Theragnostic imaging of micelle-mediated rosiglitazone delivery to atherosclerotic plaques

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Introduction: Atherosclerosis is an inflammatory disease of the arteries causing considerable morbidity and mortality in the Western world. Rosiglitazone is an effective anti-diabetic drug that also has anti-atherosclerotic potential, but its use is controversial because of reported cardiac side effects. Therefore, a theragnostic nanomedicinal micelle formulation of this drug was developed to improve delivery to the plaque and to enable the in vivo follow-up of therapy efficacy. The objectives of this study were: (1) to assess whether micelle-encapsulated rosiglitazone could be delivered preferentially to sites of atherosclerotic plaques, (2) to assess whether the gadolinium-labeled micelles allowed a noninvasive follow-up assessment of the effect of rosiglitazone delivery on plaque progression. (3) to assess whether plaque progression could be inhibited by micelle-mediated rosiglitazone therapy.

Methods: *Micelles* incorporating rosiglitazone and Gd-DTPA-BSA were prepared using lipid film hydration. Micelle-mediated delivery of rosiglitazone was validated in vitro on a rosiglitazone-responsive lcell line.

In vivo, therapeutic effects were tested in ApoE-/- and ApoE-/--eNOS-/- mice. Both genotypes were divided in 4 groups (n=5) and treated for 6 weeks with:
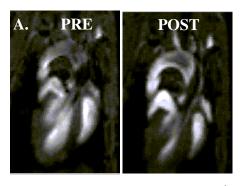1. Western diet (control)
2. Western diet with oral rosiglitazone (10 mg/kg bw/day)
3. Western diet with control micelles through mini-osmotic pumps
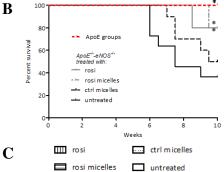4. Western diet with rosiglitazone micelles in mini-osmotic pumps(~10 mg/kg bw/day)

As significant mortality was expected for the ApoE-/-eNOS-/- mice, 10 mice were initially included per treatment group. Of the surviving animals, 5 mice per group were randomly selected for analysis after the treatment period.
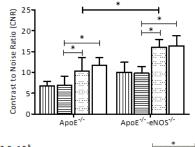
Contrast-enhanced MRI using a T1-weighted retrospectively-gated FLASH sequence (Intragate) was applied after 6 weeks of treatment to assess micelle targeting and determine the therapeutic efficacy longitudinally. Experiments were done on a 9.4T vertical bore Bruker system. CNR of plaque regions in the aortic arch were determined by drawing regions of interest in the vessel wall, the surrounding muscle, and the noise (Fig. A).
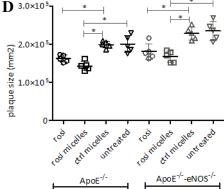
Histology was performed post-mortem on the aortic arch and carotid arteries to determine plaque size and macrophage load (CD68+). Data are represented as the mean ± standard deviation. **Statistical analyses** were done in SPSS 17.0.2 using one-way analysis of variance (ANOVA), followed by a Bonferroni correction for multiple testing in case of significance.

Results & discussion: In vivo MRI showed that Gd labeled rosiglitazone-micelles specifically accumulated in atherosclerotic plaque, visible as increased contrast 24 h post micelle administration (Fig. A). Significant anti-atherosclerotic effects (Fig. C,D) and a reduction in mortality (Fig. B) were observed after 6 weeks of treatment with both rosiglitazone micelles and rosiglitazone compared to the control groups. However, conventional rosiglitazone treatment resulted in known side effects: 27% more weight gain and 9.5% higher plasma cholesterol values than controls (p<0.05 for both). In contrast, rosiglitazone micelles did not show any significant differences in weight or cholesterol levels. In vivo MRI of plaque severity using the vessel wall CNR as a pseudomarker (Fig. C) showed a good correlation with post-mortem histological assessment of plaque size (Fig D) and macrophage load (data not shown).

Conclusions: This study shows a theragnostic nanomedical therapy of atherosclerotic plaques and the application of non-invasive molecular imaging to monitor drug delivery and therapeutic responses. We demonstrate anti-atherosclerotic effects in atherosclerotic lesions after micelle-mediated rosiglitazone therapy, while reducing the negative side effects associated with conventional rosiglitazone treatment.