

DOCTORAL THESIS

Biochemical and pharmacological effects of Chinese medicines on homocysteine and insulin-like growth factor-1 in health and in disease

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**Biochemical and Pharmacological Effects of Chinese Medicines
on Homocysteine and Insulin-like Growth Factor-1 in Health and in Disease**

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ABSTRACT

The biochemical and pharmacological effects of a single Chinese medicinal (CM) herb, Danshen (丹参, *Radix Salviae miltiorrhizae*) and CM herbal formulae, VI-28 and Vitalliver suppositories on homocysteine or insulin-like growth factor 1 (IGF-1) were investigated at the cellular level, in healthy aging subjects and in patients with chronic liver diseases respectively.

Hyperhomocysteinemia increases oxidative stress, diminishes the vasodilation by nitric oxide, stimulates the proliferation of vascular smooth muscle cells and leads to oxidant injury to the endothelium. As a result, hyperhomocysteinemia is regarded as an independent risk factor for cardiovascular diseases, hypertension, stroke and dementia etc. The protective effect of Danshen extract against homocysteine-induced endothelial dysfunction using human umbilical vein endothelial cells (HUVEC) as a cell model was studied. Homocysteine (5 mmol/L) not only decreased cell viability but also caused disruption of capillary-like structure formation *in vitro*. The protective effect of Danshen aqueous extract and its active compounds on endothelial cell function was demonstrated through an *in vitro* tube formation assay using the HUVEC system. The active components in the aqueous extract of Danshen were characterized by HPLC. Danshen extract and its pure compounds showed different effectiveness in protecting HUVEC against homocysteine-induced injury in the following descending order: Danshen aqueous extract, Danshensu, protocatechuic acid, catechin and protocatechualdehyde¹.

With aging, there is a decline in growth hormone and IGF-1 and this contributes to altered metabolic changes and body composition in aging. In order to investigate whether the levels of homocysteine and/or IGF-1 would also be regulated by Chinese medicines, an exploratory 12 weeks open trial on VI-28, a Chinese herbal suppository formulated according to the anti-aging concept of traditional Chinese medicine, was conducted to examine its biochemical, immunological, hormonal and antioxidant effects on 31 healthy Chinese male subjects. Dose and time – dependent alterations in blood markers, such as HDL-cholesterol, cortisol, IGF-1, CD4 cell, RBC-CuZnSOD and plasma-DPPH scavenging equivalent were observed, with most of them showing beneficial changes after treatment².

The effects of VI-28 suppository on serum homocysteine and IGF-1 in 36 healthy Chinese males were studied over 16 weeks. IGF-1 levels of the whole cohort increased significantly during a 3-month treatment period, but no change was observed in serum homocysteine. However, when the cohort was divided into 2 groups using

homocysteine level of 13.0 $\mu\text{mol/L}$ as the cut-off value that has been considered as getting towards hyperhomocysteinemia, a significant difference in IGF-1 was observed between the 2 groups at week 12. Furthermore, only the group with homocysteine level < 13.0 $\mu\text{mol/L}$ showed significant elevation of IGF-1 throughout the 12 weeks of treatment with VI-28³.

As the liver is the major organ for the metabolism of homocysteine and the production of IGF-1, the study on the effects of Chinese medicines on homocysteine and IGF-1 was extended to patients with chronic liver diseases (CLD), namely chronic hepatitis B (HBV) carriers with or without liver cirrhosis, using Vitalliver, a herbal suppository, similar but not identical to the VI-28 suppository in composition and function. Forty non-cirrhotic and 9 cirrhotic HBV carriers were treated with Vitalliver and observed for 3 months. Vitalliver reduced homocysteine levels in all 5 CLD patients with baseline homocysteine level > 13.0 $\mu\text{mol/L}$, and in 40 non-cirrhotic HBV carriers (changes dominated by 25 males) with mean homocysteine level < 13.0 $\mu\text{mol/L}$. Furthermore, Vitalliver was able to increase the IGF-1 levels of the non-cirrhotics with hepatitis B significantly at 1 month and 3 months after treatment, but those levels in the cirrhotics were not affected, despite the fact that the baseline IGF-1 levels of the cirrhotics were far lower than those of the non-cirrhotics. In addition, female non-cirrhotics had lower levels of homocysteine but higher levels of IGF-1 while the male patients showed the opposite pattern. This may confirm literature observations on lower levels of homocysteine in female population⁴.

The findings suggested that Chinese medicines, either a single herb (e.g. Danshen) or herbal formulae (e.g. VI-28 and Vitalliver) might produce beneficial effects on the regulation of homocysteine and/or IGF-1 either at the cellular level or in the systemic environment.

The present work has also illustrated that it is possible to carry out evidence-based research in general practice combining the expertise of Chinese medicine and the use of western medicine research methodologies and approaches in verifying the efficacy of Chinese medicine formulae. However, more studies will be needed to consolidate and substantiate the findings due to limitations such as the relatively small sample size of the study subject and not using a randomized, placebo-controlled approach when the work was conducted. Some future studies have been proposed towards such direction in the final chapter of the thesis.

Publications:

1. (1) Chui SH, Chan K, Wong RNS, Kong TK, Chen KJ. The inhibitory effect of homocysteine on the tube formation of endothelial cells can be reversed by the Danshen (*Radix Salviae miltiorrhizae*) extract. An abstract presented at the 2nd World Integrative Medicine Congress (2002) in Beijing (Appendix 1).

(2) Chan K, Chui SH, Wong DYL, Ha WY, Chan CL, Wong RNS. Protective effects of Danshensu from the aqueous extract of *Salvia miltiorrhiza* (Danshen) against homocysteine-induced endothelial dysfunction. Accepted for publication in Life Sciences.
2. (1) Chui SH, Ko KM. Biochemical, immunological, hormonal and antioxidant effects of a Chinese herbal suppository, Vigconic VI-28, on elderly male subjects – an anti-ageing solution? An abstract presented at the Anti-Aging World Conference (2003) in Paris (Appendix 2).

(2) Chui SH, Ko KM. Anti-ageing effects of a Chinese medicine preparation on elderly male subjects. An abstract presented at the 2nd Asia Pacific Conference & Exhibition on Anti-Ageing Medicine (2003) in Singapore (Appendix 3).

(3) Karlberg J, Chui SH, Ko KM, Ansaii MT. An open label 12-week trial on endocrine, metabolic, immunologic and antioxidant effects of a natural ‘anti-aging’ product (VI-28). Submitted for publication.
3. (1) Chui SH, Chan K, Wong RNS. Effects of a Chinese medicine suppository, Vigconic VI-28, on growth hormone and homocysteine in healthy men. An abstract presented at The First World Congress on Chinese Medicine (2003) in Melbourne (Appendix 4).

(2) Chui SH, Chan K, Wong RNS. A panel study on the effects of a Chinese medicinal suppository, Vigconic VI-28, on insulin-like growth factor-1 and homocysteine in healthy men. Methods and Findings in Experimental and Clinical Pharmacology 2004; 26(5) : 349 – 355 (Appendix 5).
4. (1) Chui SH, Chan K. A report on the rapid suppression of multiplication of Hepatitis B virus by a Chinese medicine product, Vitalliver. An abstract

presented at the International Conference & Exhibition of the Modernization of Chinese Medicine & Health Products (2003) in Hong Kong (Appendix 5).

- (2) Chui SH, Chui AKK, Shek LSL, Wong RNS, Chan K. The effects of a Chinese medicinal suppository (Vitalliver) on insulin-like growth factor 1 and homocysteine in Chronic Hepatitis B carriers. An abstract presented at the Tri-conference SCBA Bio/Pharm Meeting (2004) in Shanghai (Appendix 6).
- (3) Chui SH, Chui AKK, Shek LSL, Wong RNS, Chan K. The effects of a Chinese medicinal suppository (Vitalliver) on insulin-like growth factor 1 and homocysteine in Chronic Hepatitis B carriers. Submitted for publication.

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