Development of an LC-MS method for the selective detection of collagen types

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Introduction

The excess production of collagen is the hallmark of fibrosis. To date, the main parameter to quantify fibrosis is the quantification of the amount of collagen present using either hydroxyproline measurements or histology. However, besides an increase in collagen amount, changes in the composition of the collagen composition also occur. Since the determination of the different types of collagens has been difficult so far, we developed a novel LC-MS method for the selective, multiplex determination of different collagen types in tissue samples.

Methods

The pretreatment steps were designed for solubilizing cross-linked collagen and were made compatible with subsequent analyte processing steps and Ultra-Performance Liquid Chromatography – tandem Mass Spectrometry (UPLC-MS/MS) analysis (Waters Acquity / Xevo-TQS). Collagen from bovine Achilles tendon and from murine kidney, lung and liver samples was used for method development.

Target peptides were assigned for murine collagen 1α1, 3α1, 4α1, 5α1 and 6α2 on the basis of Proteome Discoverer (Thermo Scientific) analysis of experimental data and database searches. For each target peptide a stable isotope labeled internal standard (SIL IS) peptide was incorporated in the method to obtain accountable LC-MS results.

After pretreatment of the murine lung samples, the remaining material was solubilized and subjected to reduction/denaturation, alkylation, tryptic digestion and LC-MS analysis.

Time course bleomycin-induced lung fibrosis

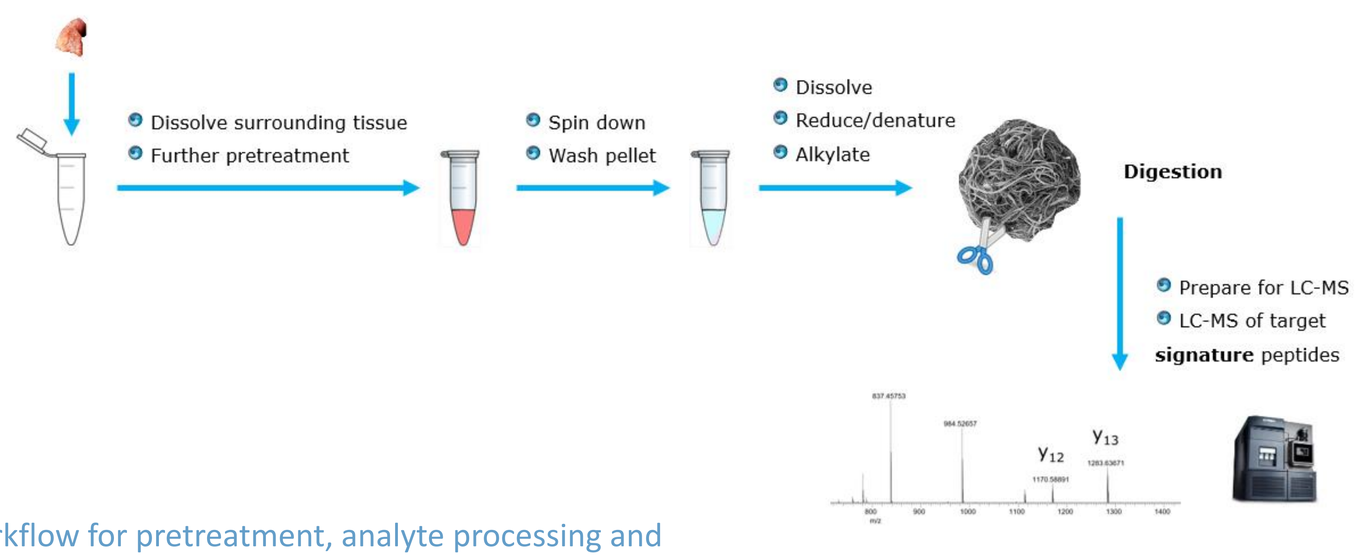
Lung fibrosis was induced in 12 week old male C57Bl/6 mice by oropharyngeal administration of bleomycin. Mice were sacrificed weekly (n=12) to determine the level of fibrosis at each time point. Naïve (n=5) and PBS-induced animals (n=7; sacrificed after 21 days) were used as controls. The caudal lung lobe was used to determine lung wet weight, collagen content (Quickzyme collagen assay) and different collagen types. The left lung lobe was used for histological analysis

Conclusion:

This method allows the quantification of specific murine collagen types in (fibrotic) tissue samples, thereby allowing to further investigate the role of changes in collagen composition on fibrosis development and opportunities for better defined disease analysis. Based on the homology of the target peptides between mouse and human, this method should also be applicable for human tissue.

Results:

Figure 1



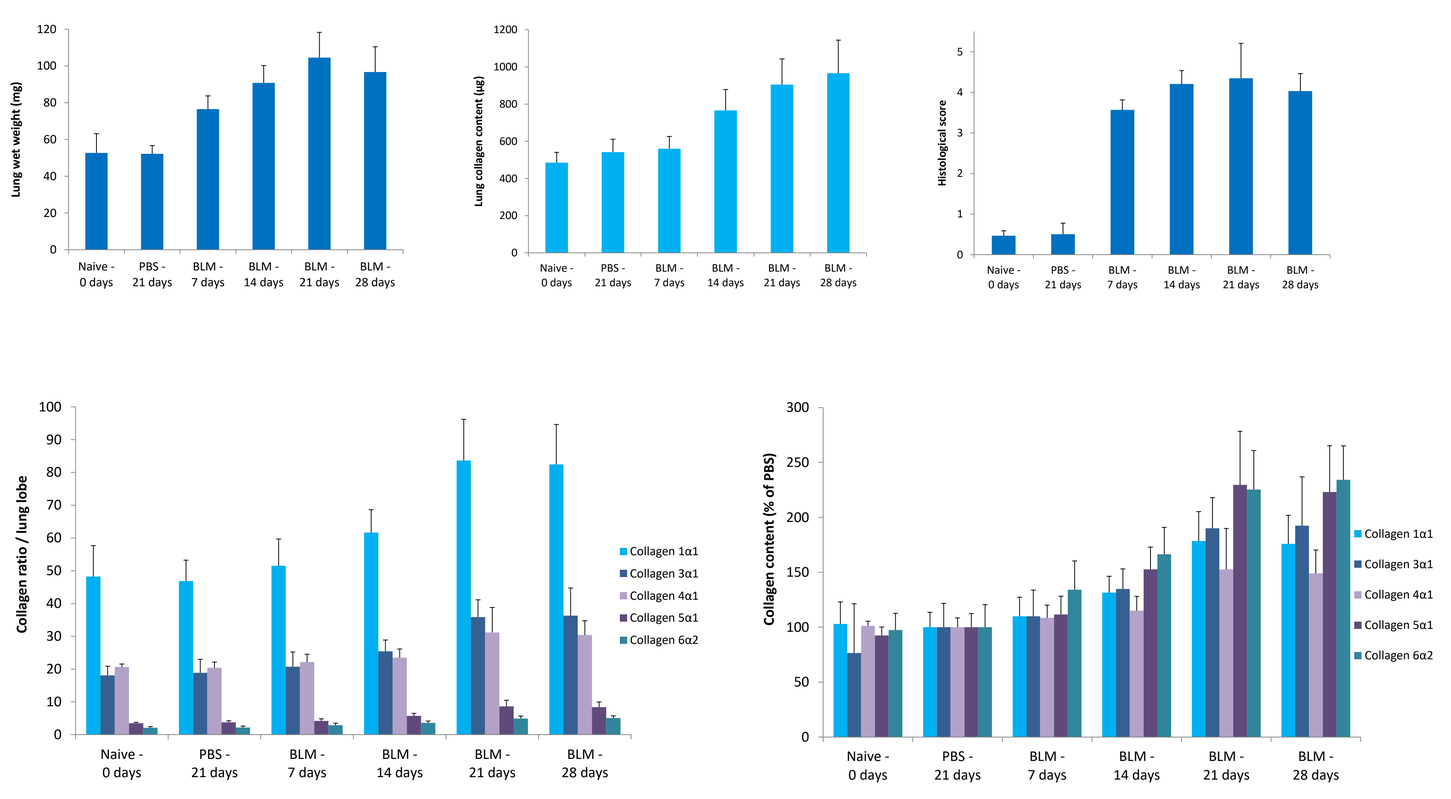
Workflow for pretreatment, analyte processing and LC-MS determination of target peptides

Figure 2

Peptide	D1 kidney	E1 lung	F1 liver	Achilles tendon
	Murine			Bovine
Col1a1_GFSGLDGAK (0 Hyp)	40203	39648	11330	1033700
Col1a1_GLTGPIGPPGPAGAPGDK (2 Hyp)	139982	148283	24941	1103753
Col1a1_GLTGSPGSPGPDGK (2 Hyp)	428862	469647	151984	10500006
Col1a1_GSPGEAGRPGEAGLPGAK (3 Hyp)	158783	150062	50509	4769747
Col1a2_GFPGTPGLPGFK (4 Hyp)	105308	102552	31231	3187440
Col1a2_VGAPGPAGAR (1 Hyp)	60415	69430	24271	1232414
Col4a1_GPPGGVGFPGSR (2 Hyp)	395022	350515	22569	10099
Col6a2_VFAVVITDGR (0 Hyp)	111529	73149	19055	25759
Col6a3_ALEFVAR (0 Hyp)	16007	8657	1927	5490

Target peptides homologous between *Mus musculus* and *Bos taurus* collagen were used to compare the results obtained for bovine achilles tendon with the results obtained for murine kidney, lung and liver samples. The color gradient was set per peptide from red (low peak area) to green (high peak area).

Figure 3 Changes in collagen types during the course of bleomycin induced lung fibrosis



Time course effects of a single o.p. bleomycin administration on caudal lung wet weight and collagen content, left lung lobe histological score and content of five different collagen types in the caudal lung lobe presented as ratio versus SIL IS and as percentage from PBS average.