Pharmacoprotection of the ovaries during chemotherapy using microRNAs technology delivered by gold nanoparticles

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MiRNA-based therapy is tested as an innovative pharmacological approach to prevent chemotherapy-induced ovarian damage and preserve the fertility of female cancer patients. Previously, we showed that let-7a replacement therapy using liposomes was able to prevent cyclophosphamide metabolites (4HC)-induced apoptosis in PND3 ovaries in vitro but in vivo delivery of miRNAs remains a major challenge. Gold nanoparticles (AuNPs) system appears to be attractive for their biocompatibility and their ability to be multi-functionalized, allowing the grating of the desired cargo and a future selected ligand for the recognition of a targeted tissue. The synthesis of the AuNPs is concentrated on the chemical bond that link let-7a to the surface of the AuNP. Classically, thiol (sulfur-hydrogen) was used in most of the studies that grafted miRNAs to AuNPs (AuNPs-S-Let-7a). Here, we exploited the macrocyclic molecule calix[4]arene for their robust carbon-gold bond and the possibility to add functional groups, allowing the attachment of a potential control number of miRNAs (AuNPs-X_4-Let-7a) and other biomolecules. First, we showed that 3 nM of AuNPs-S-let-7a and AuNPs-X_4-Let-7a were not toxic compared to the control (no AuNPs) in PND3 ovaries culture in vitro for 48h by the analysis of the expression of caspase-3 (RT-qPCR), TUNEL assay (double strand DNA breaks detection) and LDH assay (relative secretion of lactate dehydrogenase) (one way ANOVA, $n \geq 4$). Then, AuNPs-X_4-Let-7a were functionalized with a fluorochrome that allow the imaging of internalized AuNPs in the PND3 ovaries after 48h of transfection. After observing the safety and the ability of AuNPs-X_4-Let-7a to enter the tissue, we transfected PND3 ovaries with these AuNPs 24h prior to a 24h-treatement with 4-HC (AuNPs+4-HC group) to study the protective effect of let-7a delivered by AuNPs. This condition was compared to untreated ovaries (control), ovaries treated with 4-HC for 24h and ovaries cultured with 3 nM of AuNPs-X_4-Let-7a for 48h. Using the three same techniques to assess the toxicity of the 4-HC (caspase-3 expression level, TUNEL assay and LDH assay; $n \geq 4$), only the TUNEL assay reported a significant higher apoptosis rate in the 4-HC group compared to control ($p<0.05$, one-way ANOVA, $n = 5$), although a trend of a higher relative LDH secretion, released by death cells, was observed for the 4-HC group compared to the control ($n = 4$). Regarding the effect of let-7a to protect the ovaries from 4-HC toxicity, no significant difference was reported between the 4-HC group and the AuNPs+4-HC group, suggesting a need for improving let-7 a delivery as not optimal based on let-7 and two of its target genes expression analysis (Hmga2 and Stat3) ($n = 4$). To facilitate access to let-7a, 2 different strategies are currently tested (i) the addition of a spacer (10 uracils) to the passenger strand (ii) the combination of adding a spacer and introducing mismatches in the double strand of let-7a that are endogenously presented. The let-7a delivery is compared to a negative control with a non-targeting sequence. Finally, additional techniques is used to better assess the toxic effect of 4-HC and the potential protective effect of let-7a such as the analysis of other apoptotic genes expression levels and the evaluation of proteins involved in DNA repair, apoptosis and follicles activation.