Effects of uremic toxin p-cresol on proliferation, apoptosis, differentiation and glucose uptake in 3T3-L1 cells

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Background
- Malnutrition is commonly seen in chronic dialysis patients. Previous prospective study revealed that body fat mass was markedly decreased 2 years after the initiation of dialysis therapy [1].
- 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” [2].
- Chronic kidney disease (CKD) patients, even if they have neither obesity nor diabetes, exhibit the insulin resistance, which has a close relationship with arteriosclerosis and CV event [3].
- P-cresol, one of uremic toxins, is highly associated with CV event in CKD patients [4].
- Thus, we examined the effects of p-cresol on adipocyte proliferation, apoptosis, differentiation and glucose uptake.

Results
- Effects of p-cresol on proliferation of 3T3L1 cells

> **[Cell count]**

![Graph showing cell count](image)

Number of cells treated with 100µM and 200µM p-cresol decreased at day 3 and day 7. Brd-U antibody detection showed p-cresol disturbed normal cell cycle.

- Effects of p-cresol on glucose uptake

> **[Uptake of H-labeled 2-deoxyglucose]**

![Graph showing glucose uptake](image)

H-labeled DOG uptake was remarkably inhibited by 100µM and 200µM p-cresol in the presence and absence of insulin.

- Inhibition of adipogenesis of 3T3-L1 cells by p-cresol

> **[Oil red-O staining]**

![Image of oil red-O staining](image)

High concentration p-cresol inhibited the differentiation of preadipocytes into adipocytes. Total cell number was apparently decreased in this group.

Summary
- High concentration of p-cresol disturbed normal cell cycle, induced apoptosis, inhibited the differentiation of preadipocyte into mature adipocyte, and decreased glucose uptake at basal and after insulin stimulation.

Conclusion
- p-cresol inhibited proliferation and differentiation, 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.
- Further investigation is required, since it is recently suggested that main metabolite is p-cresylsulfate but not p-cresol in human body [5].

Materials and Methods
- **Preadipocyte**

![Graph showing transcription factor](image)

We cultured preadipocyte cell line 3T3-L1 cells and which were differentiated with 500µM IBMX, 25µg/mL dexamethasone, 10µg/mL insulin after 90% confluency. Treatment with p-cresol was performed in various concentrations (2, 20, 100 and 200µM). Cell proliferation was determined by cell count and Brd-U antibody detection method. The maturity of adipocyte was investigated by oil red-O staining and by real-time PCR to see the mRNA expression of PPARγ. Apoptosis was measured by ELISA kit. We also examined glucose uptake in the presence and absence of insulin using radiolabeled 2-deoxyglucose.

- **Adipocyte**

![Graph showing mature adipocyte](image)

- **Mature adipocyte**

![Graph showing mature adipocyte](image)

- **Adipogenin**

![Graph showing adipogenin](image)

References