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SUPPLEMENTAL DATA

Digoxin attenuates LPS-induced acute lung injury in mice via NF-κB and HIF-1α inhibition

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Full article is available at the following link: Digoxin attenuates LPS-induced acute lung injury in mice via NF-κB and HIF-1α inhibition

Table S1. Allocation of experimental groups and lungs for wet-to-dry ratio, histological, and biochemical analyses

Groups		*n* of mice (lungs)	Right lungs (W/D)	Left lungs (histology)	Lungs (homogenate assays)*	Same animals used across endpoints?
Control	C1	6 (12)	5	1	5	Yes, W/D and homogenate assays used overlapping samples; histology sample came from a separate animal
	C2	6 (12)	5	1	5	Yes, W/D and homogenate assays used overlapping samples; histology sample came from a separate animal.
Prophylactic groups	LPS-PU	7 (14)	5	2	6	Yes, right lungs for W/D, left lungs for homogenization; homogenization also included right lung from one histology animal.
	LPS-PDX	10 (20)	6	4	6	Yes, W/D and homogenate assays used overlapping samples; histology samples came from separate animals.
	LPS-PDG	10 (20)	6	4	6	Yes, W/D and homogenate assays used overlapping samples; histology samples came from separate animals.
u ti	LPS-	7 (14)	5	2	6	Yes, right lungs for

TU					W/D, left lungs for homogenization; homogenization also included right lung from one histology animal.
LPS-TDX	10 (20)	6	4	6	Yes, W/D and homogenate assays used overlapping samples; histology samples came from separate animals.
LPS-TDG	10 (20)	6	4	6	Yes, W/D and homogenate assays used overlapping samples; histology samples came from separate animals.

Notes: W/D refers to the lung wet-to-dry weight ratio. The homogenate assays involved total protein analysis and ELISA techniques, utilizing distinct lung samples for the nuclear NF-κB activation assay. The LPS groups comprised all mice receiving i.t. LPS (5 mg/kg) for the induction of ALI, which were further divided into prophylactic and therapeutic subgroups. The prophylactic groups included: LPS-PU (untreated), LPS-PDX (administered i.p. with dexamethasone at 5 mg/kg), and LPS-PDG (administered i.p. with digoxin at 1 mg/kg). The therapeutic groups consisted of: LPS-TU (untreated), LPS-TDX (administered i.p. with dexamethasone at 5 mg/kg), and LPS-TDG (administered i.p. with digoxin at 1 mg/kg). **Abbreviations**: ALI: Acute lung injury; ELISA: Enzyme-linked immunosorbent assay; i.p.: Intraperitoneal; i.t.: Intratracheal; LPS: Lipopolysaccharide; LPS-PDG: Lipopolysaccharide–prophylactic digoxin; LPS-PDX: Lipopolysaccharide–prophylactic dexamethasone; LPS-PU: Lipopolysaccharide–prophylactic untreated; LPS-TDG: Lipopolysaccharide–therapeutic digoxin; LPS-TDX: Lipopolysaccharide–therapeutic dexamethasone; LPS-TU: Lipopolysaccharide–therapeutic untreated; NF-κB: Nuclear factor kappa B; W/D: Wet-to-dry (lung weight ratio).

Table S2. Raw *p* values and BH-FDR q values for lung injury

Prophylactic Experiment						
Endpoint	LPS-PU vs Control		LPS-PDX vs LPS-PU		LPS-PDG vs LPS-PU	
	Raw *p* value	FDR *q*-value	Raw *p* value	FDR *q*-value	Raw *p* value	FDR *q*-value
W/D§	.17	.17	.004	.005*	.070	.08
TNF-α	.169	.17	.337	.337	.118	.118
IL-6	< .001	.001*	< .001	.001*	< .001	.001*
MPO§	< .001	.001*	.099	.113	.001	.001*
MDA	< .001	.001*	< .001	.001*	< .001	.001*
NF-kB	< .001	.001*	< .001	.001*	< .001	.001*
CXCL2/MIP-2	< .001	.001*	< .001	.001*	< .001	.001*
HIF-1α	< .001	.001*	< .001	.001*	< .001	.001*
Therapeutic experiment						
Endpoint	LPS-TU vs Control		LPS-TDX vs LPS-TU		LPS-TDG vs LPS-TU	
	Raw *p* value	FDR *q*-value	Raw *p* value	FDR *q*-value	Raw *p* value	FDR *q*-value
W/D §	.002	.002*	.077	.077	.003	.003*
TNF-α	.009	.009*	.021	.028*	.014	.014*
IL-6	< .001	.001*	< .001	.001*	< .001	.001*
MPO §	< .001	.001*	.047	.054	.002	.002*
MDA	< .001	.001*	< .001	.001*	< .001	.001*
NF-kB	< .001	.001*	< .001	.001*	< .001	.001*
CXCL2/MIP-2	< .001	.001*	< .001	.001*	< .001	.001*
HIF-1α	< .001	.001*	< .001	.001*	< .001	.001*

Notes: W/D: lung wet-to-dry ratio. § Raw *p* values from Dunn's post-hoc test following the Kruskal-Wallis test (without Bonferroni correction). The control group (n = 10) was pooled equally from C1 and C2. The LPS groups included all mice receiving intratracheal LPS (5 mg/kg) for the induction of ALI, which were subdivided into prophylactic and

therapeutic subgroups. Prophylactic groups included: LPS -PU (untreated; $n = 6$, except $n = 5$ for W/D), LPS-PDX (intraperitoneal dexamethasone 5 mg/kg; $n = 6$), and LPS-PDG (intraperitoneal digoxin 1 mg/kg; $n = 6$). Therapeutic groups included: LPS -TU (untreated; $n = 6$, except $n = 5$ for W/D), LPS-TDX (intraperitoneal dexamethasone 5 mg/kg; $n = 6$), and LPS-TDG (intraperitoneal digoxin 1 mg/kg; $n = 6$). **Significant $q < .05$* after HD-FDR correction across lung injury endpoints for each group comparison.
Abbreviations: ALI: Acute lung injury; BH-FDR: Benjamini–Hochberg false discovery rate; CXCL2/MIP-2: C-X-C motif chemokine ligand 2/macrophage inflammatory protein-2; HIF-1α: Hypoxia-inducible factor-1 alpha; IL-6: Interleukin-6; LPS: Lipopolysaccharide; LPS-PDG: Lipopolysaccharide–prophylactic digoxin; LPS-PDX: Lipopolysaccharide–prophylactic dexamethasone; LPS-PU: Lipopolysaccharide–prophylactic untreated; LPS-TDG: Lipopolysaccharide–therapeutic digoxin; LPS-TDX: Lipopolysaccharide–therapeutic dexamethasone; LPS-TU: Lipopolysaccharide–therapeutic untreated; MDA: Malondialdehyde; MPO: Myeloperoxidase; NF-κB: Nuclear factor kappa B; TNF-α: Tumor necrosis factor-alpha; W/D: Wet-to-dry (lung weight ratio).

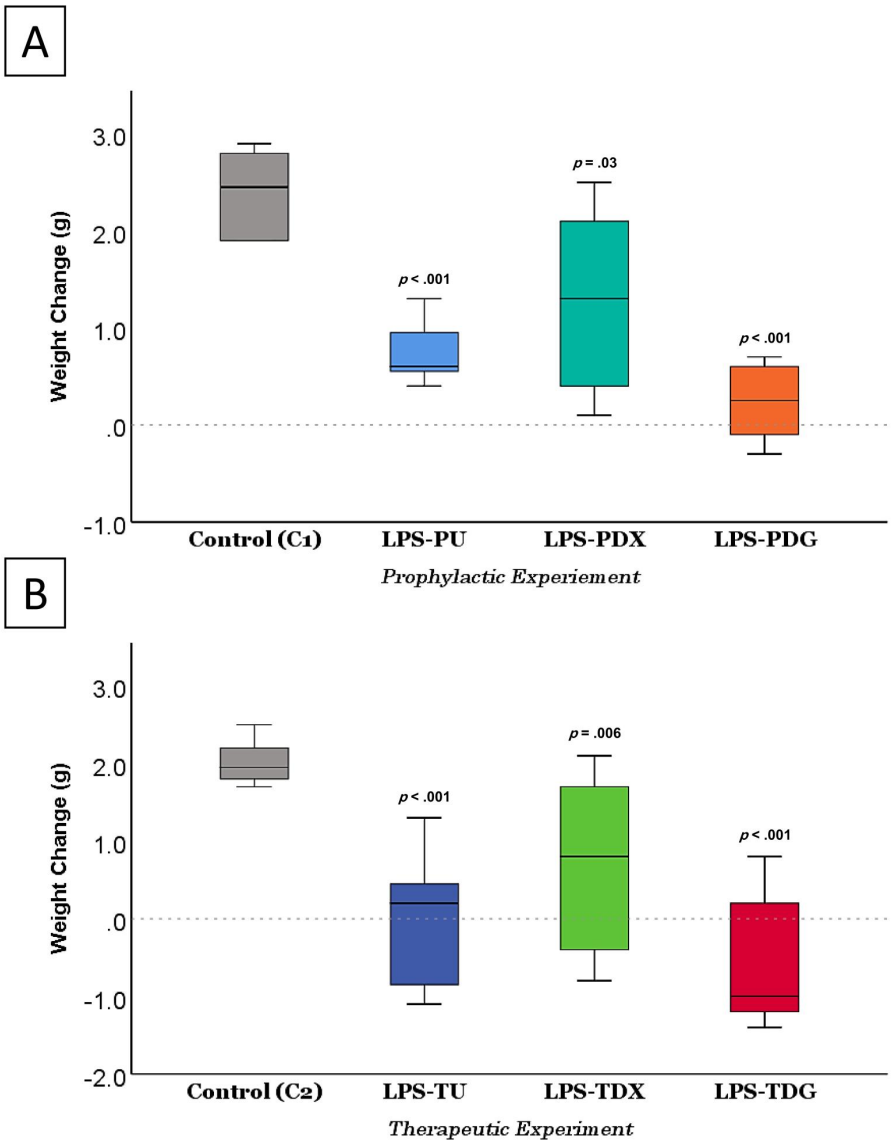

Figure S1. Weight change in mouse groups at the end of the experiment. Weight is measured in grams (g). The control group consisted of 12 mice, evenly divided between the prophylactic (C1; *n* = 6) and therapeutic (C2; *n* = 6) components of the study. The LPS groups included all experimental groups that received i.t. instillation of LPS (5 mg/kg) to induce ALI in both prophylactic and therapeutic settings. The prophylactic groups were as follows: LPS-PU (*n* = 7; untreated), LPS-PDX (*n* = 10; i.p. dexamethasone 5 mg/kg), and LPS-PDG (*n* = 10; i.p. digoxin 1 mg/kg). The therapeutic groups included: LPS-TU (*n* = 7; untreated), LPS-TDX (*n* = 10; i.p. dexamethasone 5 mg/kg), and LPS-TDG (*n* = 10; i.p. digoxin 1 mg/kg). Welch's ANOVA with Games-Howell post-hoc tests was employed to assess significance

compared to the corresponding control (C1 or C2), as indicated by the *p*-values above the boxplots. **Abbreviations**: ALI: Acute lung injury; ANOVA: Analysis of variance; i.p.: Intraperitoneal; i.t.: Intratracheal; LPS: Lipopolysaccharide; LPS-PDG: Lipopolysaccharide–prophylactic digoxin; LPS-PDX: Lipopolysaccharide–prophylactic dexamethasone; LPS-PU: Lipopolysaccharide–prophylactic untreated; LPS-TDG: Lipopolysaccharide–therapeutic digoxin; LPS-TDX: Lipopolysaccharide–therapeutic dexamethasone; LPS-TU: Lipopolysaccharide–therapeutic untreated.