HDL Inhibited Atherosclerosis Induced by Radiation Injury

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Research

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Abstract

Background-HDL inhibits atherosclerosis development from radiation damage, nonetheless, the underlying mechanism is yet to be defined.

Methods-This study used radiation patients along with cultured mouse aortic endothelial cells (MAECs) to investigate the process. Firstly, 158 patients from the oncology department of Jingzhou hospital who received radiation after neck cancers participated, and their arterial function was monitored by B ultrasound. Similarly, HDL and other blood lipid indexes were also tested. Then, MAECs were isolated and cultured and passed through MTT assay to test the HDL protective role on UVB radiation along with western blotting to test some apoptosis protein expression and possible molecules.

ResultsFirstly, those patients with high HDL levels were less likely to develop atherosclerosis, with statistical differences. We observed that MAECs treated with UVB were damaged significantly however HDL reversed the cell damage in a dose-dependent manner. In the meantime, the apoptosis process was assessed and found that HDL inhibited the apoptosis caused by UVB. Western blotting results showed that HDL enhanced phosphatidylinositol 3-kinase (PI3K) in addition to Akt phosphorylation in MAECs.

Conclusion-These results suggest that HDL protected UVB-mediated apoptosis by activation of a mechanism involving PI3K/Akt signaling pathway.

Introduction

Atherosclerosis is a disorder that affects large arteries and has been the chief cause of heart diseases [1]. It can result in hardening of an arterial wall along with further narrowing the lumen and loss of elasticity of the middle membrane thereby paving the way for serious complications which may include ischemic heart disease (IHD) such as that of myocardial infarction (MI) as well as stroke that may encompass (cerebral thrombosis and cerebral hemorrhage) in addition to gangrene of limbs. There are many factors regarded as responsible for both occurrence and development of atherosclerosis. However, hyperlipidemia is known to be the chief risk factor of atherosclerosis [2]. The incidence of atherosclerosis occurs earlier in individuals who have hypertension compared to the patients of the same age and same sex who have no hypertension. Furthermore, smoking remains one of the risk factors of atherosclerosis and has been the principal independent risk factor for coronary heart diseases. Research data reveals that heavy smoking can damage vascular endothelial cells in addition to a surge in the level of carboxyhemoglobin [3]. The two important complications of atherosclerosis are diabetes and hyperinsulinemia and they are related to secondary hyperlipidemia. When the level of insulin in the blood is elevated, the content of HDL is lowered which leads to higher morbidity and mortality of coronary heart diseases [4]. Another strong independent risk factor for atherosclerosis is family history with genetic factors. The patients who carried familial hypercholesterolemia and familial lipoprotein lipase deficiency had a relatively higher incidence of atherosclerosis when compared with the control group [5, 6]. According to the age-related pathological data, atherosclerosis is a gradually developing process from

infancy. The rate of detection, as well as the severity of atherosclerosis, are augmented with age owing to age-related changes in the arterial wall. Before menopause, the rate of incidence of coronary atherosclerosis remained lower in women than men of the same age because women had a higher level of HDL and lower level of LDL as compared with men. This difference between the two sexes vanished after menopause which may be ascribed to the effect of estrogen [7]. Whether natural or medical radiation, when exposed can damage the body and may cause atherosclerosis. Similarly, radiations at high altitude areas are responsible for causing atherosclerosis [8]. The exposure to such radiation aggravates the risk of coronary artery disease (CAD) along with atherosclerosis and is dependent upon the dose of radiation [9]. Therefore, it is to assess the risk of atherosclerosis caused by coronary angiography as well as the decisions made by patients [10]. Moreover, patients with esophageal cancer who were treated with radiations had a higher risk of heart complications which largely encompass pericardial disease along with coronary artery atherosclerosis in addition to valvular heart disease, cardiomyopathy, as well as arrhythmia [11]. So, it was an urgent need of time to know how to avoid the risk of atherosclerosis in imaging examination or radiotherapy.

Although HDL is recognized to prevent or alleviate atherosclerosis through many pathways [12, 13]. However, the role and mechanism of HDL in atherosclerosis instigated by radiation has not been identified yet. The focus of this study is to first investigate the relationship between the ratio of atherosclerosis in radiation patients and the plasma lipid level and then to study the role of HDL and its possible mechanisms by using MAECs treated by radiation.

Material And Methods

2.1. Patients

158 patients from the oncology department of Jingzhou hospital who received radiation after neck cancers participated and their arterial function was monitored by B ultrasound. Similarly, HDL and other blood lipid indexes were also tested. All patients provided written informed consent.

2.2. Materials

C57BL/6 J mice were used for MAECs isolation. DMEM medium was obtained from Gibco Company. The Institutional Animal Care and Use Committee approved the experiments involved the use of animals. The Ethics Committee of Soochow University approved the present study protocol (clearance No: 2019854268).

2.3. Cell culture

C57BL/6 J mice were employed for isolating MAECs by applying an outgrowth technique which has been described earlier [14, 15]. DMEM medium was employed which contained 10% calf serum for maintaining MAECs by providing a temperature of 37 °C within a humidified incubator provided with 5 percent CO₂. Serum-free DMEM medium was used for performing overnight (12–16 hr) serum starvation of MAECs cells after they attained their growth to that of near-confluence for all the experiments.

2.4. Preparation of HDL

Following the procedure, HDL was prepared [16]. Briefly, Human serum obtained from Jingzhou central hospital (Jingzhou, Hubei) was taken to overlay with potassium bromide (KBr) gradient solution that possessed a density of 1.063 g/mL. After that, these samples were passed through ultracentrifugation for removing low as well as very low-density lipoproteins at 35,000 rpm for a period of 18hr. The adjustment of infranatant was made to 1.21 g/mL along with solid KBr and blended with that of 1.21 g/ml buffered KBr solution, which was then passed through the process of centrifugation for a span of 48 hr at 48,000 rpm. After collecting HDL, it was dialyzed three times for 48 hr at a temperature of 4 °C against phosphate buffer saline (PBS) containing 1 mM EDTA. Finally, it was passed through PBS for dialysis with no EDTA for a period of 8 hr and then filtered with the help of a 0.22-mm filter.

2.5. MTT assay

MTT assay was performed for measuring cell growth. A 96-well plate was employed for sowing cells at a density of 5×10^3 cells/well which were cultured for specific time intervals. Besides that, a fresh cell culture medium that contained 0.5 mg/mL MTT for 4 hr replaced the medium at each time interval. Then we added 150 μ L DMSO to each well and shook on the low-speed rotation for 10 min until the crystal was fully dissolved, then measurement at 490 nm was performed by employing a Multiskan MS ELSA reader (xMark Microplate Absorbance Spectrophotometer, BioRad, CA, USA). For normalizing the relative cell number, the absorbance from control cells was used.

2.6. SDS-PAGE and Western blotting

Western blot assay was performed over total protein by adopting the standard western blotting protocol (Molecular Clone, Edition II). The concentrations that were employed for primary antibodies: PI3K (1:1500, sc-374534, Santa Cruz, USA), p-ERK (1:500, sc-7383, Santa Cruz, USA), ERK (1:500, sc-94, Santa Cruz, USA), p-AKT (1:500, sc-7985R, Santa Cruz, USA), AKT (1:500, sc-8312, Santa Cruz, USA), cleaved caspase 3 (1:500, CST 9579, Boston, USA), ATF-4 (1:500, abcam, ab-25331, London, England) and GAPDH (1:1000, Santa sc-575, Cruz, USA). Furthermore, we had to apply secondary antibodies that were already labeled with respective horseradish peroxidase (HRP) which was followed by performing enhanced chemiluminescence (ECL) detection while adopting the company's instructions (Pierce, Rockford, IL, USA). For the analysis of the integrated density mean grey value of the band, the ImageJ software was employed, and calculation was performed for the corresponding relative expression ratio.

2.7. Statistical analysis

Data were expressed as means $\pm$ SE. The two-tailed Student's t-test (for 2 groups) was used to assess the differences of means among multiple groups in addition to the analysis of variance (ANOVA, for > 2 groups). $P \leq 0.05$ was taken as statistically significant for all analyses.

Results

3.1. HDL level related to the ratio of atherosclerosis

We discovered that 118 patients (74.7%) of the total 158 radiation patients had atherosclerosis with different degrees. Moreover, the incidence rate of atherosclerosis did not have a significant association with sex but with age. Similarly, the results of the plasma lipid test revealed that patients with no atherosclerosis had a higher level of HDL than those with atherosclerosis signifying that a high level of HDL may reduce the probability of atherosclerosis development with statistical differences (Table 1).

Table 1
Relationship between incidence of atherosclerosis and blood lipid

Parameters	AS	Non-AS	X2	<i>P value</i>
Sex (Female/male)	39/79	15/25	0.102	0.749
Age (≤ 50 / >50)	30/88	26/14	18.756	< 0.001
Total cholesterol	7.38 ± 0.82	4.25 ± 0.74	21.364	< 0.001
Triglyceride	3.85 ± 0.68	2.46 ± 0.65	11.295	< 0.001
LDL	3.57 ± 0.54	2.83 ± 0.48	7.695	< 0.001
HDL	1.35 ± 0.43	1.97 ± 0.57	7.226	< 0.001

3.2. HDL reversed radiation-cell damage

The MAECs were first irradiated with $90\mu\text{w}/\text{cm}^2$ ultraviolet rays for 10 minutes after which they were either treated with 50, 100, 200 $\mu\text{g}/\text{mL}$ HDL or left untreated thereby MTT results demonstrated that UVB caused about 52% MAECs damage however HDL reversed the phenomenon, therefore this indicated that HDL protected MAECs damage caused by UVB, Fig. 1.

3.3. HDL protected UVB-induced apoptosis

In comparison to the control group or HDL group, that of cleaved caspase 3 expression in the UVB group was increased. However, in the UVB group that was treated by 200 $\mu\text{g}/\text{mL}$ HDL, the cleaved caspase 3 expression was lowered, this indicated that HDL alleviated UVB-induced apoptosis. ATF-4 as apoptosis protein was related to apoptosis induced by endoplasmic reticulum stress. So, ATF-4 expression was elevated by UVB while it was reversed by HDL during this study, therefore, indicating that HDL may protect MAECs by inhibiting endoplasmic reticulum stress apoptosis (Fig. 2).

3.4. HDL inhibited radiation-induced atherosclerosis by PI3K/AKT signal pathway

To further explore the possible mechanism, this experiment was designed to study the influence of HDL over PI3K as well as Akt expression besides phosphorylation. Similarly, Fig. 3 shows that the total

amount of PI3K, as well as Akt proteins, was lowered by UVB while the total amount of these proteins was not altered by HDL treatment rather it enhanced their phosphorylation. However, PI3K and Akt are inhibited by LY294002. It is suggested by these results that HDL promotes cholesterol transport in MAECs via a mechanism in which the PI3K-Akt pathway is triggered, Fig. 3.

Discussion

Atherosclerosis causes many clinical problems such as angina, myocardial infarction, stroke, hemiplegia, aphasia, renal artery stenosis, or lower extremity artery stenosis. These are quite serious complications and can be fatal. There are many factors responsible for causing atherosclerosis and among them, radiation may be a potential factor because patients with tumors who were treated with radiation have also been diagnosed with atherosclerosis [17, 18]. HDL was considered to protect against atherosclerosis, however, whether it can protect against radiation-induced atherosclerosis was not fully known. Therefore, our study revealed that radiation can inflict heavy damage upon cells however such damage can be reversed when these cells are treated with HDL. Nonetheless, some studies have suggested that moderate ultraviolet radiation has the potential to prevent cardiovascular disease [19]. Notwithstanding ionizing radiation even in low doses will aggravate the risk of cardiovascular disease, primarily causing endothelial cell damage which leads to the process of atherosclerosis [20, 21]. HDL reduces the deposition of cholesterol into tissues thereby playing an anti-atherosclerotic role by preventing the occurrence and development of atherosclerosis [22]. Our clinical studies demonstrated that radiation exposure remained a high-risk factor for the occurrence of atherosclerosis however HDL could effectively reduce such risk. Radiation can induce apoptosis via oxidative stress as the apoptosis of MAECs during the cell experiment with irradiation was increased and HDL may reduce the apoptosis [23]. However, our study exhibited that HDL inhibited the apoptosis of the endothelial cell [24–26]. Although some studies have found that UVB radiation resulted in endothelial cells apoptosis primarily by inhibiting PI3K/AKT signal pathway [27, 28], and HDL can protect endothelial cells through PI3K/AKT signal pathway [29], but it remained unclear whether HDL can protect endothelial cells from apoptosis caused by UVB radiation. In this study, HDL augmented PI3K/AKT expression in the UVB-treated group, but could not eliminate PI3K/AKT inhibitor LY294002 effect, therefore this was consistent with the result that PI3K/Akt pathway is not the only driver in HDL-mediated cell protection [30]. Therefore, with further experimental confirmation, there is a high chance to translate the current result for clinical use to prevent radiotherapy-induced atherosclerosis.

Study strength and limitations

This study focused on the HDL protection of radiation -induced AS, support some evidence that how to avoid or alleviate the side effects for radiation therapy of some cancer patients, also give some help for workers from radiology department to lower the incidence of AS. But, this need some more animals model test to clarify the role of HDL and cooperate with nutritionists to give some guidelines on radiation protection.

Conclusion

AS caused by radiation get more and more attention of radiation workers or radiation therapy patients, HDL as a potential drug has important role to anti AS. This study firstly demonstrated that HDL protected UVB-mediated AS by activation of a mechanism involving PI3K/Akt signaling pathway.

Abbreviations

HDL: high-density lipoprotein; MAECs: mouse aortic endothelial cells; AS: atherosclerosis;

PI3K: phosphatidylinositol 3-kinase; MI: myocardial infarction; CAD: coronary artery disease; ATF4: activating transcription factor 4

Declarations

Ethics approval and consent to participate

The Ethics Committee of Soochow University approved our study. Similarly, the Institutional Animal Care and Use Committee of the Soochow University accorded the approval for animal experiments. Furthermore, the institutional guidelines for the care and use of laboratory animals were complied with while performing all animal experiments in addition to the employment of laboratory animals and that animals were anesthetized and killed using acceptable techniques.

Consent for publication

Not applicable.

Availability of data and materials

The data will be available on request.

Conflict of interest

None declared.

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Authors' contribution

HJ designed and supervised the study. XJ, and ZK processed the study. XJ, ZK, and HJ wrote the manuscript. WQY, ZP and PLH contributed to tables and figures. HJ revised the manuscript. HJ acquired

funding.

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Figures

Figure 1

Effects of HDL on MAECs cell viability. Results were confirmed in three independent experiments.

*compared with control group, $P < 0.05$; #compared with UVB group, $P < 0.05$.

Figure 2

HDL inhibited MAECs apoptosis caused by UVB. *compared with control group, $P < 0.05$; #compared with UVB group, $P < 0.05$.

Figure 3

HDL inhibited MAECs apoptosis mainly through activation of PI3K/Akt pathway.