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#11

The Role of Car3 in the Protection of Intestinal Epithelial Cells Against Oxidative Injury

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INTRODUCTION AND OBJECTIVES

- Reactive oxygen species (ROS; H_2O_2, O_2^- and OH·) are the main cause of inflammation mediated oxidative damage in inflammatory bowel disease
- In mice with induced chemical colitis, insulin treatment decreases damage associated with disease
- Proposed mechanism of insulin's therapeutic effect is insulin mediated upregulation of carbonic anhydrase 3 (Car3)
- Car3 has not been well characterized in intestinal epithelial cells (IEC)
- **Objective: to understand how Car3 plays a role in insulin treatment by investigating how ROS affect IEC with and without induced Car3 expression**

HYPOTHESIS

LS147T colon carcinoma cells, a model for IEC, expressing Car3 under a tetracycline induced promoter will show an increased survival in the oxidative environment compared to control cells

METHODS

EXPERIMENT 1: Determine linear detection range of MTT assay → Induce oxidative damage in LS147Tdoxy- cells and Caco2 cells → Measure effects of oxidative damage over 16 hours

EXPERIMENT 2: Culture differentiated and undifferentiated Caco-2 cells → PCR to measure levels of Car3 expression in Caco2 cells → Western blot to visualize Car3 expression levels in Caco2 cells → Western blot to visualize Car3 expression Caco2/doxy+ cells

RESULTS

EXPERIMENT 1:

Figure 1:

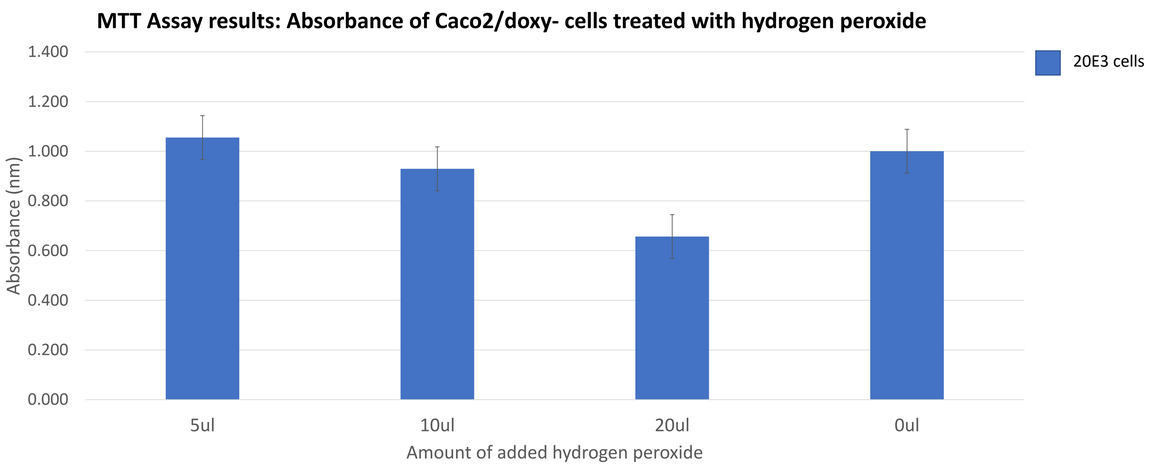


Figure 2:

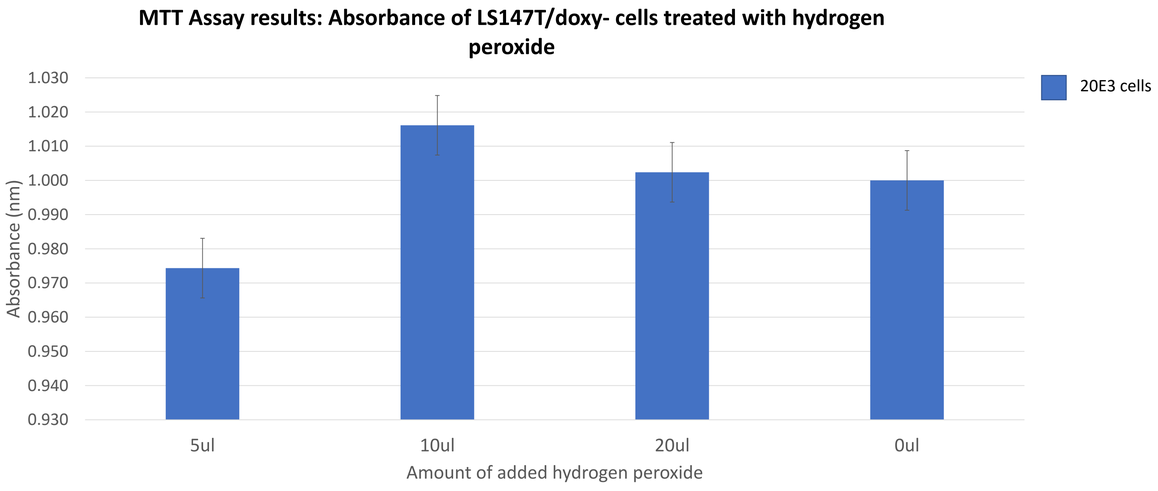
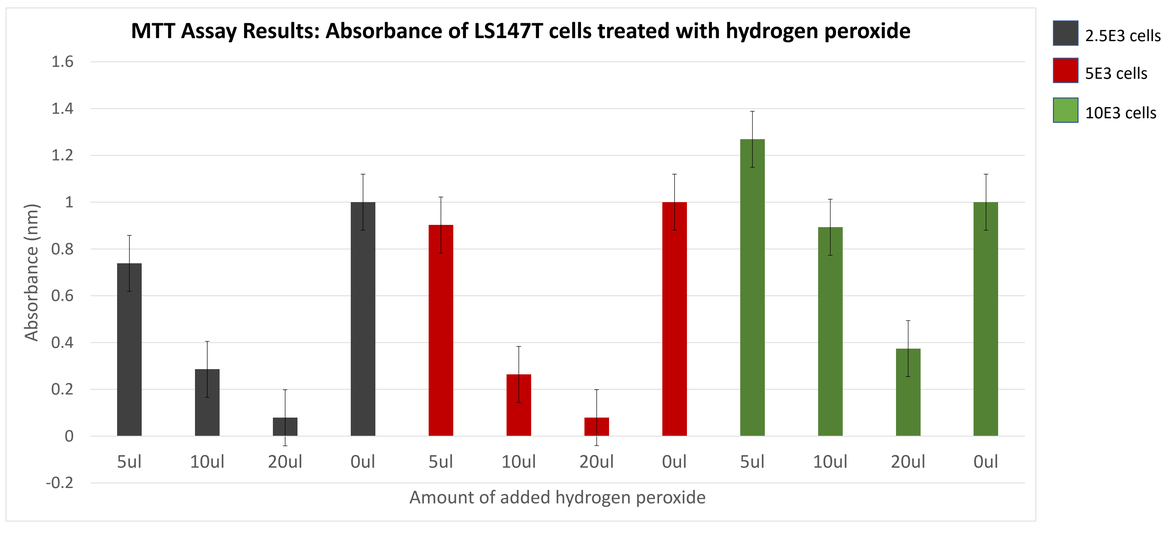


Figure 3:



EXPERIMENT 2:

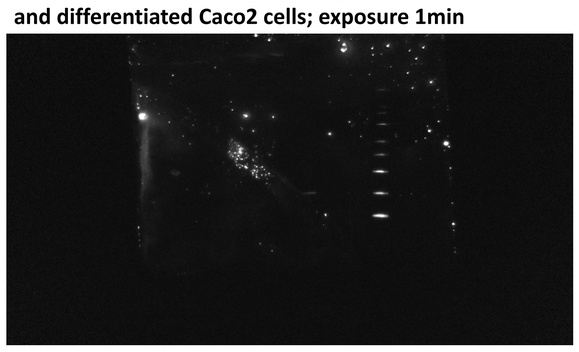
Table 1:

PCR comparing Car3 expression in undifferentiated and differentiated Caco2 cells

PCR comparing Car3 to PSMB8	Car3 Concentration	PSMB8 concentration	Car3/PSMB
water	1.57E+01	4.31E+01	3.64E-01
standard	1.00E+05	1.00E+05	1.00E+00
undifferentiated Caco2	1.81E+04	2.60E+04	6.96E-01
undifferentiated Caco2	1.65E+04	2.38E+04	6.93E-01
undifferentiated Caco2	1.51E+04	2.34E+04	6.45E-01
undifferentiated Caco2	1.58E+04	2.37E+04	6.67E-01
differentiated Caco2	5.59E+04	7.23E+04	7.73E-01
differentiated Caco2	5.88E+04	8.38E+04	7.02E-01
differentiated Caco2	5.02E+04	6.60E+04	7.61E-01
differentiated Caco2	6.54E+04	5.81E+04	1.13E+00

Figure 4:

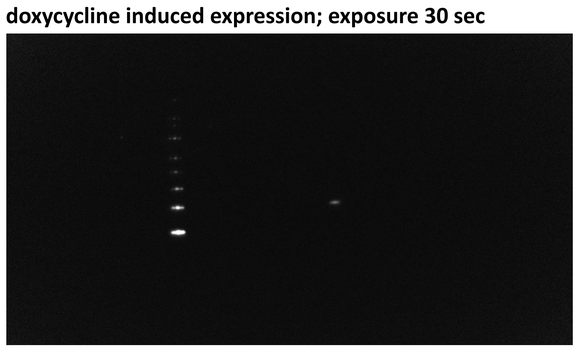
Western Blot comparing Car3 expression in undifferentiated and differentiated Caco2 cells; exposure 1min



Lane 1: undifferentiated Caco2
Lane 2: undifferentiated Caco2
Lane 3: differentiated Caco2
Lane 4: differentiated Caco2
Lane 5: mouse cells (positive control)
Lane 6: HeLa cells (negative control)
Lane 7: SKIP
Lane 8: magic marker
Lane 9: color marker

Figure 5:

Western Blot comparing Car3 levels in Caco2 cells with doxycycline induced expression; exposure 30 sec



Lane 1: color marker
Lane 2: magic marker
Lane 3: SKIP
Lane 4: Caco2
Lane 5: Caco2, Car3 (no dox)
Lane 6: Caco2, Car3 + 1mg/ml dox
Lane 7: Caco2, Car3 + 1mg/ml dox
Lane 8: Caco2, Car3 + 3 mg/ml dox
Lane 9: Caco2, Car3 + 3 mg/ml dox
Lane 10: positive control (mouse skeletal muscle) – band visible

CONCLUSIONS

- MTT assay was able to reliably detect effects of oxidative damage on survival and maturation
- While increase in Car3 expression in differentiated Caco2 cells compared to undifferentiated Caco2 was detectable in PCR, level was too low for Western Blot detection
- Results were inconclusive for whether there was an increase in Car3 in the Caco2/doxy+ system
- **Understanding the role of Car3 in IEC may provide new treatment options for patients with inflammatory bowel diseases**
- **Remains unclear how Car3 interacts with the insulin receptor in IEC and whether this plays deterministic role in the course of colitis**

ACKNOWLEDGEMENTS

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