

Original Research Article

Comparing efficacy and safety of plasmapheresis versus atorvastatin in pathological progression of atherosclerosis in a rodent model

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Abstract

Purpose: To evaluate the effect of plasmapheresis versus atorvastatin in pathological progression of atherosclerosis in a rodent model.

Method: A total of 90 male adult rats of up to 300 g were randomly distributed in three groups ($n = 30$): group 1 (plasmapheresis up to 1.5 ml daily); group 2 (atorvastatin 0.1 mg/kg per day), and group 3 (hypercholesteremic rats). The following variables were assessed for 24 weeks: plasma and hepatic lipid and anti-oxidant profiles; atherosclerotic abrasions/lesions; coronary atherosclerosis/coronary stenosis score (CSS), composition of atherosclerotic lesions, incidence of xanthoma, arch and thoracic surface involvement including arch and thoracic area occupied by lesion; and thoracic aorta (I/M) ratio.

Results: Compared to rats administered with atorvastatin, the rats treated with plasmapheresis had significantly greater improvement in levels of triglycerides (132 vs 124 mg/dl, $p < 0.05$), total cholesterol (201 vs 189 mg/dl, $p < 0.05$), low-density lipoproteins (134 vs 123 mg/dl, $p < 0.05$), very-low-density lipoprotein (11 vs 9 mg/dl, $p < 0.05$) and high-density lipoprotein (36 vs 39 mg/dl, $p < 0.05$) levels. Plasmapheresis after 24 weeks of treatment improve CSS in all coronary arteries than atorvastatin (22 vs 24 respectively; $p < 0.05$). Furthermore, lesioned composition, I/M ratio and xanthoma incidence were significantly lower in plasmapheresis group than in atorvastatin group ($p < 0.05$).

Conclusion: Plasmapheresis is a better alternative than atorvastatin in preventing pathological progression of atherosclerosis.

Keywords: Atherosclerosis, Plasmapheresis, HMG-CoA reductase inhibitor, Atorvastatin, High cholesterol diet-induced atherosclerosis

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INTRODUCTION

Atherosclerosis is a one of most common metabolic condition characterized by high level of cholesterol, triglyceride, LDL, VLDL and low level of HDL, accounted for financial burden to patient and their family. In most case, the main cause of

atherosclerosis is sedentary life style [1-3]. In severe cases of atherosclerosis, increased level of LDL (oxidized form) develop lesion in intima of coronary artery, and gradually form an atheromatic plaque which causes narrowing of coronary artery, that results in angina pectoris. Untreated serious conditions may lead to

myocardial infraction which is the most common cause of death. Angina pectoris is the key characteristics of acute cardiac attack and one of the main reasons for the hospitalization [3-6].

Currently, statin group of drugs are being widely used to manage dyslipidemia and related disorders such as atherosclerosis, hypertriglyceridemia, hypercholesteremia [4-8]. The statin drug targets the rate limiting step of cholesterol synthesis by inhibiting key enzyme which is mainly involved in cholesterol synthesis. Higher levels of LDL are known to cause damage coronary artery by blocking adequate blood supply to coronary tissues. Role of cholesterol in developing coronary artery disease has been well established. Thus, reducing the level of cholesterol within the normal range is advisable as recommended by treatment guidelines. Safety of drugs used to control lipid levels, especially TG and LDL are also equally important. Although statin group of drugs are the standard treatment option, however, exerting serious adverse events after long term use. The most common adverse events of statin drugs are hepatic and renal damage. Thus, there is an unmet need of better treatment alternative in preventing pathological progression of atherosclerosis.

There were few reports showing efficacy of plasmapheresis in controlling blood lipid [9-12]. However, there is no study comparing safety and effectiveness of statin drugs and lipid removal using plasmapheresis or apheresis technique established. Since there is no comparative data available on effect of plasmapheresis versus HMG-CoA reductase inhibitor in high cholesterol diet-induced atherosclerosis. Therefore, this study designed to evaluate the effect of plasmapheresis versus atorvastatin in pathological progression of atherosclerosis in a rodent model.

EXPERIMENTAL

A total of 90 male adult rats of upto 300 g (n = 30 in each group) were divided three groups: group 1 (plasmapheresis up to 1.5 mL daily); group 2 (atorvastatin 0.1 mg/kg per day), and group 3 (hypercholesteremic rats). Diet containing cholesterol (0.5 %) and taurocholate (1.0 %), carbohydrates (55 %), fat (5 %), protein (25 %), and mineral (10 %) was administered to each group to induce hypercholesteremia. All the rats were placed in a separate cage in day and night cycle (12 h duration for each), with access of water as and when required. The study was approved by animal ethic committee board of Hubei University [approval number: HU/AECB-

2020/12-8/234] and followed the local regulation in conducting this experiment.

Body weight and feed intake of each rat were recorded every 3rd day during the 24 weeks of study period. After 24 weeks of treatment, all rats were sacrificed using cervical dislocation and blood sample was collected using cardiac puncture to measure blood lipid profile using ELSIA kit. Thoracic aortae were used for histological examination using RT-PCR and ELSIA kit. Histological examination includes measurements of atherosclerotic abrasions/lesions including superoxide anion (O²⁻) and eNOS mRNA. Plasma sample were centrifuged and liver sample was also excised. Plasma sample, aortae/heart tissue and liver were frozen (-80 °C) until analysis. Also, antioxidant enzyme of cardiac tissue was assessed using ELSIA kit. Total liver cholesterol and liver triglyceride were measured. Moreover, atherosclerosis of the thoracic aorta of rats was used to assess coronary atherosclerosis/coronary stenosis and compared between both the treatment group. Furthermore, the area of lesioned components such as collagen, SMCs (smooth muscle cells), macrophage, lipid deposits for aortic and coronary lesion was compared between both the groups. Moreover, xanthoma was compared between both the groups as measured using Forelegs, incidence of Hind legs including severity of xanthoma was also assessed. Atheroma area was also assessed and compared. Also, arch and thoracic surface involvement including arch and thoracic area occupied by lesion and intima/media (I/M) ratio of arch and thoracic aorta was assessed between both the group.

Since this is pre-clinical study and designed to compare the effect of plasmapheresis versus HMG-CoA reductase inhibitor in preventing the progression of atherosclerosis in high cholesterol diet-induced atherosclerosis in rat model. Therefore, no formal calculation of sample size performed as the present study is kind of pilot investigation to know whether plasmapheresis is effective and safe in preventing the atherosclerosis and atherosclerosis induced damage to coronary tissue.

Statistical analysis

Data were analyzed using appropriate statistical tests. Numerical data with normal distribution were analyzed using unpair t test or Mann Whitney test based on the results of normality test. Categorical data were analyzed using chi-square test or fisher exact test based on the size

of data in each cell. Statistical analysis of data was performed using SPSS statistical analysis software (version 26.0).

RESULTS

Plasma lipid profile

At baseline, lipid profile (triglyceride, total cholesterol, LDL and VLDL levels) was found comparable between both the groups. Biochemical analysis of plasma samples showed significant reduction in triglycerides, cholesterol (total), LDL and VLDL levels from baseline after 24 weeks of treatment of plasmapheresis. A similar trend of decreased triglycerides, cholesterol (total), LDL and VLDL levels were observed from baseline after 24 weeks of treatment of atorvastatin. On between group comparison, it was observed that the rats treated with plasmapheresis had significantly greater reduction in levels of triglyceride, cholesterol (total), LDL and VLDL as compared to atorvastatin after 24 weeks of treatment. Plasma samples showed that significant improvements in HDL levels from baseline after 24 weeks of treatment of plasmapheresis. Similar trend of improvements in HDL levels was observed from baseline after 24 weeks of treatment of atorvastatin. On between group comparison, it was observed that the rats treated with plasmapheresis had significantly greater improvement in levels of HDL as compared to atorvastatin after 24 weeks of treatment (Figure 1).

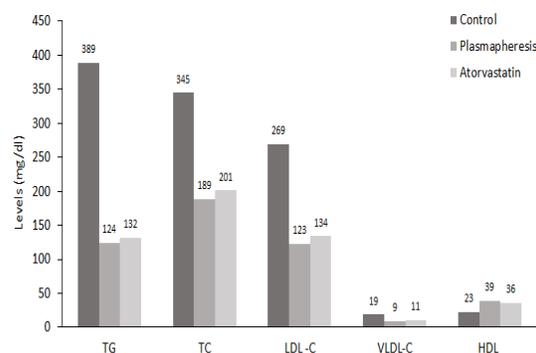


Figure 1: Plasma lipid levels in rats of all groups

Table 1: Plasma levels of antioxidant enzymes in rats

Antioxidant enzyme	Control (n=30)	Plasmapheresis (n=30)	Atorvastatin (n=30)
Catalase (nmH ₂ O ₂ decomposed (per s/g, mean, SD)	12 (7.1)	32 (4.2)*#	30 (3.3)*#
SOD (U/mg protein), mean (SD)	4.4 (1.1)	8.3 (2.1)*#	7.5 (2.2)*#
Ascorbic acid (ug/g), mean (SD)	121 (21.2)	189 (12.4)*#	184 (13.2)*#

*P < 0.05 compared to control; #P > 0.05 compared to plasmapheresis/atorvastatin

Liver lipid profile

Further, it was observed that the rats treated with plasmapheresis had significantly greater improvement in liver lipid levels when compared to atorvastatin after 24 weeks of treatment (Figure 2).

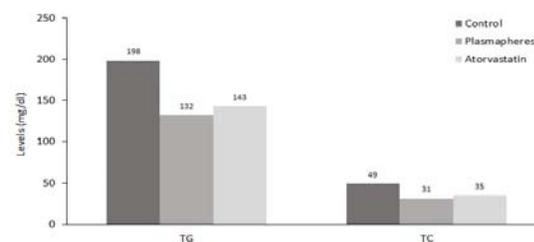


Figure 2: Liver lipid levels in rats of all groups

Plasma levels of antioxidant enzymes

Antioxidant analysis of cardiac tissue showed that there was no statistically significant difference in plasma levels of antioxidant enzymes, however, plasmapheresis after 24 weeks of treatment showed marginal improvement of anti-oxidant status when compared to rats treated with atorvastatin (Table 1).

Hepatic levels of antioxidant enzymes

The level of antioxidant enzymes (catalase and SOD) were significantly improved from baseline after weeks of treatment of plasmapheresis and atorvastatin. However, between group comparison showed that there is no statistically significant in improving the levels of SOD and catalase, although plasmapheresis treatment numerically improves the level of anti-oxidant enzymes. Similar trend of results was observed for levels of ascorbic acid, both the treatments showed significant improvement from baseline after 24 weeks of treatment, however, there was no statistically significant difference exists when compared plasmapheresis and ascorbic acid, although the plasmapheresis treated rats had numerical improvement in level of ascorbic acid when compared to ascorbic acid.

Table 2: Liver level of antioxidant enzymes in rats ps

Antioxidant enzyme	Control (n=30)	Plasmapheresis (n=30)	Atorvastatin (n=30)
Catalase (nmH ₂ O ₂) decomposed per Sec per g, mean (SD)	12 (7.1)	32 (4.2)**	30 (3.3)**
SOD (U/mg protein), mean (SD)	4.4 (1.1)	8.3 (2.1)**	7.5 (2.2)**
Ascorbic acid (micrograms per gram), mean (SD)	121 (21.2)	189 (12.4)**	184 (13.2)**

*P < 0.05 compared to control; #p > 0.05 compared to plasmapheresis/ atorvastatin

Similar trend of result was observed for liver tissue for which antioxidant analysis was performed (Table 2).

Overall, plasmapheresis and atorvastatin significantly improved the level of anti-oxidant enzyme as compared to hypercholesteremic rats.

Coronary stenosis score of coronary arteries

Coronary atherosclerosis was measured using coronary stenosis score (CSS) for all the coronary arteries namely left coronary main artery (LMT), right coronary artery (RCA), left circumflex artery (LCX), and start of right artery (RCO). The CSS in each coronary artery was measured and compared between both the group. The analysis results showed that there was no statistically significant difference between both treatments, however, treatment with plasmapheresis showed marginal improvement in CSS for all coronary arteries namely LMT, RCA, LCX and RCO when compared to rats treated with atorvastatin (figure 3). Overall, plasmapheresis and atorvastatin significantly reduce the coronary stenosis score as compared to hypercholesteremic rats.

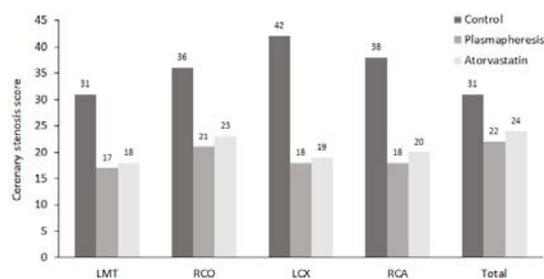


Figure 3: Coronary stenosis score of coronary arteries in rats of all groups

Lesioned composition of aortic and coronary atherosclerosis

Compared to control group, rats treated with plasmapheresis and atorvastatin had significantly lower % of lesioned composition such as collagen, smooth muscle cell, macrophage, and lipid deposits (Figure 4). However, reduction in %

of lesioned composition was significantly higher in rats treated with plasmapheresis compared with atorvastatin (Figure 4). This indicated that plasmapheresis is effective in preventing the progression of atherosclerosis in high cholesterol diet-induced atherosclerosis.

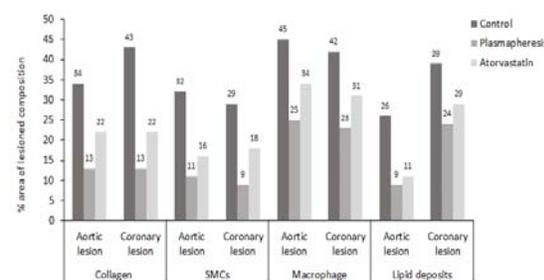


Figure 4: Lesioned composition of aortic and coronary atherosclerosis in rats of all groups

Histological features of atherosclerotic area

Compared to control group, rats treated with plasmapheresis and atorvastatin had significantly lower arch and thoracic surface involvement including lesion area and I/M ratio (Figure 4). However, % lesion was significantly higher in rats treated with plasmapheresis compared with atorvastatin (Figure 4).

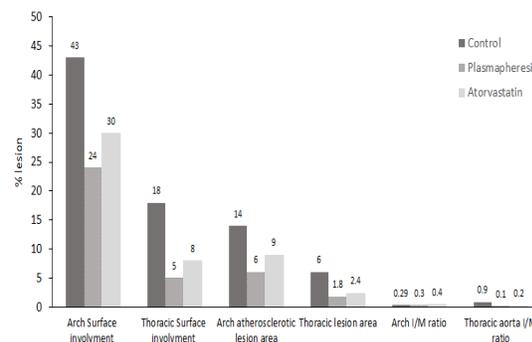


Figure 5: Histological assessment of atherosclerotic area in rats of all groups

Xanthoma

On comparing plasmapheresis vs atorvastatin,

Table 3: Comparison of xanthoma in rats of all groups

Group	Forelegs	Hind legs (incidence, %)	Total	Severity of xanthoma (%), distribution			
				-	+	++	+++
Control	100	100	100	0	59	57	13
Plasmapheresis	100	62	55	18	12	9	0
Atorvastatin	100	75	65	17	15	13	0

plasmapheresis treated rats had significantly lower incidence of xanthoma when compared to the rats treated with atorvastatin (Table 3).

DISCUSSION

This is first pre-clinical study designed to evaluate the effect of plasmapheresis versus atorvastatin in pathological progression of atherosclerosis in a rodent model. Also, compared the efficacy and safety of plasmapheresis with atorvastatin. The results of this study will serve the basis of future clinical investigation and may give a new alternative option to scientific community and researcher to think on therapy based on plasmapheresis in management of atherosclerosis and atherosclerosis induced coronary complications. It has been reported in various epidemiological studies that lipid lower drugs that effectively reduces the plasma and liver lipid levels are significantly reduces the cardiac related morbidity and mortality [13-16]. This finding of epidemiological studies was consistent with the present study, in which it was observed that the rats treated with plasmapheresis had significantly greater improvement in levels of triglycerides, total cholesterol, low-density lipoproteins, very-low-density lipoprotein and high-density lipoprotein levels. Moreover, plasmapheresis after 24 weeks of treatment significantly improved CSS in all coronary arteries than atorvastatin ($p < 0.05$). Furthermore, lesioned composition, I/M ratio and xanthoma incidence were significantly lower in plasmapheresis group than atorvastatin ($p < 0.05$ for each comparison). The results of present study pertaining to statin group were consistent with published study, where statin group of medicine was found effective in reducing lipid levels [17-20]. Moreover, the effect of plasmapheresis in reducing the lipid level such as cholesterol, and triglyceride was consistent with the previous reports [21]. Since there is no study comparing safety and effectiveness of statin drugs versus lipid removal using plasmapheresis or apheresis technique established. This study showed that plasmapheresis demonstrate significantly greater reduction plasma and liver lipid levels as compared to atorvastatin.

In the present study, histological assessment was performed to understand whether statin and plasmapheresis are effective in preventing the progression of coronary artery diseases. In the present study, plasmapheresis and atorvastatin significantly reduce the coronary stenosis score when compared to hypercholesteremic rats. The analysis results showed that plasmapheresis after 24 weeks of treatment marginally improve CSS in all coronary arteries when compared to rats treated with atorvastatin. This possibly due to antioxidant effect of plasmapheresis where improvement in level of anti-oxidant was slightly greater in rat treated with plasmapheresis when compared to statin. Similar trend of results was observed for coronary lesion (for percentage of area of lesioned composition), where hypercholesteremic rats had significantly higher % of lesioned composition when compared to rats treated with plasmapheresis and atorvastatin. On comparing plasmapheresis vs atorvastatin, plasmapheresis treated rats had significantly higher percentage of lesioned composition when compared to rats treated with atorvastatin. Moreover, compared to hypercholesteremic rats, rats treated with plasmapheresis and atorvastatin had significantly lower arch and thoracic surface involvement including lesion area and I/M ratio.

Overall, the results of the present study showed that plasmapheresis is found effective in preventing the progression of atherosclerosis in high cholesterol diet-induced atherosclerosis, in fact better than atorvastatin. This indicates that plasmapheresis could be as effective as atorvastatin and can be considered as better alternative in management of atherosclerosis and atherosclerosis induced long term complications such as coronary artery disease. The results of this study recommended the use of plasmapheresis as a better alternative in the management of atherosclerosis. However, pilot clinical study followed by large clinical study with adequate sample size to be conducted to provide recommendation for clinical use. The results of this study will serve the basis of future clinical investigation and may give a new option to scientific community and research to think on therapy based on plasmapheresis in

management of atherosclerosis and atherosclerosis induced coronary complications.

Limitations of the study

The findings of the present trial cannot be generalized to the Chinese population since the study was conducted at a single study centre in China. A large clinical trial with an appropriate sample size is needed to confirm the present findings.

CONCLUSION

Plasmapheresis may be considered a better alternative in the management of atherosclerosis as it is effective in preventing pathological progression of atherosclerosis-induced long-term complications such as coronary artery disease.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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