Autophagy protects against contrast induced tubular epithelial injury

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Backgound

Radiocontrast-induced nephropathy (RCN) is common cause of acute kidney injury in hospital. However, preventing and treating strategies against developing RCN were very limited. Since the role of autophagy in the pathogenesis of RCN has not yet been elucidated, we investigated its role in RCN.

Methods

We measured the viability of the cells for 48 hours after exposure of the renal tubular epithelial cells (RTEC) to contrast media, and examined the extent of expression of autophagic and apoptotic protein over time. The contrast medium was exposed at two concentrations (50 mg / ml and 100 mg / ml) in order to confirm the difference in cell viability according to the contrast medium concentration. To confirm the role of autophagy in preventing the development of CIN, we measured changes in cell viability after autophagy inhibition with small interference RNA (siRNA) for ULK1 and measured the expression of apoptotic and autophagy protein for 48 hours

Results

Cell viability gradually increased over time in RTEC controls not exposed to contrast media, whereas cell viability decreased after 3 hrs after RTEC exposure to contrast media, but increased after 24 hrs and 48 hrs. This phenomenon was observed in both contrast medium concentrations (50 mg / ml and 100 mg / ml). (Fig. 1).

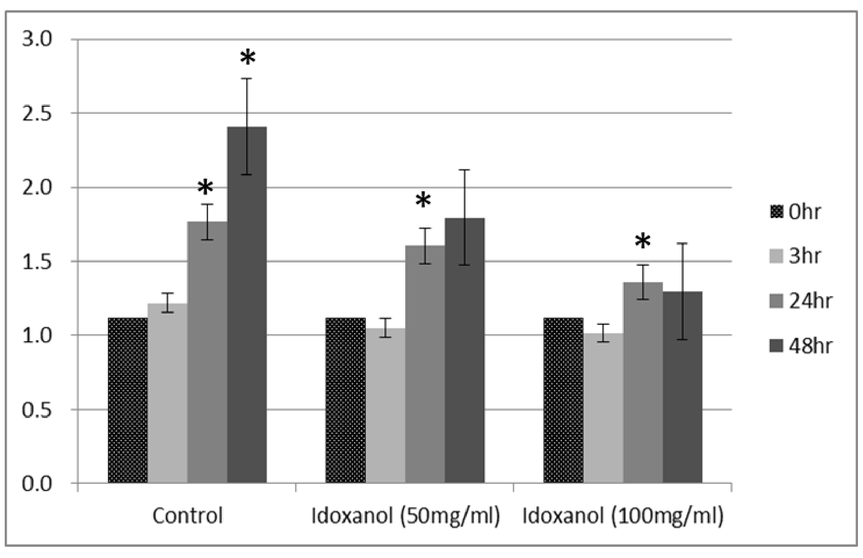


Figure 1. cytotoxicity and change of survived cell after exposure to contrast (CCK-8 array)
* P <0.05

Apoptosis, measured by Caspase 3 and 8, began to appear within 1 hour after exposure to contrast media and gradually increased until 24 hours after exposure. (Fig. 2).

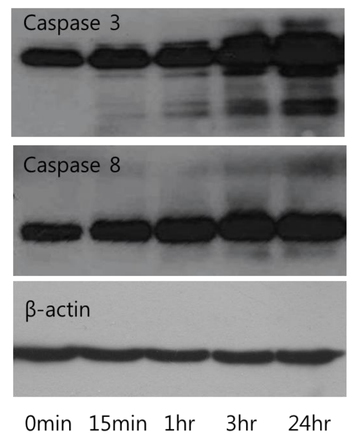
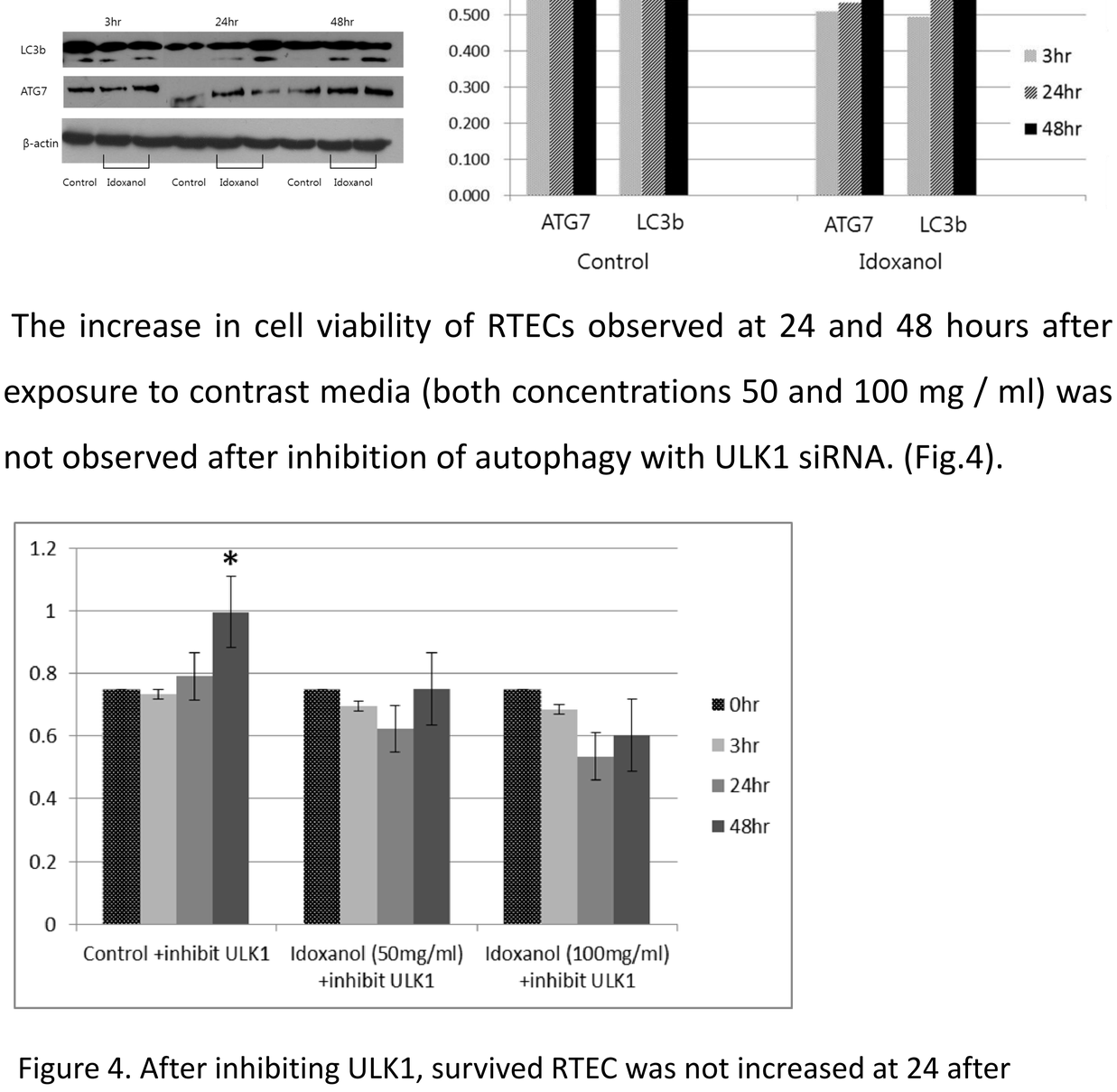


Figure 2. immunoblot analysis of Caspase 3, 8 and β-actin (loading control). Apoptosis is induced in RTEC during contrast cellular toxicity

On the other hand, autophagy measured by LC3 and autophagy related gene protein 7 (ATG7) was detected 3 hours after the contrast medium exposure, and LC3 and ATG7 induction was further increased by 48 hours. (Fig. 3).



The increase in cell viability of RTECs observed at 24 and 48 hours after exposure to contrast media (both concentrations 50 and 100 mg / ml) was not observed after inhibition of autophagy with ULK1 siRNA. (Fig.4).

Figure 4. After inhibiting ULK1, survived RTEC was not increased at 24 after contrast exposure (CCK-8 array), * P <0.05

Conclusion

Autophagy plays cytoprotective role and it's inhibition delays cellular recovery in contrast induced RTEC injury and it may occur independently of apoptosis.

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