Quantification Of Collagenogenesis In Experimental Early-stage Alcoholic Liver Fibrosis Using cis-4- [^{18}F]fluoro-L-Proline MicroPET

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1. Early-stage alcoholic liver fibrosis (early-stage ALF) is difficult to diagnose, as patients are largely asymptomatic, and there are no non-invasive imaging techniques available.
2. Hepatic stellate cells (HSC) play a central role in the liver fibrosis by secreting fibrogenic factors to produce collagen and thereby propagate fibrosis. Proline is the key amino acid in collagen synthesis.
3. Previously, we have identified [^{18}F]fluoro-proline that could serve as a PET imaging biomarker for detecting alcoholic liver fibrosis in a LPS induced acute steatohepatitis mice model (PUBMED: 32500081).

AIMS:

To establish *cis*-4-[^{18}F]fluoro-L-proline ([^{18}F]fluoro-proline) microPET technique for quantification of collagenogenesis in HSC of experimental early-stage ALF.

METHODS:

Early-stage ALF models were setup by modified Lieber-DeCarli liquid ethanol diet for 8 weeks along with intraperitoneal (IP) injection of CCl4(0.5ml/kg, biw) at 5-8th weeks and controls received isocaloric liquid diet and IP injection of PBS.

In Vitro [^{3}H]proline uptake by HSC isolated from rat livers and collagen type 1 production in culture medium were tested using liquid scintillation counter and Elisa.

Ex Vivo liver tissue collagen type 1 and proline transporter protein were compared between ALF rats and mice. [^{3}H]proline uptake ex vivo was quantified in liver tissues tested with unlabeled proline and transporter inhibitor benztropine at multiple increasing doses.

In Vivo Dynamic and static [^{18}F]fluoro-proline microPET/CT imaging was performed in ALF mice and controls.

RESULTS:

1. *In Vitro* HSC experiments

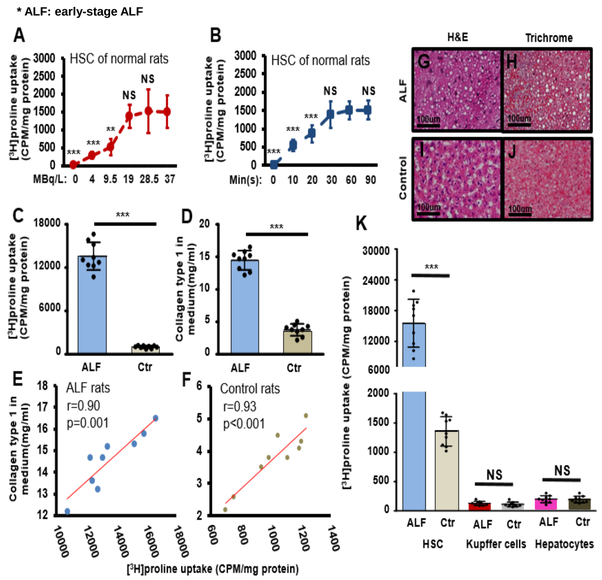

Figure 1. Correlation of [^{3}H]proline uptake to collagen type 1 production.

2. *Ex Vitro* liver tissue experiments

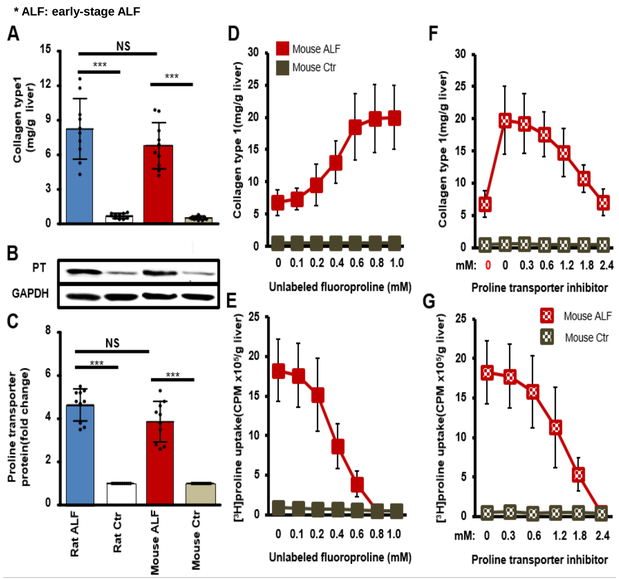

Figure 2. Collagen level and proline transporter protein expression in the livers of rats and mice with ALF and controls. and determination of proline transporter specificity of [^{3}H]proline uptake by using unlabeled fluoro-proline and its transporter inhibitor.

3. *In Vivo* mice experiments

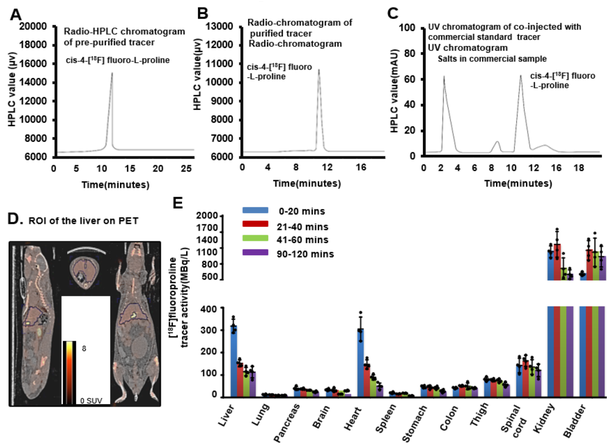

Figure 3. Dynamic and static [^{18}F]fluoro-proline PET imaging.

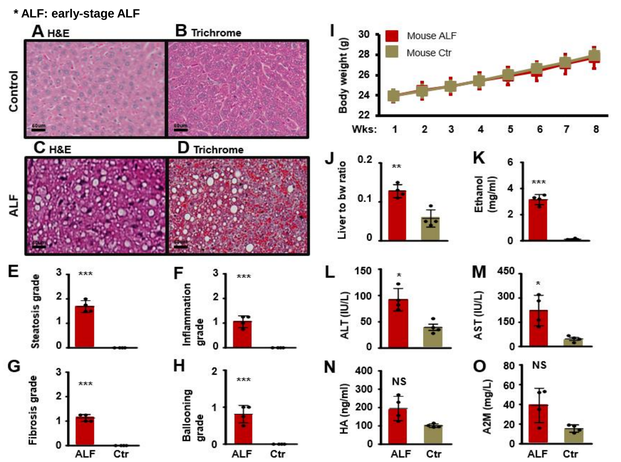

Figure 4. Histopathology, body weight and blood biochemistry changes in ALF mice and control mice.

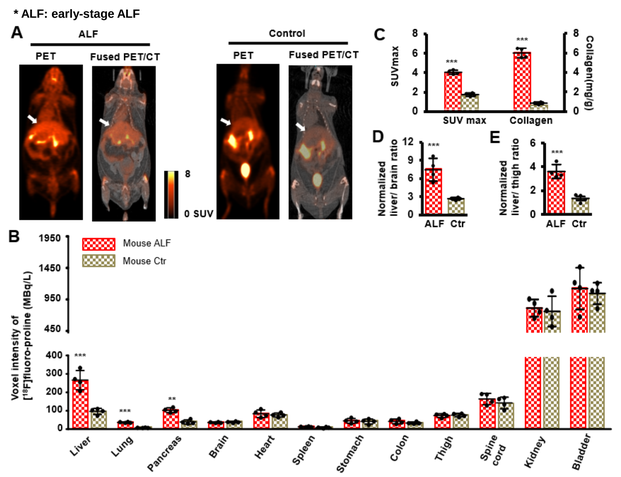

Figure 5. Static [^{18}F]fluoro-proline fused PET/CT imaging in ALF mouse model compared with control mice.

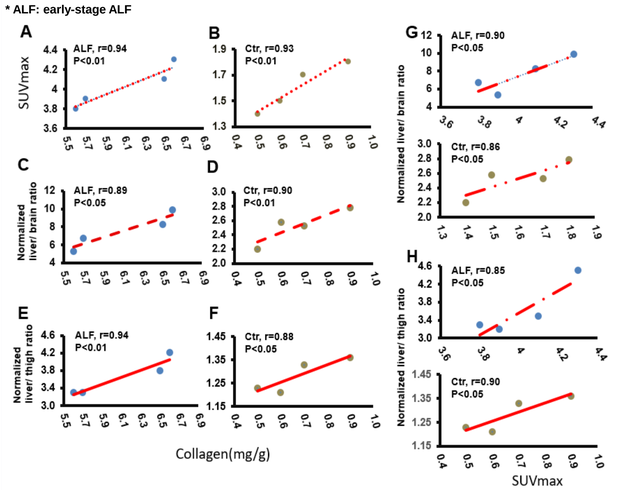

Figure 6. Correlation between collagen in liver, SUVmax, Normalized liver to brain ratio and Normalized liver to thigh ratio in ALF mice and control mice.

CONCLUSIONS:

In Vitro [^{3}H]proline uptake can be used to quantify collagenogenesis in HSC from rat model.

Ex vivo liver tissue collagen type 1 levels were almost equal between rat and mice models, so was liver proline transporter protein expression.

In vivo [^{18}F]fluoro-proline microPET/CT imaging in mouse model showed the similar tendency for quantifying collagenogenesis as in in vitro HSC in rat model.

[^{18}F]fluoro-proline microPET/CT is feasible for quantifying collagenogenesis in HSC of experimental early-stage ALF, which may be used as a promising noninvasive diagnostic tool.

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