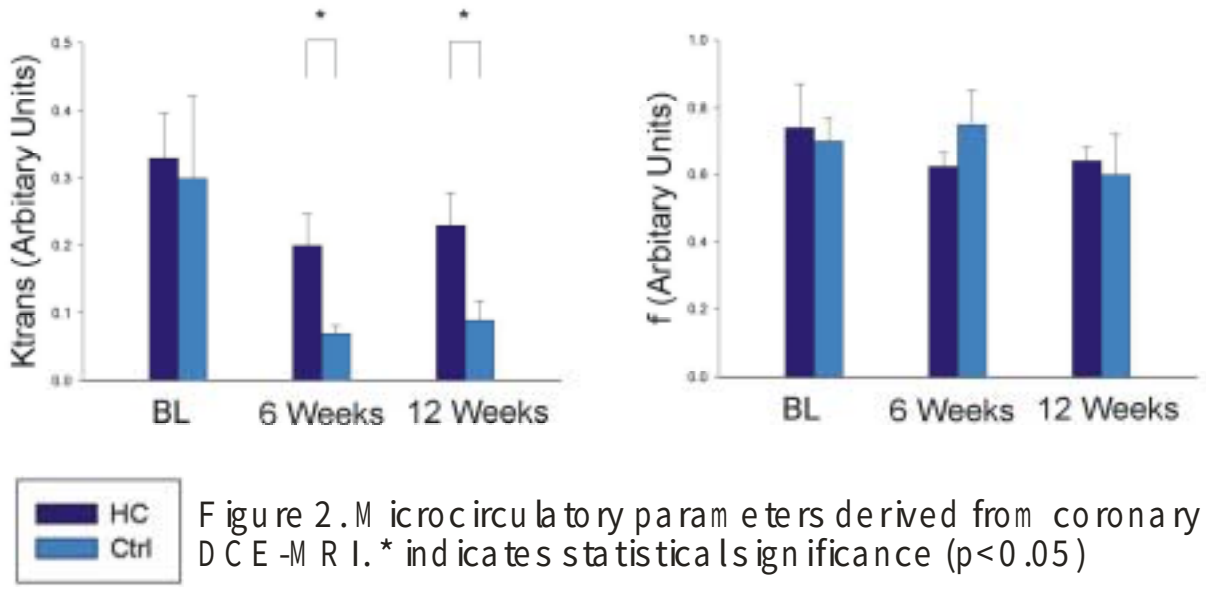

Figure 2. Microcirculatory parameters derived from coronary DCE-MRI. * indicates statistical significance ($p<0.05$)

Hearts from the HC group showed greater density of isolectin- and smooth muscle α-actin bound microvessels as shown in Figure3a (HC vs Ctrl, lectin: 24.0+5.0 vs 18.0+4.0; a-actin: 0.98+0.27 vs 0.63+0.23 per 100 square mm, $p<0.001$) Since VEGF is an important trigger for neovascularization, we examined spatial distribution of VEGF expression. Patchy expression of VEGF in perivascular and interstitial space of myocardium was noted (Figure 3b).

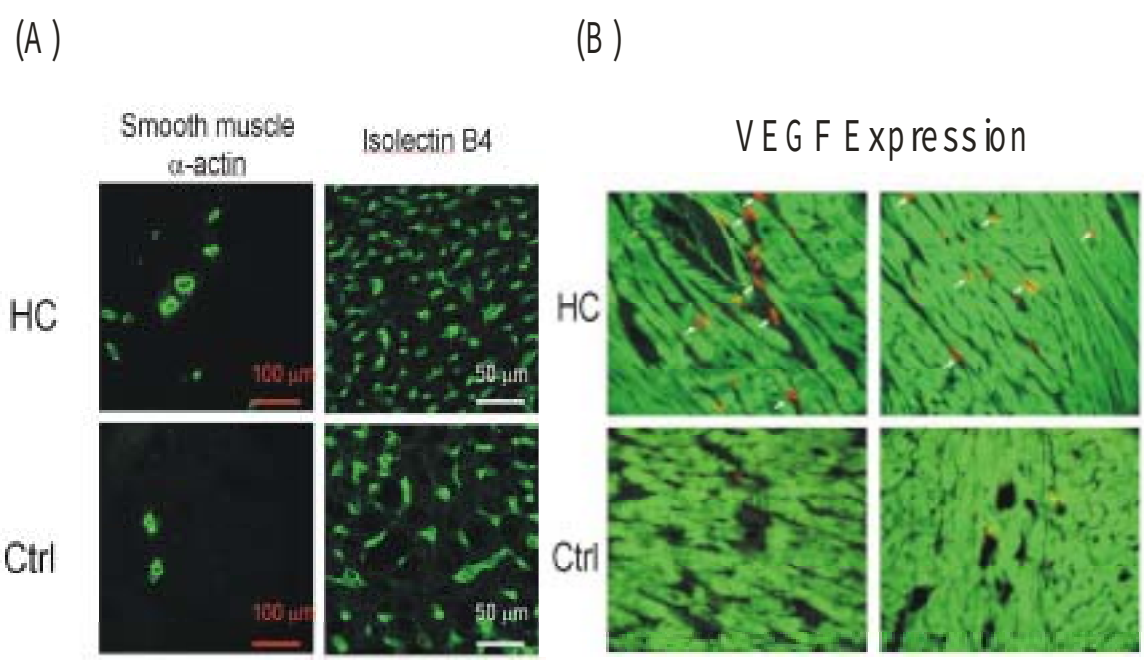

Figure 3. Comparison of microvessel density (A), and VEGF expression pattern (B) between hearts from HC and Ctrl rabbits. Increased microvessels, pericyte-bound microvessels, and VEGF expression could be seen in the HC group. Representative figure from 5 rabbits in each group.

【Discussion】

Hypercholesterolemia was associated with increased coronary leakage without evident perfusion defect. Increased microvessel density, either pericyte-bound or not, suggested microvascular remodeling. VEGF could be induced in the setting and served as a possible underlying mechanism. However, the trigger for VEGF expression remained to be elucidated. With DCE-MRI, longitudinal aspects of coronary microvascular remodeling could be unraveled, and we could have further insights into myocardial adaptation to hypercholesterolemia.