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

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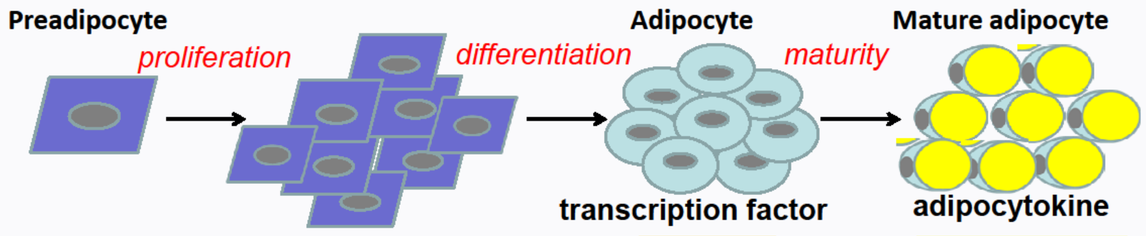
Background

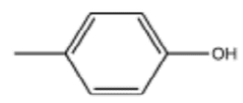
- Malnutrition is commonly seen in chronic dialysis patients. Previous prospective study revealed that body fat mass was markedly decreased 2 years after the initiation of dialysis therapy [1].
- Dialysis patients with obesity have a better survival rate and a less cardiovascular (CV) death rate compared 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.

Materials and Methods



***p*-cresol(PC)**

C_7H_8O MW 108

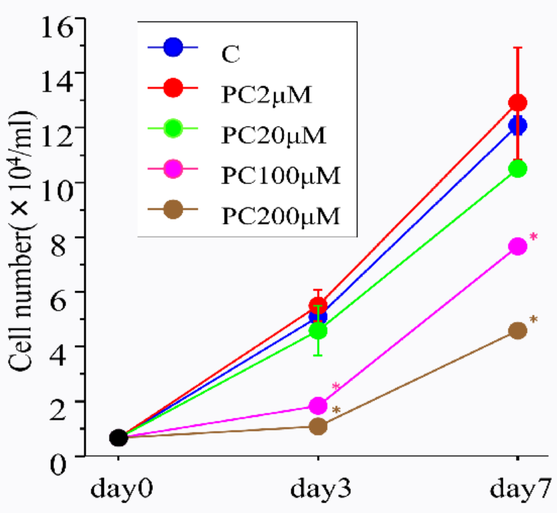


◆We cultured preadipocyte cell line 3T3-L1 cells and which were differentiated with 500μM IBMX, 250nM dexamethasone, 10μg/ml Insulin after 90% confluency. Treatment with *p*-cresol was performed in various concentrations (2, 20, 100 and 200μM). Cell proliferation was determined by cell count and Brd-U 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.

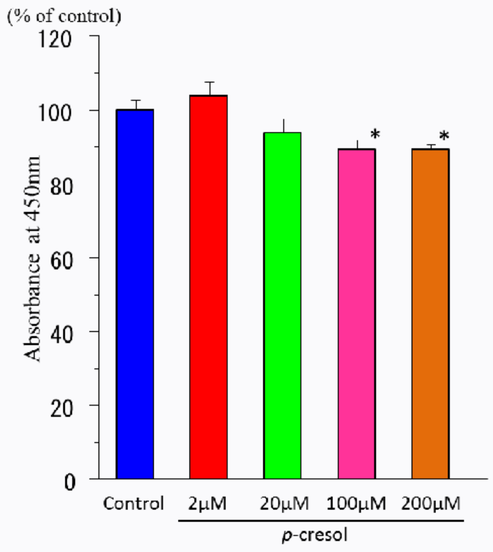
Results

◆ *Effects of p-cresol on proliferation of 3T3L1 cells*

【Cell count】



【Brd-U】

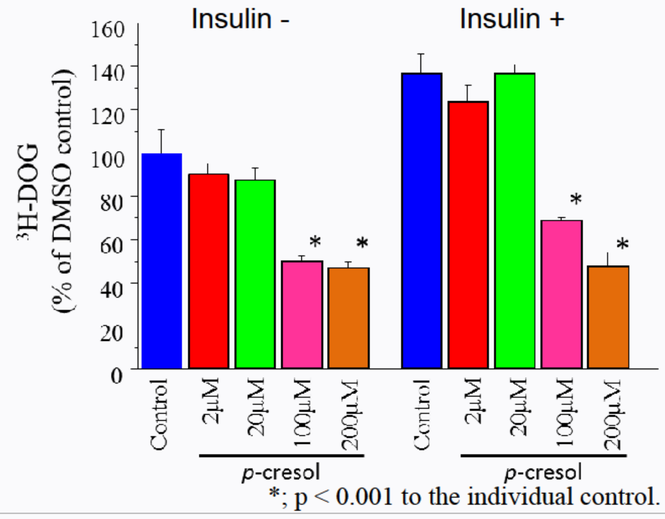


*p<0.0001; compared to the control. *p<0.05; compared to the control.

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

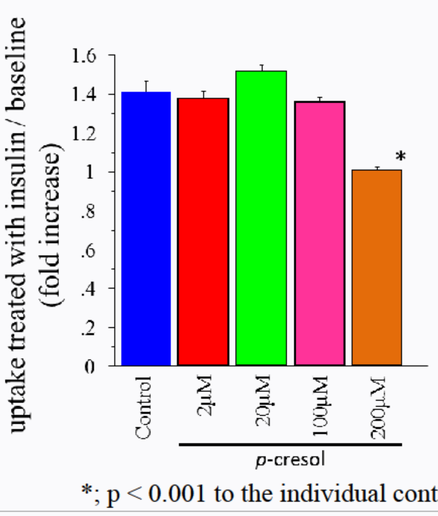
◆ *Effects of p-cresol on glucose uptake*

【Uptake of ^{3}H-labeled 2-deoxyglucose】



*; p < 0.001 to the individual control.

【response to insulin】

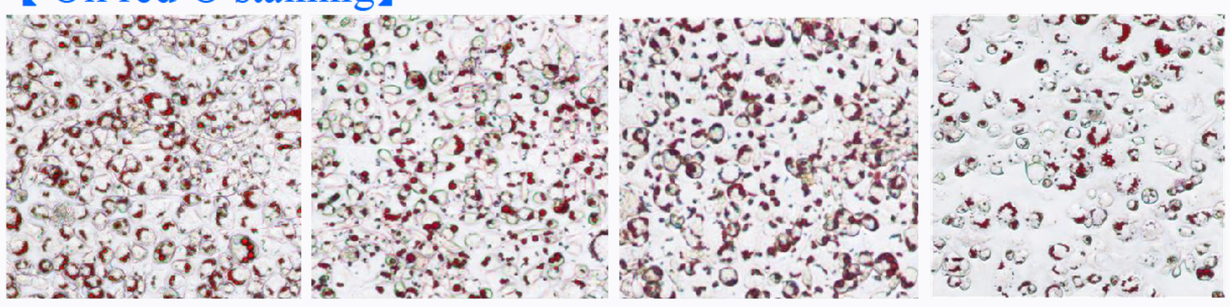


*; p < 0.001 to the individual control.

^{3}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*

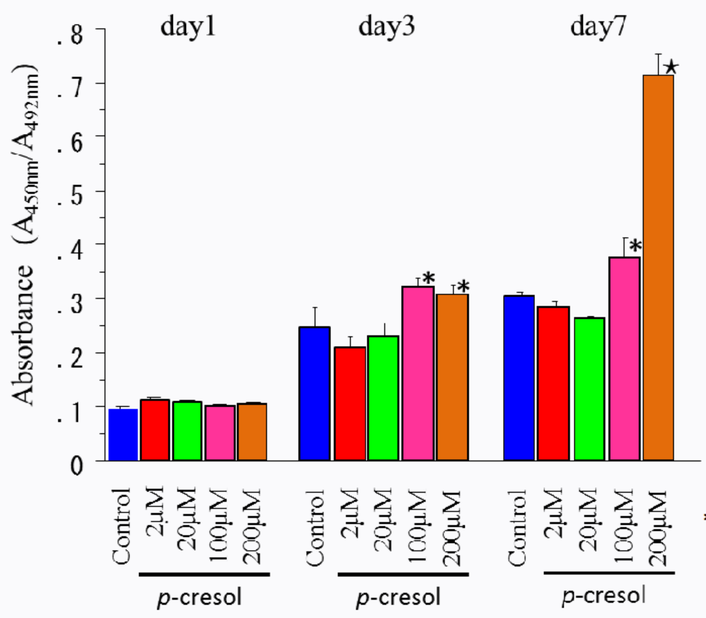
【 Oil red-O staining】



control *p*-cresol 2μM *p*-cresol 20μM *p*-cresol 200μM

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

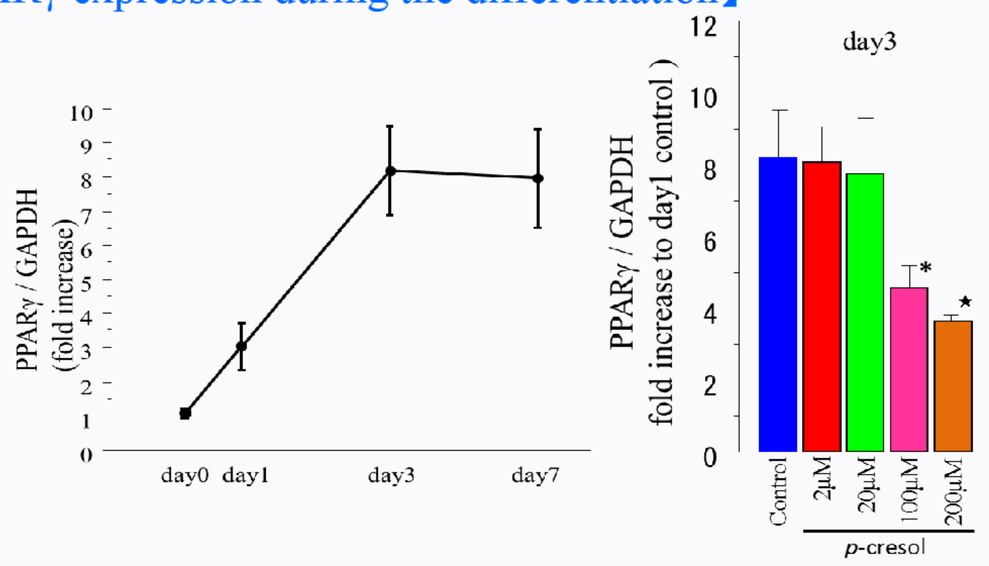
【Apoptosis】



An apoptotic cell death was significantly promoted by 100μM and 200μM *p*-cresol at day3 and day7.

*; p<0.05, and ⋆; p<0.0001, compared to control.

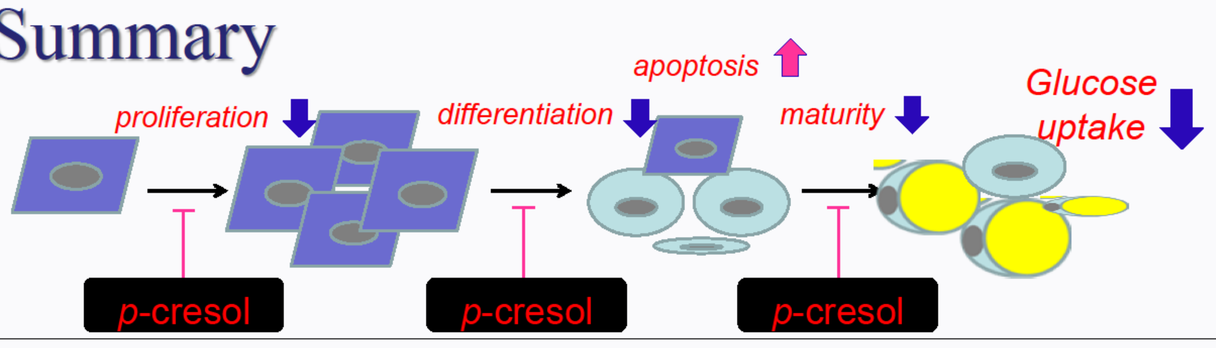
【PPARγ expression during the differentiation】



*; p<0.05, and ⋆; p<0.0001, compared to control.

In the control cells, approximately 8-fold increase in PPARγ mRNA expression was shown at day 3 and the similar level at day 7. The PPARγ mRNA level was significantly decreased in 100μM and 200μM *p*-cresol treatment.

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].

References

1. Fujino Y, et al. C-reactive protein is a significant predictor of decrease in fat mass in hemodialysis patients. *Biochem Pharmacol*.2005;59:264–268.
2. Kalantar-Zadeh K et al. Association of morbid obesity and weight change over time with cardiovascular survival in hemodialysis population. *Am J Kidney Dis*. 2005;46:489–500.
3. Becker B et al. Renal insulin resistance syndrome, adiponectin and cardiovascular events in patients with kidney disease: the mild and moderate kidney disease study. *J Am Soc Nephrol* . 2005;16:1091-1098.
4. Meijers BK et al. p-Cresol and cardiovascular risk in mild-to-moderate kidney disease. *Clin J Am Soc Nephrol*. 2010;5:1182-1189.
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