行政院國家科學委員會補助專題研究計畫成果報告

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※ **飲食治療對高膽固醇血症誘發之血管內皮細胞通透性變化之影響** ※
※ The Effect of Diet Therapy on the Hypercholestrolemia-induced Vascular Permeability Change ※
※ in Rabbit　Iris ※
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中文摘要

關鍵詞：血管內皮通透性，高膽固醇血症，飲食治療，螢光光度測定儀，眼虹彩。

近年來，血管內皮細胞功能失全，由於會導致多種疾病而引起廣泛的注意。過去的研究顯示血管內皮乃動脈管腔中物質通過或沉積到血管壁上的一個屏障，動脈血管壁通透性的增加會導致或加速動脈硬化瘢的形成。在心血管系疾病的危險因子中，高膽固醇血症可說是眾所皆知與動脈粥狀硬化性疾病相當有關的重要危險因子。已有許多研究顯示高膽固醇血症會促使血管內皮功能失全。過去也已有大型的研究顯示要治療或預防動脈硬化性心血管疾病時應包含一完善的飲食治療，且單單飲食治療便可確實降低此疾病的罹病率及死亡率。然而，由於方法之缺乏，至今仍鮮少有研究探討飲食治療是否可以改變血管通透性，其有關之功能及病理組織形態學變化之詳細序列研究亦付闕如。

在這一年的研究計劃中，我們使用序列的眼部前房螢光光度計測量法來探討飲食治療對以高膽固醇血症所誘發之血管內皮通透性變化之影響。我們將對 60 隻紐西蘭白兔，餵食 12 週的高膽固醇飲食(0.5% cholesterol-enriched diet)誘發高膽固醇血症後，改以一般正常飲食餵食 80 週，來降低其血中膽固醇值；同時我們亦以一般兔飼料來餵食另 60 隻的紐西蘭白兔共 92 週 (對照組)。這兩組兔子我們均在基礎時及其後每兩週測量其眼部的血水屏障功能變化、血中膽固醇及三酸甘油酯濃度、及眼虹彩部粥狀硬化瘢之出現及消失狀況。我們比較血中脂質濃度、血管通透度及虹彩部粥狀硬化瘢之序列變化，並找出其在飲食治療後的變化排序及其相關性。我們發現，血管通透性在粥狀硬化的相當早期便已發生且可為飲食治療來改善。形態檢查發現此功能變化與細胞間隙的改變有關。

Abstract

To study the effect of diet therapy on the vascular permeability, serial anterior chamber fluorophotometric examinations were carried out on 60 controls (group I) and 60 diet-induced hypercholesterolemic (group II) rabbits. Functional and morphopathological changes of the blood-aqueous barrier associated with total serum cholesterol (CHO) levels were studied during the 12 weeks of standard (group I) or 0.5% cholesterol-enriched diet (group II) feeding followed by 80 weeks of standard rabbit chow feeding schedule.

The blood-aqueous barrier (F60 value) in group II was more permeable than that in group I since the second week (group I: 491.0$\pm$35.9 ng/ml, group II: 868.4$\pm$85.7 ng/ml, respectively, $p<0.0001$). The F60 escalated further up to the 22nd week; thereafter it declined slowly to its baseline at 32nd week of the experiment. The rise and decrease of F60 was relatively parallel to, however, lagged behind the change of CHO level. In group I, most of the interendothelial junctions of the iridic microvasculature were interdigitating type. In contrast, many of the interendothelial junctions became not only the overlap type but also the end-to-end typed or even open junction after 12 weeks of lipid-enriched diet feeding in group II. These endothelial changes in the hyperlipidemic animals gradually recovered while the CHO level decreased after the diet therapy.

In conclusion, the vascular permeability change occurred in the very early stage of atherosclerosis and could be reversed by the diet therapy. This functional and morphological study clarified how hypercholesterolemia increased the vascular permeability.

Key words: Vascular permeability, hypercholesterolemia, diet, interendothelial junction.

A cardinal characteristic of the development of atherosclerosis is the accumulation of LDL within the arterial wall.[i, ii, iii, iv, v, vi, vii] Changes in arterial permeability are important in the initiation and development of atherosclerosis. The vascular endothelium forms a highly selective permeability barrier.[viii] Recently, the endothelium is of great interest because its damage leads to a variety of diseases.[ix] Normally vascular endothelial cells attach to each other by tight, gap and adherence junctions, which may serve as potential sites of increased endothelial transport, particularly when they have been injured in diseases such as atherosclerosis or hypertension.[x, xi,xii,xiii]

Many recent studies have shown an improvement of arteriolar vasodilatory function after aggressive lipid-lowering therapy.[xiv, xv] It has been shown that a comprehensive strategy to decrease cardiovascular morbidity and mortality should include primarily a cardioprotective diet.[xvi] In spite that dietary therapy is always tried first in the management of dyslipidemia, there are, however, few studies showing that diet therapy could reverse the vascular permeability change,[x] and the functional and morphological changes have never been studied simultaneously and sequentially in detail because of the methodological limitation.[xvii] Change of the iridic vessels in the eye is thus a good indicator of the general status of vascular system. In our previous study, using the noninvasive anterior segment fluorophotometry, we could evaluate the vascular endothelial integrity in vivo chronologically.[xi] In this study, taking the advantage of not needing to sacrifice the animals, we used fluorophotometry chronologically to detect the effect of diet therapy on the hypercholestrolemia-induced vascular permeability change.

Materials and Methods

Animals, diets and examination schedules A total of 120 male New Zealand white rabbit, aged about 8 weeks, were randomly divided into 2 groups. Sixty animals were fed standard rabbit chow (Purina 5321, St. Louis, MO, U.S.A.) for 92 weeks and served as controls (group I). Another 60 rabbits (group II) were fed the same diet enriched with 0.5% cholesterol (Wako Co., Japan) and coconut oil (Yeali Co., Taiwan) for 12 weeks. The lipids composed 40% of the total energy source of the diet. Afterwards, the diet for group II rabbits was shifted to standard chow for another 80 weeks. Both the salt and vitamin mix were American Institute of Nutrition Standards. All animals were allowed food and water ad libitum during the experiment except an overnight of fasting before blood sampling. Six animals in each group were sacrificed at 4, 8, 12, 16, 20, 24, 36, 48, 60 and the end of 92-week's experiment period, respectively, for the histopathological studies.

Biochemical measurements Blood was sampled at the time of fluorophotometric examination. Serum total cholesterol (CHO) and triglyceride (TG) levels were determined by automated enzymatic methods (Merk: 14366 and 14354, respectively).[xviii,xix]

Permeability Examination Anterior chamber fluorophotometry was done at the beginning, every 2 weeks up to the 24th week, and then every 4 weeks up to the end of the feeding schedule. The anterior chamber fluorophotometry was performed as previously descibed.[xi] Briefly, the rabbits were anaesthetized with an intramuscular injection of 2:3 mixtures of xylazine (2%, Bayer, Leverkusen, Germany) and ketamine (50mg/ml,

Parke-Davis Co., R.O.C.). Fluorophotometry was performed using the Fluorotron Master II (Coherent Co.) fitted with an optical anterior segment adaptor. After measurement of the lens and corneal autofluorescence, each rabbit received an intravenous injection of 10% fluorescein sodium (15 mg/kg of body weight). Anterior chamber fluorescein concentration 60 minutes after intravenous injection of sodium fluorescein was measured. The mean values of the anterior chamber fluorescence along the visual axis over a 2.0 mm band positioned in the anterior chamber were averaged (F60). We used the F60 to represent the status of the blood-aqueous barrier. All fluorophotometric results were expressed as total fluorescence in terms of equivalent concentrations of fluorescein sodium.

Morphology and Pathology At the time of fluorophotometric examination, two independent observers examined both eyes of each animal for the presence of iris atheroma, with and without slit-lamp biomicroscope. Initially the atheroma plaque appeared as yellowish streak-like deposition on the iris while it protruded into the posterior chamber from the posterior aspect of the iris at advanced stage. The iridic atheromatous plaques were scored as follows: 0: no evidence of atheromatous plaque even with slit-lamp biomicroscope; 1: streak-like deposition detected under slit-lamp biomicroscope but not detectable by naked eyes; 2: iridic plaque visible by both slit-lamp biomicroscope and naked eyes. Serial external eye photographs were taken for future reference before each fluorophotometric examination. The time when first evidence of the iridic deposition appeared and disappeared, both with and without biomicroscopic aid, were recorded. All animals were sacrificed by an overdose of intravenous pentobarbital at the above mentioned time points. Both eyes of each animal were enucleated immediately. A small piece of the iris was sampled and processed for transmission electron microscope (EM).

Data analysis All the values were expressed as mean ± SEM. The data in both eyes of each animal were averaged and analyzed to avoid over-estimation. The levels of CHO, TG, F60 and iridic atheromatous plaque (IAP) score in each group were averaged at different interval. Chronological changes in the CHO, TG, F60 and IAP scores were examined by two-way analysis of variance (ANOVA) for groups I and II, respectively. Under biomicroscopic examination, the time when IAP first became detectable or totally disappeared was averaged. The difference between these two groups at various experimental time-point was examined by Student's *t* test. A p value of less than 0.05 was considered statistically significant.

Results

Biochemistry The chronological changes of CHO and TG were summarized in Fig. 1. In group I, there was a slight decrease in the CHO level in the first 4 weeks and remained rather steady thereafter. However, in group II the CHO level increased rapidly above its baseline measurement after two weeks of cholesterol-enriched diet feeding. This increase reached a peak level at the 14th week. After shifted to normal diet feeding, the CHO level in group II declined slowly to its baseline level at the 32nd week of the experiment, i.e. 20 weeks after normal diet feeding schedule. The TG level decreased progressively in group I during the experiment. In contrast, it increased significantly above baseline level after 8 weeks of cholesterol-enriched diet feeding in group II. The TG level decreased more rapidly, however, to its baseline level at the 20th week of the experiment, i.e. 8 weeks

after shifted back to normal diet feeding.

Permeability In group I, there was a slight decrease in F60 in the first 10 weeks and remained rather steady after the 12th week of the experiment. In group II, the initial decrease in F60 was not seen. The blood-aqueous barrier in group II was more permeable than that in group I since the second week (group I: 491.0±35.9 ng/ml, group II: 868.4±85.7 ng/ml, respectively, $p<0.0001$) and increased significantly above its baseline level after 8 weeks' cholesterol-enriched diet feeding. The F60 escalated further up to the 22nd week, 10 weeks after the diet was changed to standard rabbit chow. Thereafter, it declined slowly to its baseline at the 32nd week of the experiment. The rise and decrease of F60 was relatively parallel to, however, lagged behind the change of CHO level. The difference in F60 between groups I and II remained significant up to the 48th week of experiment, i.e., 36 weeks after shifted to standard diet feeding.

Iris Atheroma With and without the aid of slit-lamp biomicroscope, there was no iridic streak deposition detectable in group I throughout the experiment. However, in group II the first trace of iridic streak appeared in 7.2 ±0.5 weeks following cholesterol-enriched diet feeding. It increased most significantly between the sixth and tenth week. These iridic atheromatous plaques regressed to totally invisible by slit-lamp in 62.3 ±2.8 weeks after switching to standard rabbit chow feeding. The morphologic change in iridic atheroma was much slower compared to the changes in the CHO, TG, and vascular permeability.

Ultrastructural examination In group I, most of the interendothelial junction of the iridic microvasculature was interdigitating type. However, the intracytoplasmic vacuolation increased significantly in the endothelium after 4-8 weeks of lipid-enriched diet feeding in group II. During that period, the intercellular junctions started to become the overlap type. After 12 weeks of lipid-enriched diet, many of the interendothelial junctions became not only the overlap type but also the end-to-end typed or even open junction. These endothelial changes in the hyperlipidemic animals gradually recovered while the CHO level was decreased following diet therapy. The simple or open junctions could be hardly found at the 36th week, i.e. 24 weeks after normal diet therapy. Most of the interendothelial junctions turned back to be the interdigitating type at the 48th week although there were still abundant extravascular lipid droplets and cholesterol clefts in the iridic matrix.

Discussion

In this study, we reconfirmed that hypercholesterolemia could induce the vascular permeability alteration during the very early stages of atherosclerosis. After diet control, the vascular permeability could be reversed slowly back to its baseline level. Although the initial increase and subsequent decrease of vascular permeability lagged behind the change of CHO level, it paralleled with the CHO alteration and always preceded the iridic macroscopic change.

In the study of Roscoe and Vogel,[xvii] in which rabbits were fed 1% cholesterol, there was a linear increase in the total iridic cholesterol during the first 2 months of cholesterol feeding. Nevertheless, the biggest increase in total iridic cholesterol was in the third month. In this study, while the serum total cholesterol reached a peak in the

12th to 14th week, the F60 level peaked in 20th to 22nd weeks, i.e. 8 to 10 weeks after the diet was changed to standard rabbit chow and when the serum cholesterol has become much lower than the peak level. These results demonstrated that the vascular permeability change was relatively parallel to, however, lagged behind the change of CHO level. We believed that the increased vascular permeability was not simply due to the increased concentration of serum cholesterol. It was the high-fat, high-cholesterol diet that caused endothelial injury and altered the endothelial barrier.

Although the F60 change also correlated well with the IAP score in this study, the alteration in F60 usually preceded the visual appearance and disappearance of iridic deposits. While the F60 in group II was higher than that in group I since the second week, the first trace of the iridic streak deposition appeared in 7.2 ± 0.5 weeks. The iridic atheromatous plaques regressed to totally invisible by slit-lamp in 62.3 ± 2.8 weeks after switching to standard rabbit chow feeding, although the CHO and F60 level had decreased to normal level in 20 weeks after shifted back to the standard rabbit chow. We postulate that alterations in the permeability of local vasculature led to changes in the iridic deposits,[xx,xxi] which led to the development of sudanophilic substance accumulation and foam cells formation.[xxii] While this functional change could be quickly induced and reversed by diet manipulation, the atheromatous plaque formation needed some time to develop when the endothelial barrier had been damaged, and even a much longer time to regress after the vascular dysfunction had been reversed.[xx,xxi,xxii]

Under electromicroscopic examination, we have shown that the endothelial intracytoplasmic vacuolation increased significantly and the intercellular junction changed from the interdigitating type in the normal rabbits to the overlap type, end-to-end type or even open junction in hypercholesterolemic rabbits. These endothelial changes in the hyperlipidemic animals gradually recovered while the CHO level was decreased by the diet therapy although abundant extravascular lipid droplets and cholesterol clefts would persist much longer in the iridic matrix. These pathological changes of the iridic stroma and microvasculature could explain well the sequential functional change of the vascular permeability.

In summary, using the anterior segment fluorophotometry, we could evaluate the vascular endothelial integrity in vivo. The vascular permeability change occurred in the very early stage of atherosclerosis and could also be reversed by the diet therapy. Hypercholesterolemia would make the interdigitating-typed interendothelial junctions progressively replaced by the overlap, simple end-to-end or even open junctions, which were more permeable for the particles. This functional and morphological study clarified how hypercholesterolemia increased the vascular permeability.

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