Multiple administrations of bleomycin induce more chronic lung fibrosis

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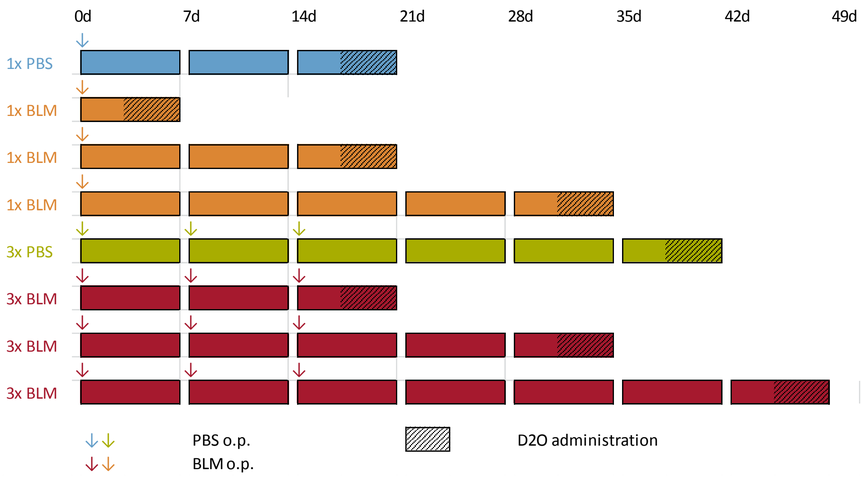
Introduction

Bleomycin-induced lung fibrosis is self-limiting and resolves over time. We investigated whether multiple administrations of bleomycin results in more chronic fibrosis development as characterized by an augmented molecular pro-fibrotic response and a prolonged formation of new collagen.

Methods

C57BL/6 mice received either one (d0) or three doses (d0, 7, 14) of bleomycin (BLM). Animals were sacrificed after 1, 3 or 5 weeks after final BLM dose. The transcriptome was determined using gene microarray analysis. New collagen formation was quantified using a proteomics approach in which deuterated-water in the drinking water was used to label newly formed proteins for 4 days prior to sacrifice. Hydroxyproline and proline content was measured using HPLC.



Survival and body weight

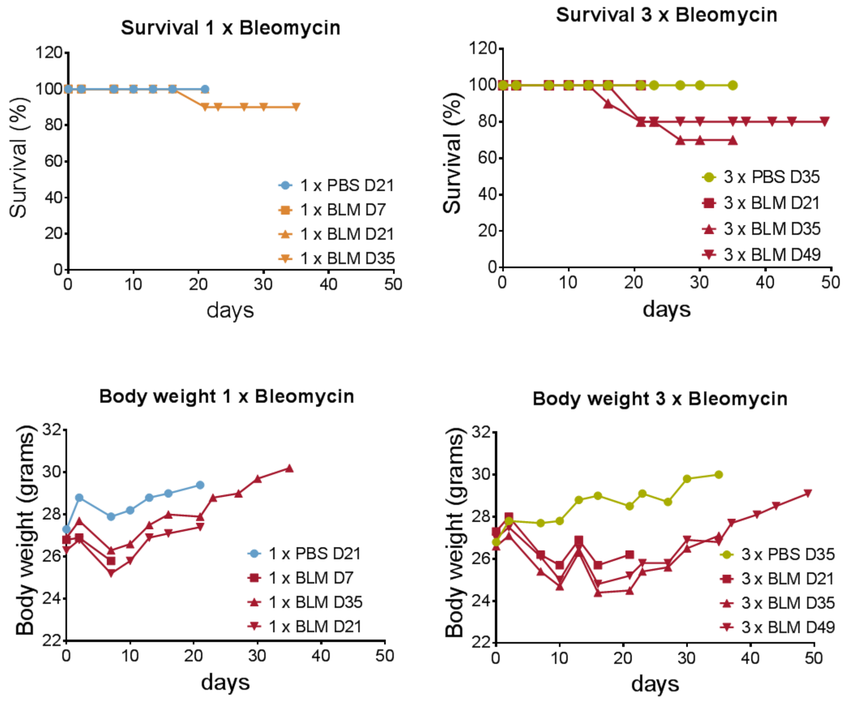


***Figure** 1: effects of single and multiple o.p. bleomycin administrations on survival and body weight*

Collagen formation, histological score and BAL

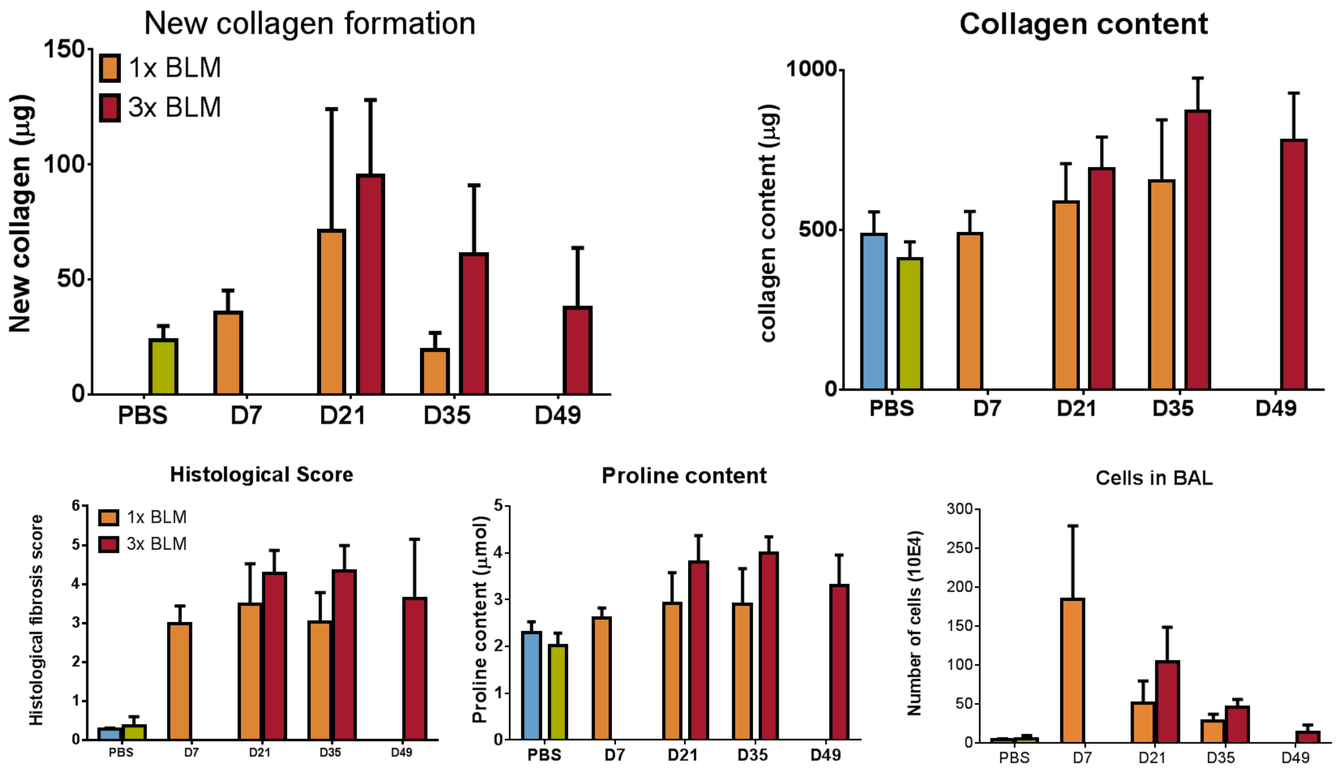
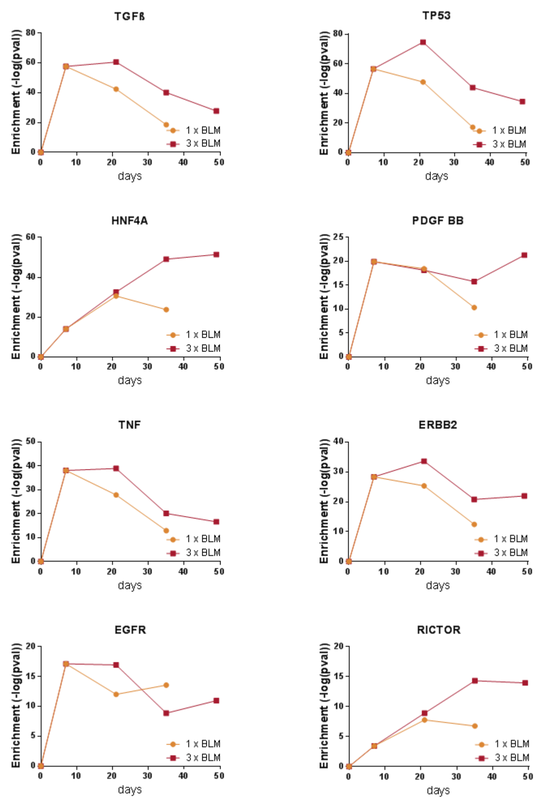


***Figure 2:** Multiple administrations of bleomycin results in prolonged collagen synthesis, higher collagen and protein content, increased histological score and a prolonged influx of cells into the BAL*

Multiple bleomycin administrations induce a stronger response on the number of differentially expressed genes

#DEG's	BLM1d7-PBS	BLM1d21-PBS	BLM1d35-PBS	BLM3d21-PBS	BLM3d35-PBS	BLM3d49-PBS	PBS3d35-PBS1d21	BLM3d49-BLM1d35	BLM3d35-BLM1d35	BLM3d21-BLM1d21
BLM1d7-PBS	3279	1818	805	2714	2008	1587	1	191	621	478
BLM1d21-PBS		3917	1533	2816	3127	2871	0	439	1026	222
BLM1d35-PBS			1826	1515	1772	1783	0	376	712	100
BLM3d21-PBS				5322	3898	3117	0	508	1369	650
BLM3d35-PBS					6316	4682	1	635	1677	403
BLM3d49-PBS						5569	1	673	1426	280
PBS3d35-PBS1d21							1	0	0	0
BLM3d49-BLM1d35								686	499	38
BLM3d35-BLM1d35									1743	144
BLM3d21-BLM1d21										923

***Table 1:** Number of differentially regulated genes based on a FDR (false discovery rate) < 0.01 compared to PBS*



Canonical Pathways	Rank -log(pvalue) 1x BLM BLM1d7-PBS	BLM1d21-PBS	BLM1d35-PBS	3x BLM BLM3d21-PBS	BLM3d35-PBS	BLM3d49-PBS
Regulation of eIF4 and p70S6K Signaling	277	129	101,5	68,5	14	1
Mitochondrial Dysfunction	199	3	1	54	2	2
PI3K/AKT Signaling	49	34	13	10	4	3
EIF2 Signaling	473	285	114	187	83	4
IGF-1 Signaling	55	1	3	6	17	5
ERK/MAPK Signaling	143	11	66	56	18	6
mTOR Signaling	212	266	200	36	10	7
Sertoli Cell-Sertoli Cell Junction Signaling	35	8	11	73	1	8
Oxidative Phosphorylation	473	83	5	158	34	9
iNOS Signaling	181	85	35	93	88	10
B Cell Receptor Signaling	75	78	308	61	6	11
Germ Cell-Sertoli Cell Junction Signaling	42	18	27	65	9	12
Prostate Cancer Signaling	25	13	9	5	15	13
Tight Junction Signaling	52	57	21	34	12	14
CD28 Signaling in T Helper Cells	125	46	82	55	40	15
CD40 Signaling	103	21	28	39	52,5	16
Molecular Mechanisms of Cancer	10	4	118	21	30	17
Protein Kinase A Signaling	159	79	138	22	22	18
CDK5 Signaling	211	45	251	96	86	19
Role of Osteoblasts, Osteoclasts and Chondrocytes	107	17	176,5	85	137	20

***Figure 4.** top 20 canonical pathway ranked on basis of 49 days after 3x BLM*

***Figure 3.** Upstream regulator enrichment was used to determine time resolved activation state between single and multiple BLM administrations.*

Conclusions

- **Multiple administrations of bleomycin result in:**
  - **Prolonged collagen production indicating a more chronic fibrosis**
  - **Larger therapeutic window**
  - **Increased number of genes under regulation of various upstream regulators and involvement of novel pathways at later time points**