Analysis of fibrosis in acute and chronic fibrosis models: total collagen versus dynamic collagen

Anita van den Hoek[1]
Jessica Snabel[1]
Nanda Keijzer[1]
Hans Princen[1]
Jan Verheijen[2]
Reinout Stoop[1]
Arianne van Koppen[1]
Natascha van Lent[2]
Roeland Hanemaaijer[1,2]

[1]TNO Metabolic Health Research, Leiden, The Netherlands
[2]QuickZyme Biosciences B.V., Leiden, The Netherlands

Background

Quantification of extracellular matrix production is of key importance in fibrosis research, with collagen being one of the major matrix components. In tissues and tissue extracts collagen solubilization is poor, and therefore the best way to quantify collagen in tissues is via measurement of hydroxyproline upon tissue hydrolysis.

Aim

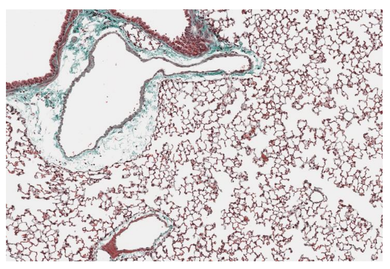
In this study we compared **total collagen analysis** (Hyp) and **dynamic collagen analysis** (deuterated Hyp) in acute and chronic in vivo fibrosis models.

Methods

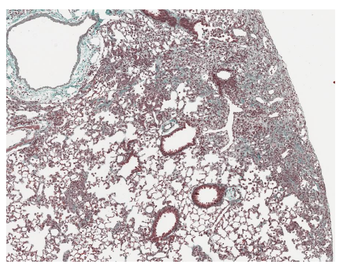
- We performed total collagen analysis using the colorimetric QuickZyme Total Collagen assay (microtiter plate format). Dynamic (newly synthesized) collagen analysis was assessed by the incorporation of deuterium into hydroxyproline by supplying D_2O in the drinking water and analysis by LCMS.
- Both methods were compared using an **acute fibrosis model** (bleomycin-induced lung fibrosis; 21 days; labelling with D_2O for last 2 weeks) and **a chronic fibrosis model** (high fat high cholesterol diet-induced model for NASH with associated hepatic fibrosis in ApoE*3Leiden.CETP mouse; 25 weeks; labelling with D_2O for last 2 weeks).
- Histology, total collagen and collagen synthesis rate were assessed.

ACUTE FIBROSIS MODEL

Clear fibrosis was induced



PBS



Bleomycin

Figure 1. Fibrosis induction in bleomycin-induced lung fibrosis model vs. vehicle controls.

In the acute bleomycin-induced lung fibrosis model 21 days after o.p. administration of bleomycin clear fibrosis was observed in the left lung lobe (Fig. 1).

Increased induction of new collagen vs. total collagen

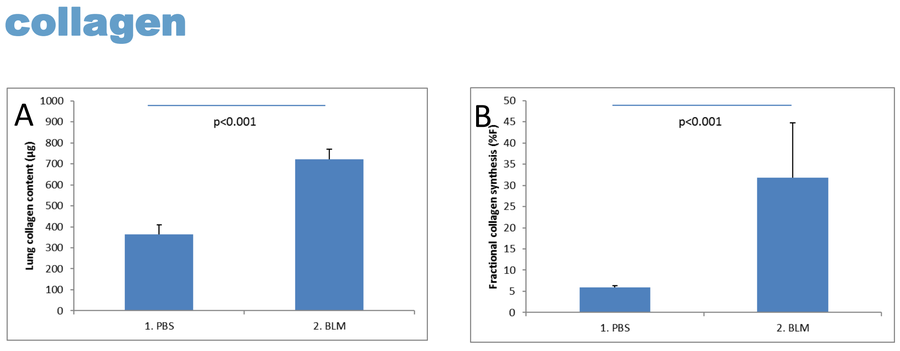


Figure 2. Total collagen content of the medial and accessory lung lobes (A) and new collagen content in the cranial lung lobe (B) of bleomycin induced C57Bl6J mice as compared to vehicle controls. 2B Values are presented as the mean ± SD.

Using total collagen analysis a significant (P<0.001) 2.0-fold increase in total collagen was observed in the lung lobes observed upon bleomycin administration (Fig. 2A). Using dynamic protein analysis a significant (P<0.001) 5.6-fold induction in new collagen formation was observed (Fig. 2B).

CHRONIC FIBROSIS MODEL

Clear fibrosis was induced

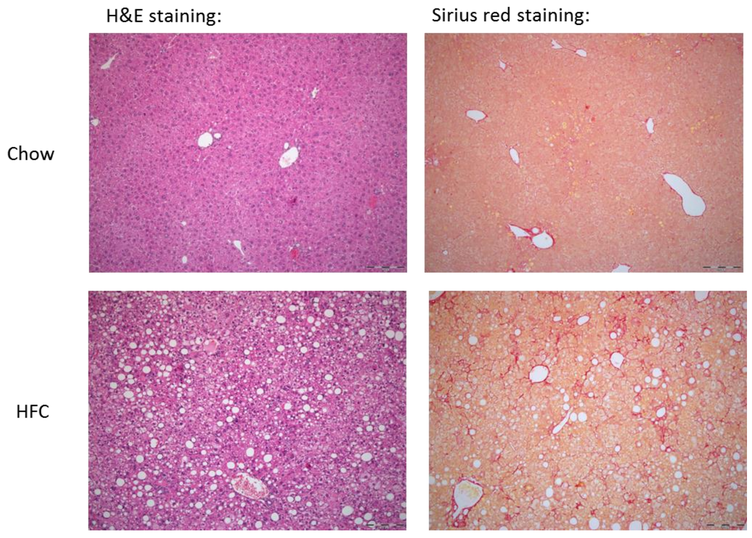


Figure 3. Fibrosis induction after high fat/high cholesterol (HFC) diet in chronic NASH/fibrosis model vs. mice fed a healthy chow diet.

Clear hepatic steatosis and fibrosis was observed after feeding animals a high fat/high cholesterol diet for 25 weeks as compared to feeding a healthy chow diet.

Similar induction of new collagen vs. total collagen

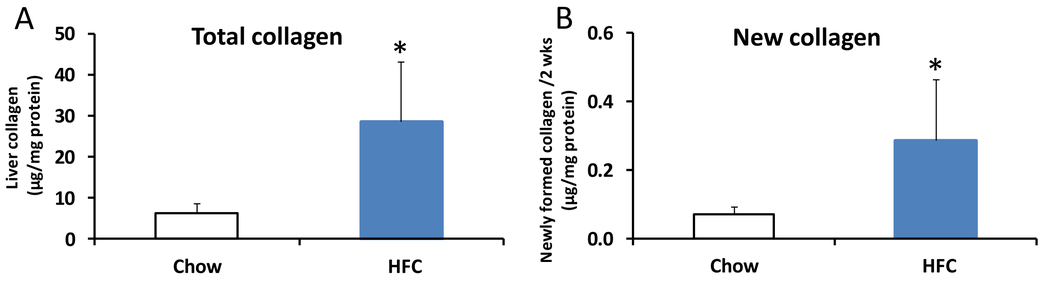


Figure 4. Total collagen content (A) and new collagen content (B) in liver from high fat/high cholesterol fed ApoE*3Leiden.CETP mice vs. mice fed a healthy chow diet. Values are presented as the mean ± SD. * P<0.001 vs. Chow control.

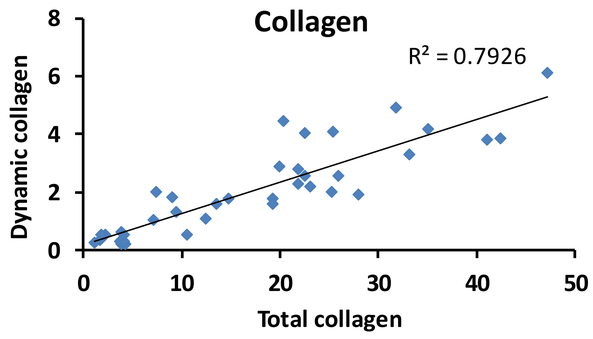


Figure 5. Correlation between total collagen levels and new (dynamic) collagen levels in livers of chow and high fat/high cholesterol fed ApoE*3Leiden.CETP mice.

Total collagen analysis showed a significant (P<0.001) 4.5-fold increase in collagen in HFC –fed animals. Dynamic collagen analysis upon 2 weeks administration with D_2O showed a significant (P<0.001) 4.2-fold increase in new collagen (Fig. 4).
A strong correlation was observed between the total collagen and the dynamic collagen analyses (Fig. 5), indicating that in chronic fibrosis models such as diet induced NASH/fibrosis, both collagen analyses give a similar window for assessing therapeutic intervention.

Conclusions

- In acute fibrosis models, especially targeted on organs with high intrinsic collagen levels such as lung, the use of dynamic collagen analysis increases the window for assessing prophylactic and therapeutic intervention.
- In chronic fibrosis models such as diet-induced NASH/fibrosis, both total collagen and dynamic collagen analyses give a similar window for assessing therapeutic intervention, but the chromogenic total collagen analysis is much faster, easier and cheaper.