MASTER'S THESIS

Investigation on the correlation between redox changes and oxidative stress in diabetes, and their role in transcription factors activation in vitro and in vivo

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Investigation on the Correlation Between Redox Changes and Oxidative Stress in Diabetes, and their Role in Transcription Factors Activation *In Vitro* and *In Vivo*

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Abstract

Diabetic mellitus is the most common endocrine disease. This chronic disorder is characterized by high blood glucose level (hyperglycemia) and long-term complications of eyes, kidneys, nerves and blood vessels, resulting in much morbidity and mortality in diabetic patients. However the underlying mechanism of pathogenesis of these long-term complications is complex and still largely unknown.

Many hypotheses, such as polyol pathway, advanced glycation end products, activation of PKC, have been suggested in an attempt to explain the development of the diabetic complications. According to recent researches, oxidative stress induced by the hyperglycemia seems to be a common pathway of various hypotheses, hence it is the most possible candidate in causing the diabetic complications. In the past *in vitro* experimental findings, high glucose could lead to oxidative stress in porcine endothelial and vascular smooth muscle cells. Transcription factors NF-κB and AP1 were also activated.

Therefore the main objectives of this project were to study the change of the reduced glutathione (GSH), which is the primary non-enzymatic antioxidant in cytosol, and malondialdehyde (MDA), which is the end products of lipid peroxidation, in the vascular smooth muscle cells (VSMC) under high glucose cultured condition (*in vitro* experiment), and in the eyes, aortas and kidneys of diabetic rats (*in vivo* experiment). Furthermore, time course studies were also carried out to establish the temporal relationships between redox status and oxidative stress. In addition the binding activities of transcription factors NF-κB and AP1 in different time points were also studied in *in vitro* and *in vivo* models. GSH and MDA were measured by the colorimetric method using spectrophotometer with 400nm and 586nm. While the binding activities of the transcription factors, NF-κB and AP1, were determined by electrophoretic mobility shift assay (EMSA). The specificity of the band(s) were verified by the specific competition and non-specific competition in the gel. Finally the effectiveness of Trolox C, a water soluble derivative of vitamin E, in reversing the change of GSH and MDA of VSMC cultured in high glucose condition was investigated.

In brief, we have successfully isolated and cultured the VSMC and endothelial cells from the aorta of S.D. rats. However only VSMC were studied in details in the *in vitro* experiments. We found that significant decrease (0.045 ± 0.003 μmol/mg in high

glucose condition Vs. 0.063 ± 0.004 μmol/mg in normal glucose condition) ($P < 0.01$) of GSH was observed from Day 2 onwards. Similarly significant increase (0.1 ± 0.012 nmol/mg in high glucose condition Vs. 0.06 ± 0.005 nmol/mg in normal glucose condition) ($P < 0.05$) in MDA was observed from Day 2 onwards. In Day 10, GSH and MDA levels of VSMC cultured in high glucose condition were significantly less and more ($P < 0.01$) than that of the cells grown in the normal glucose cultured condition respectively. Furthermore these depletion and increment of GSH and MDA were due to high glucose environment only, as demonstrated using other controls. In regard to transcription factors, only binding activity of NF-κB, but not AP1, was increased (about 50%). In addition Trolox C had only partial effect in normalizing the GSH and MDA concentrations.

We have also successfully set up the *in vivo* model using S.D. rats injected with streptozotocin. Their serum glucose elevated rapidly within a week after the injection, and the percentage of glycated hemoglobin increased with time. GSH and MDA concentrations in eyes and aortas of diabetic rats were found to be significantly decreased and increased ($P < 0.01$) respectively in the first and the eighth week onwards after the injection. However there was a significant increase ($P < 0.01$) in GSH concentration in the kidneys of diabetic rats comparing with the normal control. No significant change was found in MDA concentration in the kidney of diabetic rats in the experimental period except the 4^{th} week with significant increase ($P < 0.01$). Interestingly the binding activities of the NF-κB and AP1 of the above three tissues were all found to be increased in the 8^{th} week but not in the 4^{th} week after the injection.

To conclude, high glucose cultured condition lead to a reduced level of GSH in VSMC in the early period. And this depletion would then lead to oxidative stress occurring in the cells (as the MDA level was increased). These oxidative change signals may subsequently lead to activation of NF-κB, but not AP1 in VSMC. Trolox C had partial effect in normalizing the GSH and MDA of VSMC cultured in high glucose condition. Similarly, hyperglycemic condition may lead to the cells of the eyes and aorta of the diabetic rats, generating more free radicals. These reactive oxygen species may then attack the cell membrane and stimulate the activation of NF-κB and AP1. However, the complications occurring in the kidney may primarily due to expansion of extracellular matrix rather than oxidative damage.

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