Lysophosphatidic acid protects against endotoxemia-associated acute kidney injury

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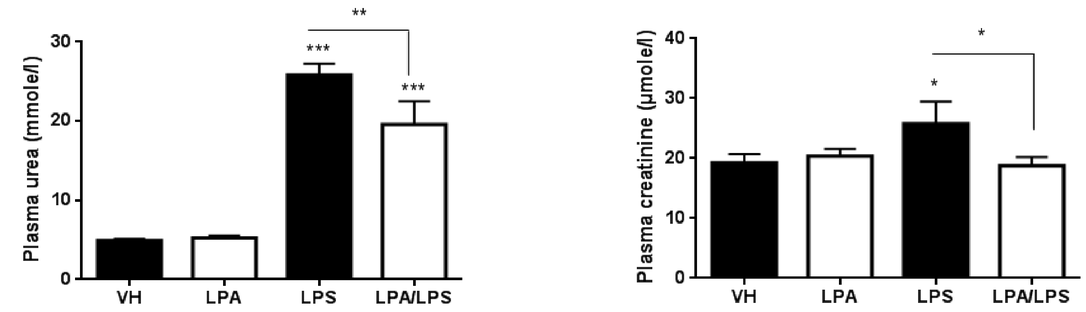
Objective. Lysophosphatidic acid (LPA) is a lysophospholipid present in various biological fluids that exerts biological activities through the activation of specific G protein coupled receptors (LPA1 to LPA6). Injection of lysophosphatidic acid (LPA) was shown to prevent AKI development in animals exposed to ischemia-reperfusion injury [1, 2]. It was also shown that administration of LPA to mice exposed to severe sepsis increases their survival and reduces damage of liver, lung, neuromuscular junction [3, 4]. Moreover, exposure to lipopolysaccharide (LPS) or ischemia was shown to alter LPA production in plasma and bronchoalveolar lavage fluid. We aimed here to study whether LPA could exert a protective action against LPS-induced AKI *in vivo* and *in vitro*.

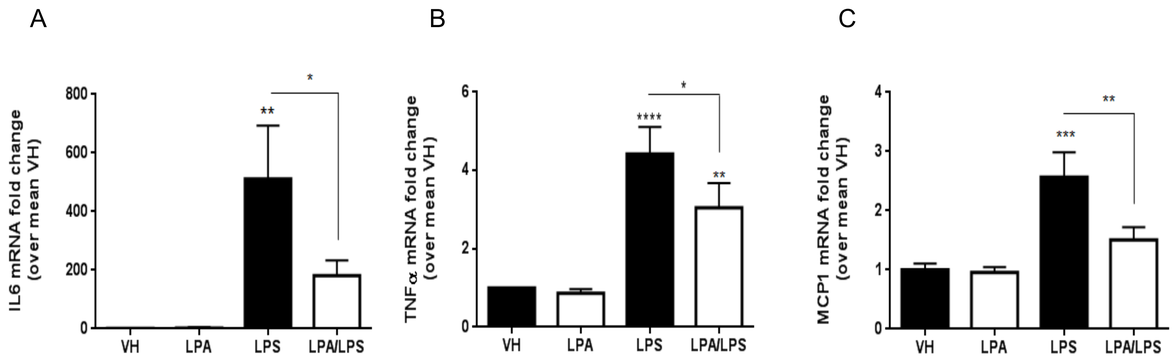
I. LPA counteracts LPS-induced AKI *in vivo*

1) LPA attenuates LPS-induced kidney dysfunction



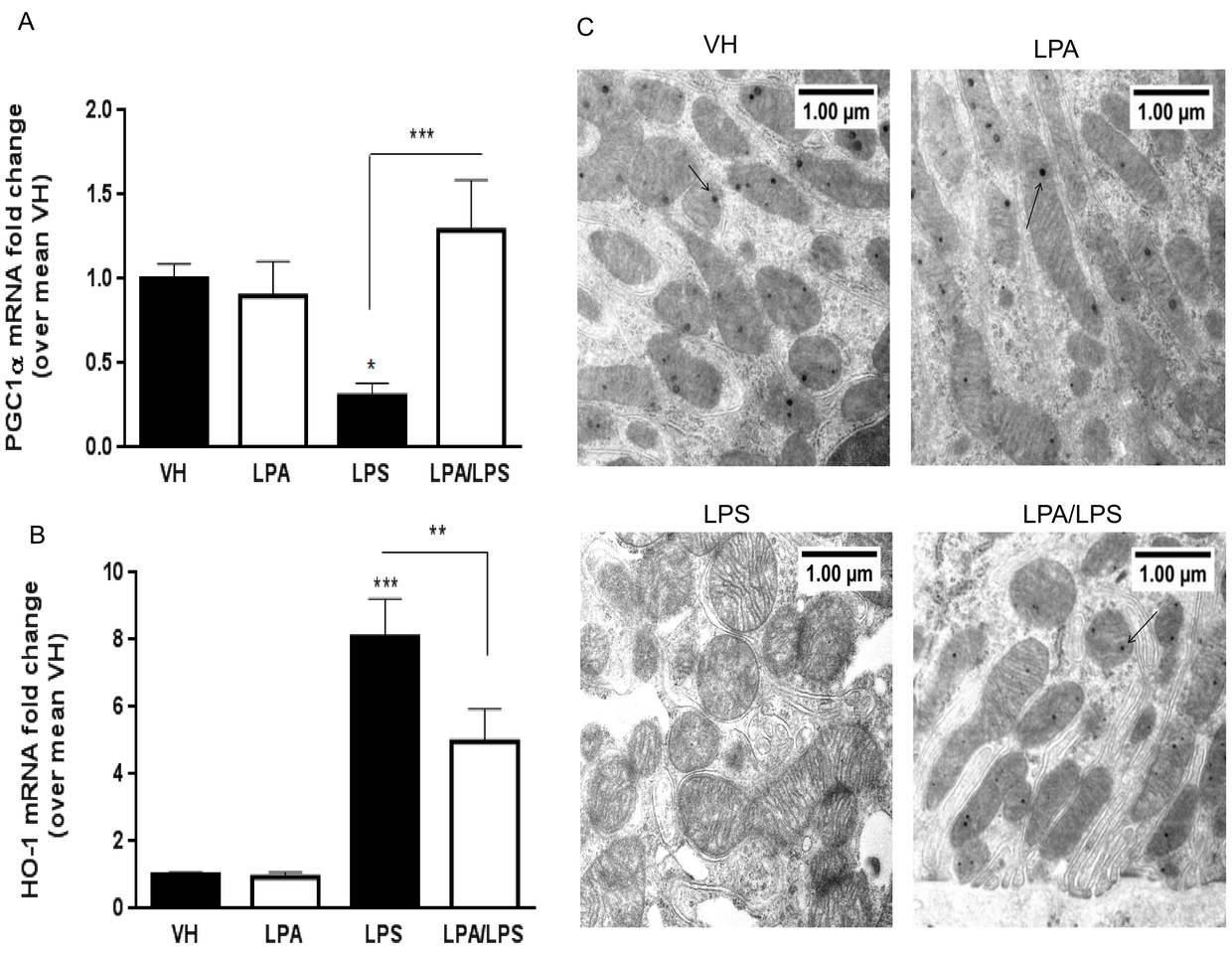
Mice were pretreated with LPA 18:1 (5mg/kg, i.p.) 1 hour before injecting LPS (10 mg/kg, i.p.). Control animals received 100µl of 1% BSA/PBS (VH). Blood was collected after 24h to measure plasma urea (A) and plasma creatinine (B). The number of animals per group was 9, 9, 9 and 10 for VH, LPA, LPS and LPA/LPS respectively. Values are means ± SEM. ANOVA analysis: *p<0.05, **p<0.01 and ***p<0.001.

2) LPA reduces LPS-induced outburst of inflammatory cytokines in kidney



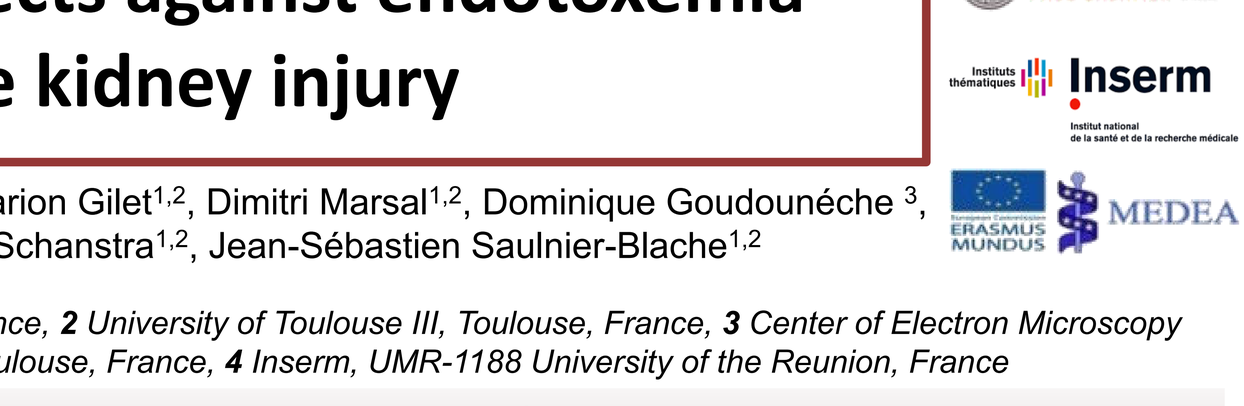
Mice were pretreated with LPA 18:1 (5mg/kg, i.p.) 1 hour before injecting LPS (10 mg/kg, i.p.). Control animals received 100µl of vehicle (VH). Kidney cortex was collected after 24h to measure the gene expression of IL-6 (A), TNFα (B) and MCP-1 (C). The number of animals per group was 9, 9, 9 and 10 for VH, LPA, LPS and LPA/LPS respectively. Values are means ± SEM. ANOVA analysis: *p<0.05, **p<0.01 and ***p<0.001, ****p<0.0001.

3) LPA attenuates LPS-induced alteration of mitochondria



Mice were pretreated with LPA 18:1 (5mg/kg, i.p.) 1 hour before injecting LPS (10 mg/kg, i.p.). Control animals received 100µl of vehicle (VH). Kidney cortex was collected after 24h to measure the gene expression of PGC1α (A) and HO-1 (B). The number of animals per group was 9, 9, 9 and 10 for VH, LPA, LPS and LPA/LPS respectively. Values are means ± SEM. ANOVA analysis: *p<0.05, **p<0.01 and ***p<0.001. (C) Transmission electron microscopy of kidney slices (magnification x7000). Representative of 5 to 7 images from 1 animal per group (arrows indicate dense granules).

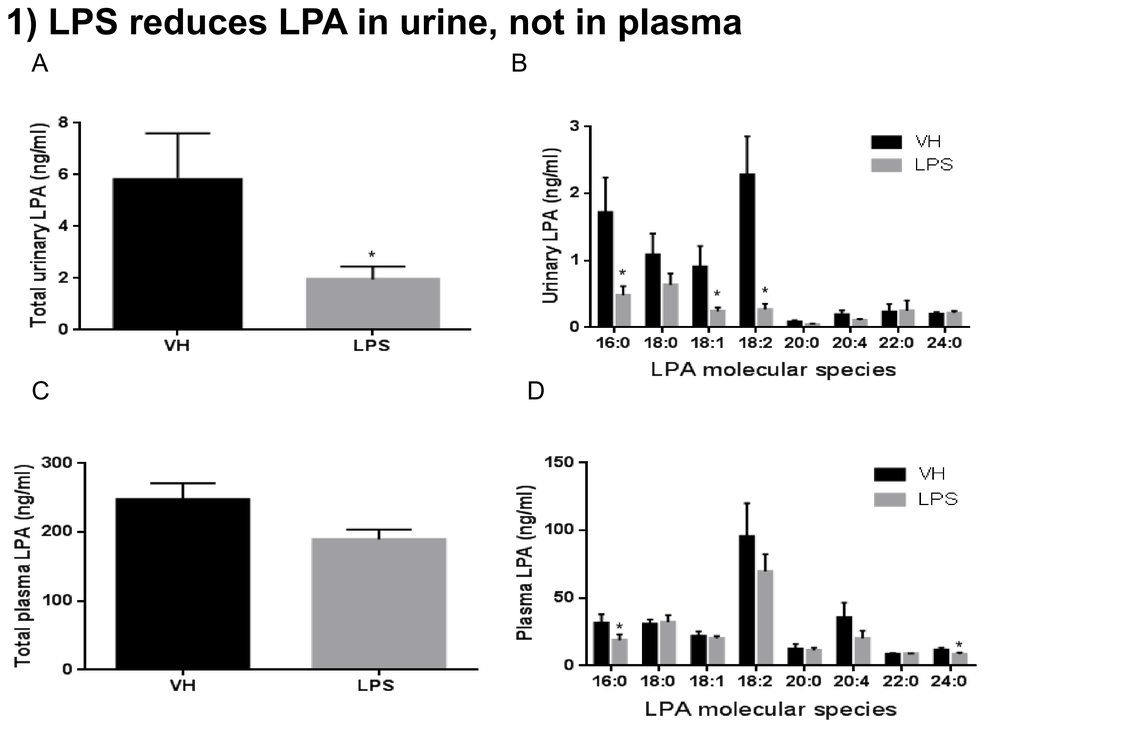
II. Anti-inflammatory effects of LPA on macrophages *in vitro*



Serum deprived RAW264.7 marcrophages in culture were pretreated with LPA18:1 (10 µM) for 15 minutes before being exposed to LPS (100ng/ml) or vehicle (VH) for 4 hours. Total RNA was extracted to measure the expression of IL-6 (A), TNFα (B) and MCP-1 (C). Values are means ± SEM of 4 separate experiments. ANOVA analysis: **p<0.01 and ***p<0.001

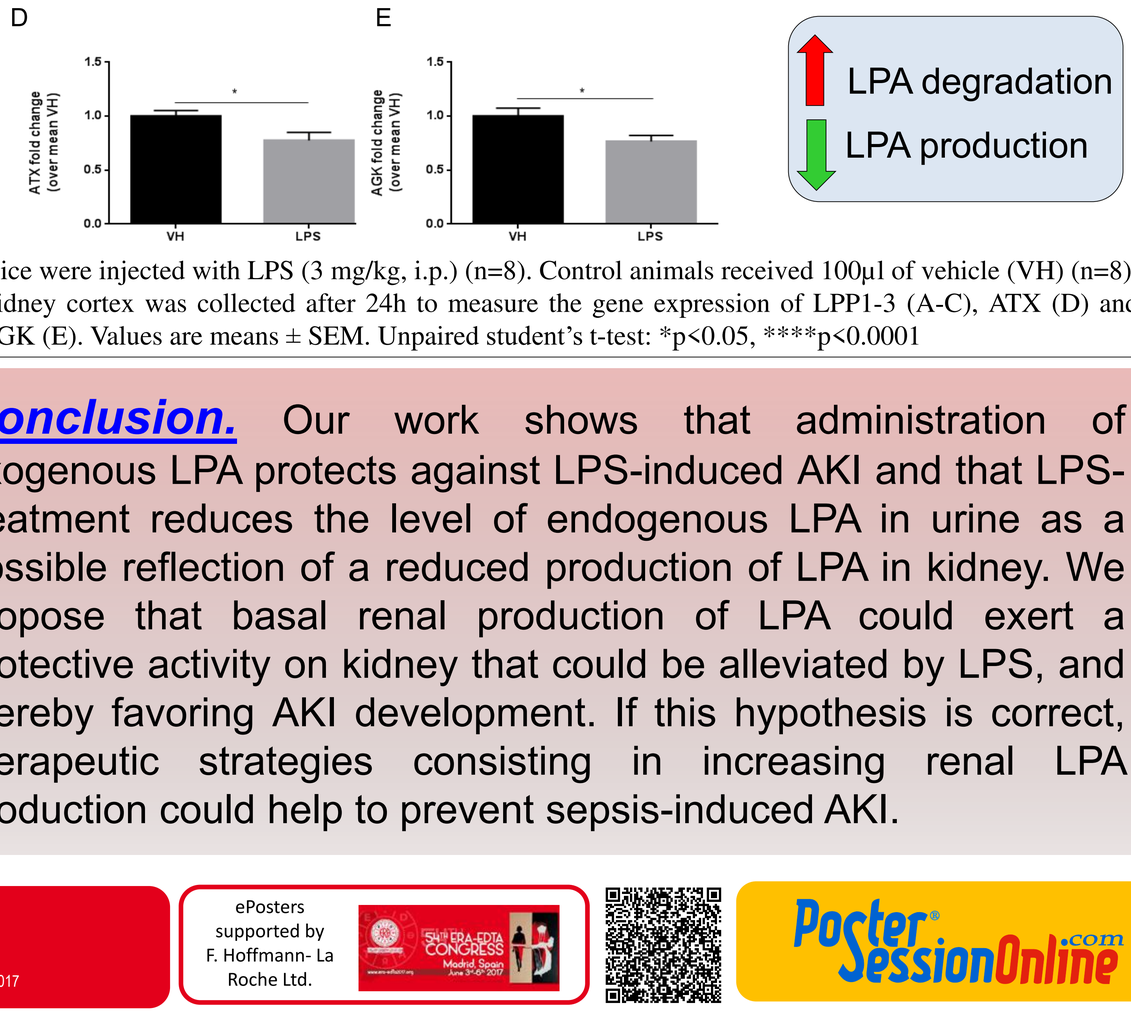
III. LPS reduces LPA production

1) LPS reduces LPA in urine, not in plasma



Mice were injected with LPS (3 mg/kg, i.p.) (n=8). Control animals received 100µl of vehicle (VH) (n=8). The mice were placed into metabolic cages to collect urine after 24h. Blood was collected after 24h and plasma was prepared. LPA was quantified in urine and plasma by LC/MS-MS. Concentration of total LPA (sum of LPA species) in urine (A) and plasma (C) and different LPA species in urine (B) and in plasma (D) from LPS-treated and control (VH) mice after 24h. Values are means ± SEM. Unpaired student's t-test: *p<0.05

2) LPS alters renal expression of LPA metabolic enzymes



Mice were injected with LPS (3 mg/kg, i.p.) (n=8). Control animals received 100µl of vehicle (VH) (n=8). Kidney cortex was collected after 24h to measure the gene expression of LPP1-3 (A-C), ATX (D) and AGK (E). Values are means ± SEM. Unpaired student's t-test: *p<0.05, ****p<0.0001

Conclusion. Our work shows that administration of exogenous LPA protects against LPS-induced AKI and that LPS-treatment reduces the level of endogenous LPA in urine as a possible reflection of a reduced production of LPA in kidney. We propose that basal renal production of LPA could exert a protective activity on kidney that could be alleviated by LPS, and thereby favoring AKI development. If this hypothesis is correct, therapeutic strategies consisting in increasing renal LPA production could help to prevent sepsis-induced AKI.

References. 1. de Vries, B., et al., *Lysophosphatidic acid prevents renal ischemia-reperfusion injury by inhibition of apoptosis and complement activation.* Am J Pathol, 2003. **163**(1): p. 47-56.
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Experimental AKI II
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