学 位 論 文 の 要 旨

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学 位 論 文 名　Effects of Uremic Toxin *p*-Cresol on Proliferation, Apoptosis, Differentiation and Glucose Uptake in 3T3-L1 Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Malnutrition and sarcopenia commonly seen in chronic dialysis patients may result from restriction of protein intake and hypercatabolism. Previous prospective study revealed that body fat mass was markedly decreased 2 years after the initiation of dialysis therapy. Moreover, dialysis patients with obesity have a better survival rate and a less cardiovascular (CV) death rate, compared with lean patients, which is so-called "reverse epidemiology".

It is also known that chronic kidney disease (CKD) patients, even if they have neither obesity nor diabetes, exhibit insulin resistance, which is closely related with arteriosclerosis and CV event. According to previous studies regarding the insulin resistance in renal failure patients, glucose uptake and gluconeogenesis in the liver are similar to those in healthy subjects whereas glucose uptake in the muscle and adipose tissue falls to 60% of the counterpart. Thus, adipocytes might play a key role in the insulin resistance and malnutrition in CKD patients.

Although uremic toxins can be involved in the insulin resistance and CV disease risk in CKD patients, little is known about their pathogenesis so far. While *p*-cresol, one of uremic toxins, is hardly detectable in healthy subjects, the blood concentration in dialysis patients is

reported to be 24 mg/L (approximately 200 μM). The blood *p*-cresol level is hardly reduced even after hemodialysis session because of highly protein-binding property. It has been reported that its concentration is highly associated with the risk of CV event. However, the underlying mechanisms remain unknown. Thus, we focused on this uremic toxin to examine the effects on proliferation, differentiation, and glucose uptake in adipocytes and the precursors.

MATERIALS AND METHODS

We cultured mouse preadipocyte cell line, 3T3-L1 cells, which were differentiated into mature adipocytes with 500 μM 3-isobutyl-1-methylxanthine (IBMX), 250 nM dexamethasone, and 1 μg/ml insulin after 90% confluency. Cell proliferation was determined by cell count and Brd-U antibody detection method 1 day after plating. The maturity of adipocyte was determined by Oil Red O staining. PPARγ mRNA expression was quantified by real-time PCR. Apoptosis of the cells was analyzed using an enzyme-linked immunosorbent assay (ELISA) system, which quantified histone-complexed DNA fragments by anti-histone-antibody and peroxdase-labeled anti-DNA antibody after induced cell death. Glucose uptake was examined in the presence or absence of insulin using ^{3}H-labeled 2-deoxyglucose. Effects of *p*-cresol on glucose uptake were evaluated in various concentrations (2-200 μM).

RESULTS AND DISCUSSION

In the cell count experiments, the number of 3T3-L1 cells treated with 100-200 μM *p*-cresol was significantly decreased at day 3 and day 7. Brd-U antibody detection showed a significant reduction at 100-200 μM *p*-cresol, suggesting that *p*-cresol disturbed normal cell cycle progression. Oil red-O staining at day 7 showed that the number of mature adipocytes was decreased by the treatment with 200 μM *p*-cresol. As consistent with this finding, apoptotic cell number at day 7 was increased by the treatment with 100-200 μM *p*-cresol. Two hundred μM *p*-cresol decreased mRNA expression of PPARγ even when corrected with GAPDH mRNA level. ^{3}H-labeled 2-deoxyglucose uptake was remarkably reduced by 100 and 200 μM *p*-cresol in the

presence or absence of insulin, which was consistent with decreased number of mature adipocytes observed during the experiment. To evaluate the response to insulin, we calculated the ratio of glucose uptake in the presence and absence of insulin. As a result, the significant reduction was observed by 200 μM *p*-cresol. This indicated that the insulin resistance might be developed in response to high concentration of *p*-cresol.

We investigated whether or not uremic toxins affect cellular function and lead to the insulin resistance in adipocytes and the precursors. In the present study, we found that *p*-cresol of the blood level reported in dialysis patients inhibited proliferation, maturation and glucose uptake in 3T3-L1 cells, suggesting that *p*-cresol is responsible for the loss of fat mass and the development of insulin resistance in spite of the absence of obesity in chronic dialysis patients. Moreover, the toxic effect was accompanied with an increase in apoptosis and decrease in the number of mature adipocytes, which are most likely responsible for the reduced glucose uptake. In addition, high concentration of *p*-cresol inhibited an insulin-induced glucose uptake, partially due to attenuated insulin sensitivity.

In our study, we firstly found that *p*-cresol might be involved in reduced fat mass and the development of insulin resistance. Hence, these findings are compatible with the concept of "reverse epidemiology", where lean patients have higher mortality rate than obese patients undergoing dialysis therapy.

CONCLUSION

p-Cresol inhibited proliferation, differentiation and glucose uptake, and induced apoptosis in 3T3-L1 cells. These findings indicate that the accumulation of uremic toxins may induce the reduction of adipose tissue, insulin resistance, and eventually poor prognosis in chronic dialysis patients. Novel therapeutic methodologies, which target reduction or removal of uremic toxins such as *p*-cresol, may lead to better prognosis in dialysis patients.