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Oxidative stress and apoptosis are strongly involved in ricin-induced intestinal cell intoxication

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INTRODUCTION & AIM

Ricin, a type 2 ribosome-inactivating protein (RIP), is a lethal toxin purified from castor bean seeds, and it has been classified as a chemical and biological weapon according to Schedule 1 by the OPCW [1]. Ricin cytotoxicity derives from its lectin chain, which facilitates cell entry, and from its active chain, which can depurinate different substrates, thus leading to multiple cell death pathways [2]. Oral ingestion is highly toxic, with symptoms appearing within 24 hours and a human lethal dose estimated at 1-20 mg/kg [3]. While the systemic effects of ricin are well-known, its localized impact on the gastrointestinal tract remains a critical concern. This study aims to elucidate ricin intoxication in two human intestinal cell lines, named HT29 and Caco-2, and to determine ricin impact on the integrity of the epithelial barrier.

METHODS

Ricin cytotoxicity was evaluated on two human intestinal cell lines, HT29 and Caco-2, in dose and time-response experiments, determining cell viability via an MTS-reduction assay. Cells (3×10^3/well) were treated with scalar concentrations of ricin for 48 and 72 h. The effect of ricin on Caco-2 cell monolayer integrity was monitored by Trans-Epithelial-Electrical Resistance (TEER) measurements at 0, 8, 24, 32, 48 and 72 h after ricin intoxication. Cell death was determined through flow cytometry analysis of Annexin V/PI positivity and the involvement of oxidative stress in ricin-treated cells was indirectly investigated after 48 h, pretreating cells with two reactive oxygen species scavengers, catalase (CAT) and butylated hydroxyanisole (BHA).

RESULTS & DISCUSSION

1 Cytotoxic effect of ricin on intestinal cells

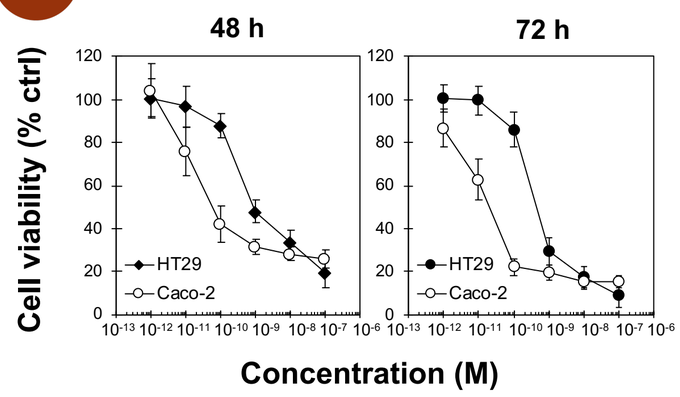

Fig. 1. Ricin strongly affected cell viability in HT29 cells, after 48 and 72 h, with viability starting to significantly decrease at 10^{-9} M. In Caco-2 cells, viability started to significantly reduce at 10^{-10} M and 10^{-11} M at 48 and 72 h, respectively.

2 Effects of ricin on barrier integrity in Caco-2 monoculture

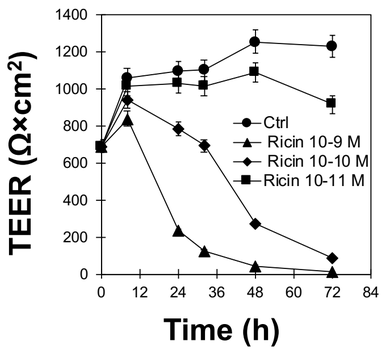

Fig. 2. At 10^{-9} M concentration, ricin strongly affected the barrier integrity after 24 h in Caco-2 monolayer, showing TEER values 5-fold lower than those of controls. At 10^{-10} M concentration, ricin was also able to reduce TEER values by about 1.3-fold with respect to control cells. At 10^{-11} M concentration, ricin has no effect, and TEER values are comparable to controls.

3 Evaluation of cell death induced by ricin in HT29 and Caco-2 cells

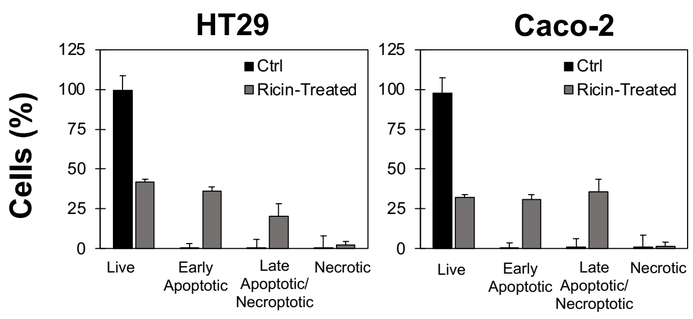

Fig. 3. At 10^{-9} M concentration, ricin was able to induce apoptosis/necroptosis after 48 h, without the involvement of necrosis.

4 Effects of ROS scavengers on ricin cytotoxicity in HT29 and Caco-2 cells

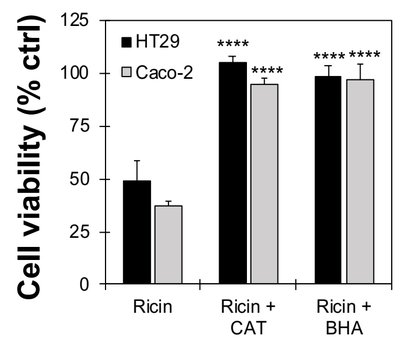

Fig. 4. Ricin cytotoxicity (at 10^{-9} M concentration) can be completely prevented after 48 h pre-incubating cells with antioxidant scavengers, demonstrating the involvement of oxidative stress in cell death mechanisms after ricin exposure.

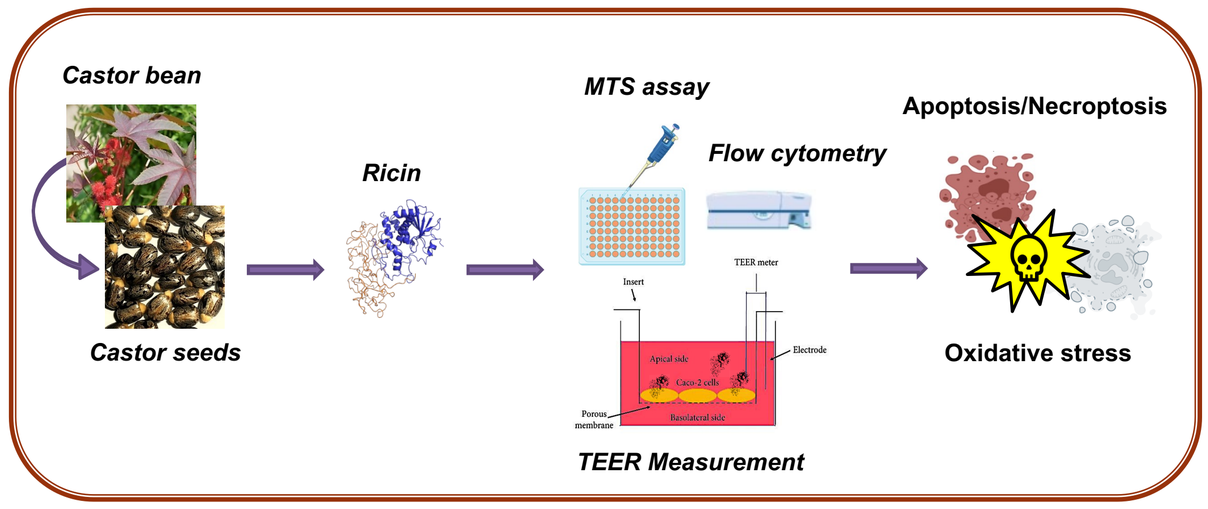

Fig. 1 Experimental Workflow

CONCLUSIONS

Our results demonstrate a dose- and time-dependent viability reduction in both HT29 and Caco-2 cells after ricin exposure.

TEER experiments demonstrate a dose- and time-dependent reduction in barrier integrity after ricin intoxication in Caco-2 cell monolayer.

Experiments on cell death mechanisms revealed a predominant role of apoptosis/necroptosis in ricin-intoxicated HT29 and Caco-2 cells after 48 h, without necrosis involvement.

Interestingly, both antioxidants used, CAT and BHA, can significantly protect HT29 and Caco-2 after 48 h of ricin exposure.

These results pave the way for new promising therapeutic strategies against oral ricin poisoning.

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