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A Novel Method Using ^2H_2O to Measure Collagen Turnover in HIV-infected Individuals

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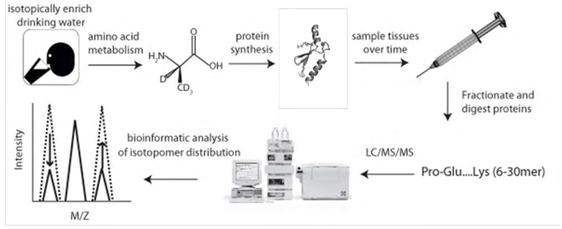
Background

- Lymphoid tissue fibrosis occurs early in HIV infection and is thought to be a central factor in the pathogenesis of HIV. Lymphoid fibrosis has been associated with impaired T cell homeostasis, chronic T cell dysfunction and chronic inflammation.
- Lymphoid fibrosis persists during effective antiretroviral therapy (ART), and has been associated with suboptimal immune reconstitution. The potential role of anti-fibrotic agents in HIV-infection is an area of active investigation.
- Collagen deposition is a dynamic process. Traditional histologic techniques cannot distinguish between new collagen deposition and any fibrosis that may have accumulated during the untreated phase of HIV infection.
- We investigated the use of a technique utilizing ^2H_2O (heavy water or D20) intake to quantify new collagen deposition in gut-associated lymphoid tissue (GALT) in treated HIV-infected individuals.

Methods

- Eighteen HIV-infected individuals on suppressive ART received outpatient oral doses of ^2H_2O for 4 weeks and subsequently underwent colorectal biopsies.
- The ^2H-labeled hydrogen atoms are incorporated into newly synthesized amino acids and proteins during the period of heavy water intake, but not into proteins synthesized prior to heavy water labeling.
- ^2H_2O enrichment in total body water (TBW) in each patient was quantified from weekly salivary swabs during ^2H_2O administration.
- Single 3mm biopsy pieces were subjected to sequential physical and chemical extraction methods to fractionate collagen molecules based on solubility in guanidine HCl.
- Guanidine-soluble collagen represents more recently synthesized, less cross-linked collagen.
- Incorporation of the ^2H_2O label into collagen in GALT was quantified by liquid-chromatography-mass spectrometry and a fractional synthesis rate (FSR; per week) was calculated for guanidine-soluble and -insoluble GALT collagen.

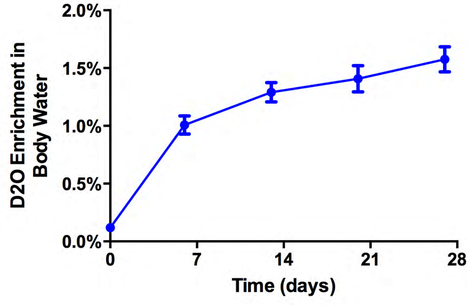
Overview of the methods of $^2H_20$ labeling and measurement



Results

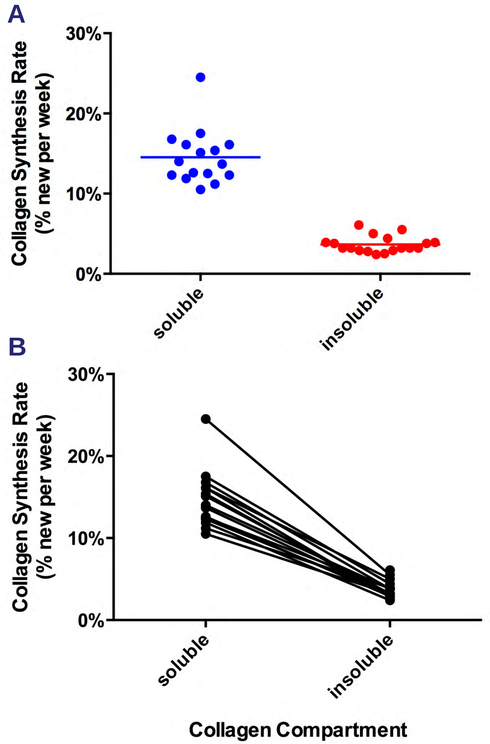
- Outpatient administration of ^2H_2O was well tolerated.
- After 28 days of ^2H_2O intake, subjects reached the target goal of 1-2% ^2H_2O enrichment in TBW.

Average percentage of $^2H_20$ enrichment in total body water over the 4 week intake period (Mean with SEM shown, n=18)



- The median fractional synthesis rate of guanidine-insoluble collagen in GALT was 3.2% per week (IQR 2.9%-3.9%), or a median half-life of ~22 weeks.
- Guanidine-soluble collagen in GALT turned over much more quickly, with a median fractional synthesis rate of 12.8% per week (IQR 12.3%-16.1%), or a median half-life of 5.4 weeks.

Fractional synthesis rate of guanidine-soluble and –insoluble collagen in GALT (A: Dot-plot with medians shown, B: paired plot)



- In comparison with other human studies where collagen fractional synthesis rate was measured by ^2H_2O labeling, the rates in GALT were 5-fold higher than the fractional synthesis rates of skin collagen in healthy volunteers, but similar to the synthesis rates of liver collagen in patients with fibrotic liver disease.

Conclusions/Implications

- Heavy water labeling is a safe, well-tolerated, and clinically feasible method to measure collagen turnover in GALT of treated HIV-infected individuals.
- The relatively high rates of collagen turnover observed in the GALT during treated HIV disease (guanidine soluble collagen half-life ~ 5 weeks) suggest that collagen deposition and turnover is ongoing and dynamic in this setting, and may therefore be amenable to intervention.
- The ^2H_2O labeling technique provides an assessment of collagen turnover rates, in contrast to static metrics from traditional histologic techniques.
- This method may be useful in future studies to evaluating the effects of anti-fibrotic therapies in HIV infection.

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