Author's response to reviews

Title: Suppression of low-density lipoprotein oxidation, vascular smooth muscle cell proliferation and migration by a herbal extract of Radix Astragali, Radix Codonopsis and Cortex Lycii

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Dear Editor,

1952403058440251 - Suppression of low-density lipoprotein oxidation, vascular smooth muscle cell proliferation and migration by a herbal extract of Radix Astragali, Radix Codonopsis and Cortex Lycii

Thank you very much for your reply dated 23 Sep, 2010 in which you advised us that you would be willing to consider a revised version of the above manuscript.

Following the reviewers’ comments, we have seriously revised our original manuscript. We are pleased in re-submitting our revised manuscript via manuscript submission website whereas the responses to reviewers are attached as follows.

Thank you very much.

Yours Sincerely,

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Comment: Are any specifics known about which component of SR10 may be more likely to be participating in the model?

Response: In atherosclerotic plaques, proinflammatory cytokines exert adverse effects on lipids thereby aggravating atherosclerosis. Recent evidence shows that tumor necrosis factor-alpha (TNF-a) can down-regulate the expression of ATP-binding cassette transporter A1 (ABCA1), which plays a vital role in reverse cholesterol transport and determines the process of atherosclerosis. In a report, it has been shown that Astragalus polysaccharide (APS), the main extract from the traditional Chinese medicinal herb Astragalus Membranaceus could protect ABCA1 against the lesion of TNF-a in THP-1 derived foam cells, which may contribute to its antiatherosclerotic properties (Ref R1). For SR10, it is the water extract of three herbs and one of them is Radix Astragali. Thus, it may contain various polysaccharides from this herb. However, whether Astragalus polysaccharide could be an component responsible for the anti-atherosclerotic action of SR10 is still needed to be verified.


Comment: How easy would it be to achieve comparable levels of SR10 in vivo as were used in this study and what is known about the beneficial effects of these doses in humans with atherosclerosis?

Response: In this study, 1.25-5 mg/ml of SR10 was used to treat vascular smooth muscle cell in vitro. In vivo experiment will be required to test if this mg/ml concentrations of SR10 can be reached in the host’s body. Actually, SR10 is a novel formulation. No previous study has been done for the effects of SR10 on anti-atherosclerosis. Moreover, SR10 is comprised of three herbs and the components in these herbs may have their own interactions. Therefore, it is difficult to predict the physiological effects and the bioavailable concentration of SR10 in human or animals. Future in vivo experiment must be done in order to access the efficacy of SR10 in atherosclerotic treatment. This issue has been addressed in the Discussion section.

Comment: There are no data exploring whether ROS and ERK signaling are involved in the effects of SR10.

Response: Firstly, data shown in this and previous manuscript provide some evidences that one of the action of SR10 is to inhibit ROS generation which is the main mechanism of LDL oxidative modification:
1. SR10 inhibited RBC hemolysis induced by AAPH which is a strong ROS generator (reported in Figure 1 of present manuscript).

2. SR10 enhanced antioxidant enzyme levels and activities in mice (reported in Table 2, Figure 3 & 4 of previous paper: Chan JY, Lam FC, Leung PC, Che CT, Fung KP. Anti-hyperglycemic and anti-oxidative effects of an herbal formulation of Radix Astragali, Radix Codonopsis and Cortex Lycii in a mouse model of type 2 diabetes mellitus. Phytotherapy Research 2009;23(5):658-65).

Secondly, to investigate the effect of SR10 on the activity of ERK 1/2 is one of the aims for our future study. We are studying the signal pathway involved in SR10 action in vascular smooth muscle cells (VSMC). Preliminary results showed that SR10 suppressed the proliferation of VSMC via MAPK pathway and phosphorylated ERK1/2 is one of the regulators affected (data shown in Figure A below). We hope to publish this data in another manuscript in near future. For the present manuscript, we want to focus on the pharmacological efficacy of SR10 in inhibiting VSMC proliferation. In fact, it has been previously reported that baicalin (an active component from Scutellaria baicalensis) and coryn oxideine (isolated from Uncaria rhynchophylla) significantly inhibited PDGF-induced ERK1/2 activation (Ref R2 and R3).


Comment: Why only cyclin D1 was selected in this study?

Response: In this manuscript, we want to emphasize the therapeutic efficacy of SR10 in inhibiting vascular smooth muscle cell proliferation. Therefore, DNA synthesis and cell cycle analysis were preformed. Mechanistic study to elucidate how SR10 modulate cell...
cycle-regulating proteins in VSMC will be our next target. Cyclin D1 is the initial and important regulator mediated in G1 cell cycle, thus the effect of SR10 in cyclin D1 expression can provide more evidences to show the inhibitory effect of SR10 in VSMC proliferation.

Comments: Wrong references for MTT assay and Western blot analysis of cyclin D1

Response: Reference for MTT assay should be [11] and this has been revised. Reference [14] is for Western blot analysis but not specific for cyclin D1. This has been corrected in the Methods section.

Comment: Copper (II) chloride in Methods section is not consistent with CuSO4 in Fig. 2 legend.

Response: The assay is performed using copper chloride. The error in figure legend has been corrected.

Comment: No statistical data in Figure 5B.

Response: Statistical analysis of Fig. 5B has been added as Fig. 5C in the revised manuscript.

Comment: Recognize the migrated cells in Fig. 6A.

Response: Arrows has been added in Fig. 6A to indicate the migrated cells.
Comment: The author mentioned the role of ERK1/2 in the Introduction. I propose to examine the effect of SR10 on the activity of ERK1/2.

Response: To investigate the effect of SR10 on the activity of ERK1/2 is one of the aims for our future study. We are studying the signal pathway involved in SR10 action in vascular smooth muscle cells (VSMC). Preliminary results showed that SR10 suppressed the proliferation of VSMC via MAPK pathway and phosphorylated ERK1/2 is one of the regulators affected (data shown in Figure A below). We hope to publish this data in another manuscript in near future. For the present manuscript, we want to focus on the pharmacological efficacy of SR10 in inhibiting VSMC proliferation. In fact, it has been previously reported that baicalin (an active component from Scutellaria baicalensis) and corynoxeine (isolated from Uncaria rhynchophylla) significantly inhibited PDGF-induced ERK1/2 activation (Ref R2 and R3).

![Figure A](image)


Comment: The Western blots should be quantitated. It is difficult to rely on these data to conclude that cyclin D1 was suppressed.

Response: The quantitation of cyclin D1 expression has been added as Figure 5C in the revised manuscript. It was found that cyclin D1 was suppressed significantly by SR10.
Comment: The introduction is not cohesive and does not clarify the objective or hypothesis of the paper.

Response: The introduction has been revised accordingly with less association with diabetes.

Comment: The concentrations used throughout the paper are not consistent.

Response: Different concentrations of SR10 were used in different experiments because different cellular models are involved. For Fig. 1, the effect of SR10 on red blood cell was tested. For Fig. 2, it was an in vitro test with the absence of living cells. For Fig. 3 to Fig. 6, smooth muscle cell line was applied. Since the sensitivity and efficacy of SR10 is different in different cell type, we used different concentration range to show the efficacy of SR10 in inhibiting atherogenesis.

Comment: Many of the discussion are statements of results and should be deleted.

Response: Statements of results which were already mentioned in Result section has been deleted from the discussion.

Comment: The action of PDGF-BB in triggering proliferation and migration of SMC should be further explained.

Response: Some content regarding to the mechanism of PDGF-BB in VSMC has been added in the revised manuscript.

Comment: Running title need to be rephrased.

Response: Running title has been rewritten in the revised manuscript.

Comment: The conclusion needs more sentence to explain the significance of SR10.

Response: The conclusion has been rewritten to describe more the significance of SR10.