ELAD C3A CELLS MAY IMPACT LIVER REGENERATION THROUGH SECRETED FACTORS
Patricia W Bedard, PhD, Jason Lapetota, BS, Jessica Van Allen, BS, Nancy Heredia, BS, George K. Michalopoulos, MD, LE Kandeen, PhD

Research and Development, Vital Therapies, Inc., San Diego, CA, 92128- Vital Therapies Employees University of Pittsburgh Medical Center, Pittsburgh, PA, United States, 15213 - Vital Therapies Scientific Advisory Board Member

BACKGROUND

The normal liver possesses enormous capacity to regenerate and replace tissue loss when damaged. However, hepatocytes of patients with hepatitis due to chronic alcohol consumption, viral infection, or other toxic insults, have both diminished replicative capacity and increased apoptotic cell death.

Liver regeneration is a highly orchestrated event involving multiple pathways and cell types. Regeneration is initiated by an innate immune response, followed by hepatocyte survival, proliferation and differentiation before the maturation of pro/fibrotic cells.

We have shown that ELAD C3A cells have been reported to respond to pro-inflammatory cytokines and key mediators of the acute-phase response, found elevated in alcohol-induced liver decompensated (AILD) patients, by expression of anti-inflammatory proteins. This has the potential to contribute to the first stage of regeneration.

Our present studies demonstrate a potential role for VTL C3A cell factors in a subsequent stage of liver regeneration, that of promoting cell survival and proliferative capacity of various liver cell types.

OBJECTIVES

The purpose of these studies was to evaluate the ability of VTL C3A cell secreted factors reported in the literature as having a beneficial effect on hepatocyte survival, replication and/or liver regeneration. Then, finding such factors, to evaluate the effects of selected factors on various liver cell types.

MATERIALS & METHODS

Vital Therapies’ (VTL) ELAD® System is an investigational human hepatic cell-based liver treatment comprised of four metabolically-active ELAD cartridges with ancillary device components and support circuitry intended to continuously treat patients with liver failure secondary to acute hepatocellular insult and alcohol use.

ELAD C3A cell spent media were assayed using contracted ELISA multiplex (Myriad) or chemiluminiscent multiplex array detection (Aushon Craplex) assays for known mitogenic, angiogenic and other regenerative factors.

A primary human hepatocyte (PHH) apoptosis model was adapted and utilized to detect apoptosis and anti-apoptotic factors in PHH (Gibco) using CD95 (Fas) and CD11a (lyo) (endothelial cells) as an example for liver failure secondary to alcohol hepatocellular insult and alcohol use.

To detect the potential effects of these factors on the cells of the liver, a series of cell-based models were developed.

PHH apoptosis model. ELAD CM administered 3 h prior to challenge of PHH cultures with a Fas-agonist antibody significantly reduced PHH apoptosis, as measured by annexin V staining (Fig. 2).

ELAD CM-treated PHH maintained a normal size and cell membrane morphology vs. Fas-agonist treated, as visualized by annexin V staining (Fig. 2).

An aortic endothelial cell (HAEC) angiogenic factor model was developed as a surrogates for liver sinusoidal EC (LSEC) by co-culture with CD95+ cells. Western blot with HAEC cells or treated with ELAD CM prepared by static incubation of Williams E medium in a mature ELAD C3A cell cartridge. ELAD CM was measured by Caspase-Glo® assay (Promega) binding to Annexin V (Roche) and Western immunoblot (primary antibodies, Cell Signaling).

Liver regeneration is a highly orchestrated event involving multiple pathways and cell types. Metabolically-active VTL C3A cells offer the potential of contributing to liver regeneration by impacting these cell types and pathways in ways that non-cell-based therapies are unlikely to achieve.

To begin to assess the potential effects of these factors on the cells of the liver and to better understand the mechanisms of action of the ELAD System, CM was administered to PHH in culture. ELAD CM was found to promote survival in both untreated cells and those induced toward apoptosis by a Fas-agonist antibody (Fig. 1).

A similar model was shown to be dependent upon AR, the most necessary factor for both liver regeneration and tumor hepatocyte growth. However, AR was not protective at 20 nM in our model (data not shown [DNS]). To determine if the pro-survival effect of ELAD CM was mediated by FGF, we evaluated cell lyse from untreated and Fas-agonist-treated VTL C3A cells with and without ELAD CM, by Western immunoblot (Fig. 3).

We found phosphorylation of AKT, ERK2/J, and STAT3, suggesting activation of the EGFR. The role of these factors in the clinical setting remains to be determined.

The pro-PHH survival effect of ELAD CM is consistent with data showing CM from HepG2 cells (parental cell line to C3A cells) contains essential factors to support human fetal hepatocyte growth in culture.

LSEC and bone marrow progenitor cells of HMSCs (BMSC) have been shown to participate in liver regeneration by increased production of HGF in response to hepatic VEGF. The effects of ELAD C3A cell secreted VEGF was evaluated in an HAEC co-culture surrogate model of LSEC (due to greater availability of HAEC). Although HGF was not significantly increased (DNS), secretion of PI GF increased 5-fold over untreated HAEC, 24 h after administration of ELAD CM. The HAEC continued to produce increased PIGF in the presence of ELAD CM for the 7-day duration of the model (Fig. 4). PIGF is proposed to recruit VEGFR1 stem cells from bone marrow for organogenesis.

Both SFC and EPO work synergistically with G-CSF, SFC to induce proliferation in cholangiocytes and hepatocytes, and EPO to increase survival in patients with decompensated cirrhosis. MSCs secreted factors increase in VTL C3A cells response to IL-18 and IL-DNS.

CONCLUSIONS

VTL C3A cells produce a variety of secreted factors with well-established roles in cell growth, survival, regeneration, and hepatopoiesis. Our cell-based models prevented PHH apoptosis and enhanced HAEC PIGF secretion. This may facilitate liver regeneration, directly by stimulation of hepatocytes, or indirectly by attraction of resident cell populations during ELAD treatment.

REFERENCES


CONTACT INFORMATION

1170 Avenue of Science, Suite 200, San Diego, CA 92128, biotrans@vitaltherapies.com

#1770