Resveralogues protect HepG2 cells against cellular senescence induced by hepatotoxic metabolites

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Introduction

Progressive liver disease and dysfunction cause toxic metabolites including ammonia and unconjugated bilirubin to accumulate in plasma. As the population ages alternatives to liver transplantation become increasingly important. One approach for use as a bridge to transplant or recovery is the use of bioartificial liver systems (BALS) containing primary or immortalised hepatocytes as ex-vivo replacements or supports for endogenous liver function. However, exposure to the hepatotoxic metabolites present in plasma causes the rapid failure of these cells to carry out their primary metabolic functions despite remaining viable [1,2]. Hypothesing that components of the hepatotoxic mixture of metabolites and cytokines that accumulate in plasma during liver failure induce cellular senescence and that this altered phenotype drives the failure to detoxify patient plasma in BALS leading to device and clinical endpoint failure.

Methods

HepG2 cell populations, grown in both standard two-dimensional tissue culture systems(2-D) and three-dimensional cultures (3-D) on novel alginate-modified HEMA-MBA cryogels, were exposed to physiologically reflective concentrations of hepatotoxic metabolites and cytokines for six hours. Following treatment, cultures were profiled for growth fraction (by EdU and Ki67 labelling), and senescent fraction (QPCR for p53,p16 and p21 as well as senescence-associated-β galactosidase). Albumin and urea synthesis was measured at 2-D and 3-D surfaces after exposure to hepatotoxins. Simultaneously the potential for SIRT1 activating and non-activating resveralogues to protect HepG2 populations from hepatotoxic metabolites were evaluated.

Results

HepG2 cells were forced into senescence by the toxic metabolites in under six hours (as measured by loss of thymidine analogue incorporation EdU or detectable Ki67 staining) which is associated with a ten to twenty-fold reduction in the capacity of the cultures to synthesise albumin or urea (figure1). This state of senescence induced by liver toxins (SILT) can be prevented by preincubation with either 2-5µM resveratrol, or a series of novel resveralogues with differential capacities to scavenge radicals and activate SIRT1.

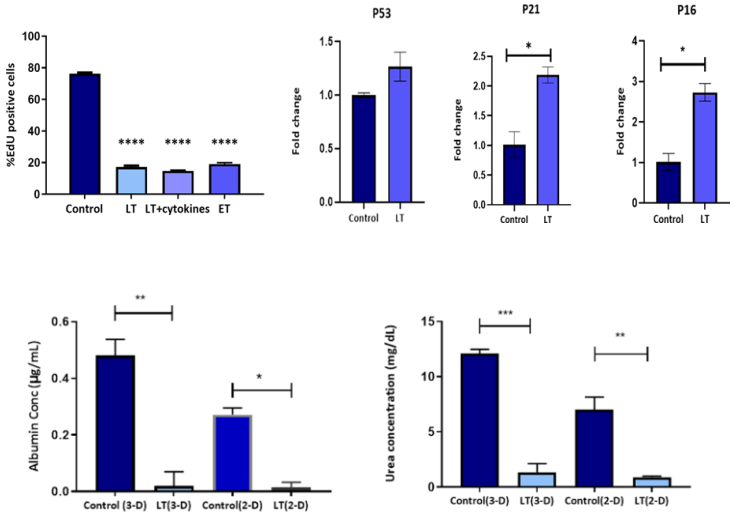

*Figure1.Six-hour treatment with liver toxins cocktail (LT) induces senescence in HepG2 cells as efficiently as etoposide treatment (ET) as measured by loss of labelling index (top left) and induction of the classical senescence markers p21 and p16 (top right). Senescence triggers loss of urea and albumin synthesis in HepG2 (bottom left & right) in both 2- (2-D) or 3-dimensional cultures (3-D). All data ±SD, *p<0.05,**p<0.01, ***p<0.001,****p < 0.0001.*

Discussion

Senescence Induced by Liver Toxins appears to be a previously unrecognised but critical barrier to the deployment of BALS and to the development of artificial organs more generally which can now be overcome using small molecules that are safe for human use at concentrations readily achievable *in vivo*.

References

1. Abrahamse et al, International journal of artificial organs, 25(10) (2002), pp. 966-974.
2. Struecker et al, Nature Reviews Gastroenterology and Hepatology. 11(3) (2014) pp166- 176.