# POSTER SESSION XI

## A565

TITLE: HEPATIC REGULATION OF FIBRINOLYSIS: IMPLICATIONS FOR COAGULOPATHY DURING LIVER TRANSPLANTATION

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Previous clinical investigations have demonstrated the occurrence and morbidity associated with primary fibrinolysis during liver transplantation. This coagulopathy has been associated with an increased activity of tissue-type plasminogen activator (tPA) and decreased activity of its inhibitor, plasminogen activator inhibitor type 1 (PAI-1) following reperfusion of the hepatic graft.[1] Rapid hepatic clearance usually regulates the action of tPA, and hepatic clearance of tPA appears to require PAI-1.[2] Because liver preservation is known to damage non-parenchymal liver cells — sinuoidal endothelial cells (E), and lipocytes (L) — to a greater extent than parenchymal cells, hepatocytes (H),[3] we measured the binding of tPA and the production of PAI-1 by these different types of hepatic cells *in vitro*.

Methods: Hepatic cell suspensions were prepared from anesthetized rats using enzymatic perfusion of the liver and purified with gradient centrifugation and elutriation. Primary cultures of H, E, and L cells were maintained in tissue culture dishes for 2 days before study. Recombinant tPA was iodinated with the chloramine-T method, and used to measure cell specific binding of tPA determined over a range of tPA concentrations = 0-32 nM. Binding was expressed as fmol of tPA /$10^6$ cells, and was determined in triplicate samples. Production of PAI-1 by cultured cells was determined by measuring the amount of PAI-1 secreted into serum-free culture medium over 24 hr using a specific enzyme linked immunosorbent assay for PAI-1. PAI-1 production was expressed as ng PAI-1 / ug of DNA (a measure of cell number) and measured in triplicate samples. Comparisons of tPA binding at 16 nM concentration and PAI-1 production between hepatocytes (H) and non-parenchymal cells (E,L) were made using ANOVA and $P < 0.05$ was considered significant.

Results: All hepatic cell types demonstrated specific binding of tPA with saturation at tPA concentrations > 8 nM. At 16 nM (when binding sites were fully saturated), H cells had greater binding than E or L cells (table). In contrast, the production of PAI-1 was greater in the non-parenchymal cells (E and L) compared to the parenchymal hepatocytes (H).

Discussion: Our results indicate that hepatic parenchymal and non-parenchymal cells have different patterns of tPA binding and PAI-1 production. In particular, the non-parenchymal cells showed more production of PAI-1 than the hepatocytes. Because PAI-1 is necessary for the binding of tPA to hepatic cells,[2] preservation injury to non-parenchymal cells may reduce the production of PAI-1 by those cells, impair the hepatic clearance of tPA and account for the development of primary fibrinolysis seen following graft reperfusion. Further studies are required to elucidate the disruption in hepatic regulation of fibrinolytic activity following liver graft reperfusion.

References:

1. Transplantation 47:978--984, 1989
2. J Biol Chem 264:7228-7235, 1989
3. Transplantation 46:178-181, 1988

Table: Binding of tPA (at 16 nM) and Production of PAI-1 by Hepatocytes (H), Sinusoidal Endothelial Cells (E), and Lipocytes (L). (Mean ± Std Dev)

| | H | E | L |
|---|---|---|---|
| tPA Binding (fMol/$10^6$ cells) | 35.8 ±4.0 | 9.7 ±2.6* | 0.9 ±0.8* |
| PAI-1 Production (ng PAI-1/ug DNA) | .17 ±.02 | .48 ±.05* | .82 ±.09* |

Mean ± SD * — $P$ <0.05 compared to H

## A566

TITLE: HALOTHANE CAUSES A BIPHASIC TENSION RESPONSE TO K+ IN ISOLATED PULMONARY ARTERY

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A growing body of evidence indicates that pulmonary vascular regulation is mediated by a complex interaction among neural, humoral and local mechanisms. Compared with the conscious state, halothane (HAL) anesthesia causes flow-independent pulmonary vasoconstriction that is not mediated by reflex neurohumoral activation, by cyclooxygenase metabolites, nor due to the effects of general anesthesia and controlled ventilation[1]. The *direct* effects of Hal on isolated pulmonary blood vessels remain largely unexplored. Our objective was to test the hypothesis that HAL attenuates developed tension in response to $K^+$ in isolated bovine pulmonary artery.

Methods: We investigated the effects of HAL (2 × MAC, 1.8%) on the dose-response to potassium ion ($K^+$) in segments of bovine pulmonary artery (30 rings from n=5 animals). Rings ≈4mm wide were cleaned of connective tissue and were mounted in organ chambers containing Krebs-bicarbonate buffer (37°C) gassed with 95%air-5%CO2. Rings were stretched to their optimal length and then exposed to high [$K^+$] (50mM) to elicit a maximal contraction ($K_{max}$). Only rings with intact endothelium, as determined by relaxation to acetylcholine ($10^{-6}$M), were studied. Incremental $K^+$ dose-response curves were performed in control (−HAL) and HAL (+HAL) groups. For each lung studied, rings were randomly assigned to both −HAL and +HAL groups. +HAL rings were first exposed to a given $K^+$ dose. After achieving a steady-state contractile response, the buffer was rapidly replaced by buffer with the same [$K^+$], pre-equilibrated with HAL. At the same time HAL was also added to the ventilating gas. Data were expressed as the percent of $K_{max}$ response using each ring as its own control. Dose-response curves were analyzed using a logistic curve-fitting model.

Results: HAL produced a transient increase in developed tension which rapidly decreased to a plateau (figure inset shows single +HAL ring response at [$K^+$]=20mM). Neither the minimum ([$K^+$]=10mM) nor maximum ([$K^+$]=40mM) contractile responses were different among the three $K^+$ dose-response curves. At low [$K^+$], HAL produced a transient vasoconstriction with no change in plateau contraction compared to control. Conversely, at high [$K^+$] HAL caused only a decreased in the plateau contraction compared to control. HAL caused an increase in the $ED_{50}$ from 25.3±0.5mM to 28.5±1.0mM in the +HAL (plateau) response ($p<0.01$, figure).

Discussion: These data indicate that HAL attenuated the steady-state contractile response of isolated bovine pulmonary artery to extracellular $K^+$. The transient vasoconstriction caused by HAL at low [$K^+$] is consistent with the observation[2] that HAL releases intracellular $Ca^{2+}$. Since high [$K^+$] opens voltage-dependent $Ca^{2+}$ channels, the vasodilation caused by HAL at high [$K^+$] could be explained by a decrease in $Ca^{2+}$ influx or altered sensitivity of the contractile apparatus. Further studies are needed to explore these possibilities.

1.Am J Physiol 259:H74-H83, 1990 2.Anesthesiology 71:409-417,1989