Nanoparticular bisphosphonate to selectively target and repolarize liver macrophages for efficient anti-fibrotic effect in CCl_4 liver mice

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EASL European Association for the Study of the Liver

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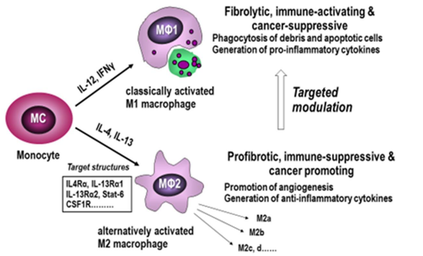
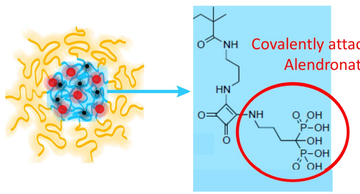
BACKGROUND

- Liver macrophages can polarize into M1-type proinflammatory or M2-type, largely anti-inflammatory, phenotypes when stimulated by key cytokines [D. Schuppan, Y. O. Kim, J. Clin. Invest. 2013, 123, 1887]
- A subset of M2 polarized macrophages share characteristics with tumor-associated macrophages (TAMs) and are associated with tumor and fibrosis progression [M. Zhang, Y. He, X. Sun, Q. Li, W. Wang, A. Zhao, W. Di, J. Ovarian Res. 2014, 7, 19]



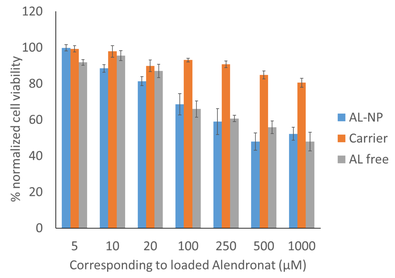
[Figure taken from D. Schuppan, Y. O. Kim, J. Clin. Invest. 2013, 123, 1887]

- Bisphosphonates such as Zoledronate exert a repolarizing effect on M2 macrophages and show anti-tumor and anti-fibrotic activity in rat hepatocellular carcinoma. However, after intravenous or oral administration, bisphosphonates are quickly sequestered in bone or are excreted via kidneys.
- Therefore, we aimed to develop biodegradable and nontoxic nanogel particles (NP) with covalently linked Alendronate (AL-NP) that primarily home to the liver.

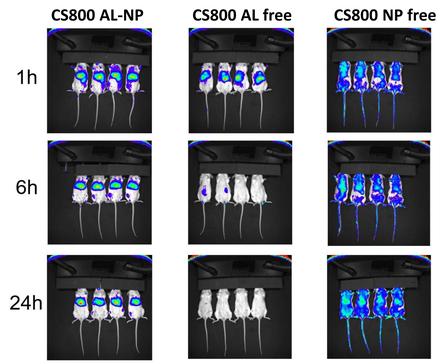


IN VITRO RESULTS

- In primary murine macrophages, unloaded NP did not show cytotoxicity even at high concentrations (~500 µM AL), while AL-NP induced a 50% reduction of cell viability at 1 mM Alendronate loading, equal to free Alendronate (AL) as determined by the MTT assay.

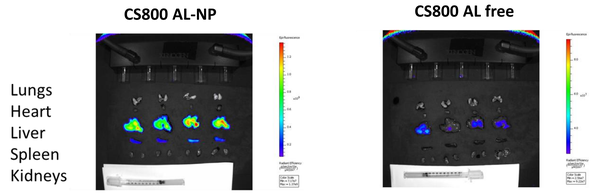


IN VIVO BIODISTRIBUTION



- Near-infrared fluorescence labeled AL-NP (CS800 AL-NP), free CS800 labeled (CS800 AL free) and free CS800 labeled NP (CS800 NP free) were injected intravenous (i.v.) in healthy Balb/c mice. Their distribution was determined by in vivo near infrared imaging. As shown below Al/NP rapidly accumulated in the liver mice already 1 h after i.v. injection, whereas free AL was readily cleared via the kidneys. Lastly NP demonstrated a prolonged circulation time.

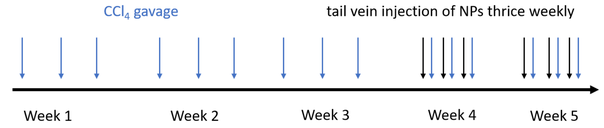
EX VIVO BIODISTRIBUTION



- 24 h after the i.v. injection, mice were sacrificed and organs were removed for *ex vivo* imaging
- CS800 AL-NP and NP (not shown) accumulated prominently in the liver and less in the lungs, spleen or kidneys, while free AL was rapidly excreted via the kidneys.

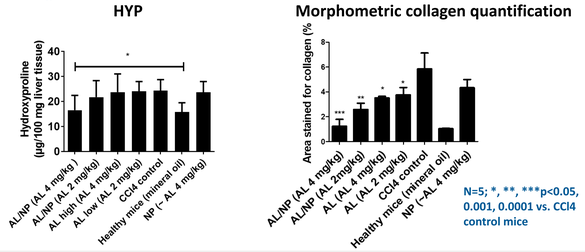
CCl_4 LIVER FIBROSIS MODEL

- Over a period of five weeks, healthy balb/c mice were gavaged with escalating doses of CCl_4 for liver fibrosis induction
- At week four, mice were injected with AL-NP and free AL thrice weekly, receiving 6 injections in total

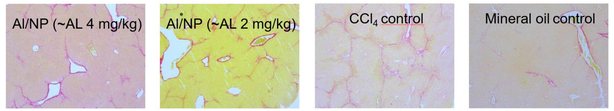


IN VIVO ANTIFIBROTIC EFFECT

- AL-NP (AL ~4 mg/kg) induced a significant ($p<0.05$) antifibrotic effect as determined by hydroxy proline quantification (HYP), while accurate morphometric collagen quantification of Sirius Red stained liver sections revealed even a highly significant ($p<0.001$, <0.0001) reduction of collagen around ~80% for both concentrations AL-NP (AL ~2 or 4 mg/kg)



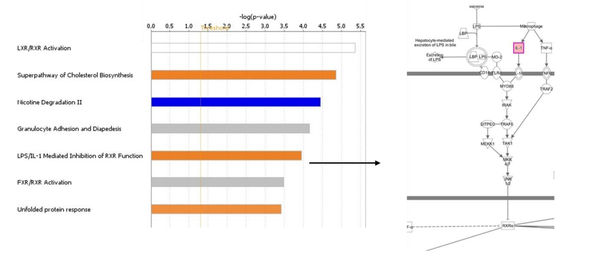
Representative liver sections stained with sirius red for collagen



RNA-SEQ ANALYSIS

- Ingenuity Pathway Analysis (IPA®) predicted upregulation of the proinflammatory pathway (e.g. LPS/Il-1 mediated inhibition of RXR function- driven by M1-type macrophages) in AL/NP treated groups vs controls

Top canonical pathways — LPS/IL-1 mediated inhibition of RXR function



CONCLUSION

- Biocompatible Alendronate coupled nanoparticles (AL-NP) induced a similar cytotoxic effect in murine macrophages comparable to free alendronate, while carries exhibited no significant effect
- AL-NP (re-)polarized *in vitro* pro-tumorous and pro-fibrotic M2- towards putative anti-tumorous and anti-fibrotic M1-type macrophages
- After i.v. injection of AL-NP accumulated efficiently in the liver, while free alendronate was rapidly excreted via the urinary tract
- AL-NP showed *in vivo* an antifibrotic effect in CCl_4 fibrotic mice as shown by HYP and sirius red analysis
- Canonical pathway analysis of RNA-Seq data revealed that AL-NP treatment upregulated signatures of fibrolytic M1 like macrophages

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