Targeted nanodrug delivery systems for the treatment of Tuberculosis

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Introduction

South Africa currently has the highest incidence of TB per 100 000 (358 per 100 000) people in the world. In 2007 alone 112 000 people died of TB in South Africa, of which 94 000 (72%) were co-infected with HIV (1). Although TB treatments exist, poor patient treatment compliance and drug resistance pose a great challenge to TB treatment programs worldwide. To improve the current inadequate therapeutic management of TB, a polymeric anti-TB nanodrug delivery system for anti-TB drugs was developed that may enable entry, targeting, sustained release over long periods and uptake of the antibiotics in the cells, hence reducing the dose frequency and simultaneously improving patient compliance.

The **aim** was to prepare functionalised polymeric nano drug delivery vehicles to target TB infected macrophage cells. Successful nano encapsulation of anti-TB drugs and a targeting agent, mycolic acids (MA) was achieved. MA (a lipid molecule on the cell wall of *M.tb,)* was explored due to its cholesteroid properties (2) that could attract it to the infected macrophages/foam cells. The nanoparticles were characterized and subjected to *in vitro* analyses in THP-1 and U937 cells to determine their uptake and localization. Cytotoxicity in different cell lines was also analysed. In another approach targeting was attempted by attaching nucleic acid aptamers (3) onto the surface of drug-carrying PLGA nanoparticles. The aptamers were prepared via the SELEX process (4), specifically against the mannose receptor (MR), which is significantly over-expressed during the activation of the macrophages in the presence of *M tb.*

Experimental and Results

Targeting ligand: Mycolic acids

Mycolic acids (MA) make up the major part of the cell envelope of *Mycobacterium tuberculosis*. MA was shown to assume a fine structure-dependent cholesteroid nature that attracts cholesterol (2, 6) and converts macrophages into foam cells (5). It is actively pumped into the extracellular matrix of *M tb.* biofilms during the drug resistant persistent phase (7), where it may possibly be targeted as a cholesterol rich zone.

THP-1 and U937 monocyte-macrophage like cell lines were used to determine whether the labeled MA nanoparticles would be taken up by the cells. The data presented in Fig 1 illustrate that labeled MA nanoparticles were taken up into the macrophage cells, demonstrating that MA-nanoparticles may serve as a suitable carrier for anti-TB drugs.

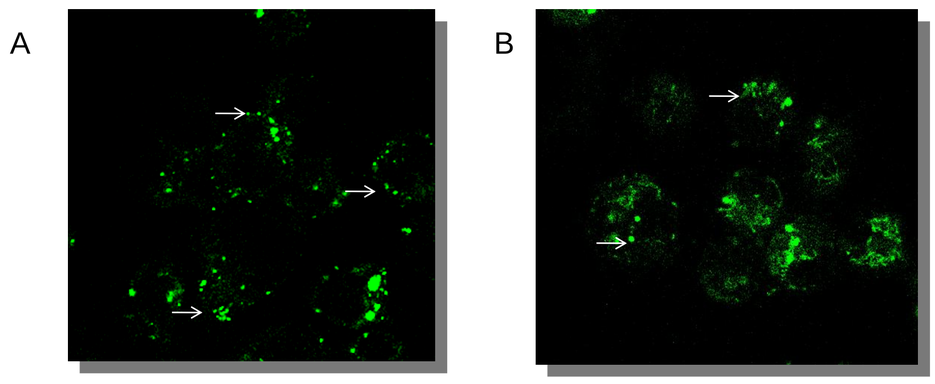

Fig.1 Live cell image of labeled MA nanoparticles taken up by a)THP-1 and b) U937 macrophages.

The mycobactericidal activity of the INH containing nanoparticles was tested, i.e. whether the drug is released from the nanoparticle, as well as whether the MA in the particles influences the efficiency of the *M.tb* inhibition in an *in vitro* study with THP-1 macrophage cells infected with *M.tb* H37Rv. Bacterial growth was monitored with a BACTEC 460 radiometric instrument. The results (Fig 2) indicate a delay in INH release as a function of the polymer that slowly degrades in the macrophage. After 1 and 2 day exposure to the nanoencapsulated INH, a significant mycobacterial growth inhibition was observed, but with reduced toxicity/efficiency compared to free INH. The effect of free INH on bacterial growth inhibition could be matched by using a ten times higher concentration of 2 µg/ml of the nanoencapsulated INH with or without MA. In this *in vitro* model, the inclusion of MA in the INH nanoencapsulated particle did not show improved efficiency of delivery or toxicity of the INH drug in the macrophage. In contrast, it appeared as if the MA actually worked against INH killing of the *M.tb* in the macrophages. This may be explained by MA serving as an external source of the MA cell wall component, overcoming the bottleneck in cell wall synthesis.

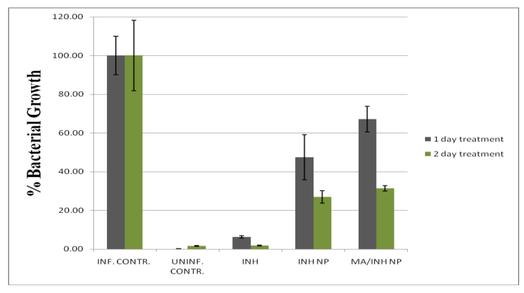

Figure 2. BACTEC growth inhibition of *M.tb* in infected THP-1 macrophages, two days after exposure to various test compounds compared to INH. The results represent the average of triplicate values. Concentration for INH shown was 0.2µg/ml as 2µg/ml INH went out of range.

Targeting ligand: Aptamers against the mannose receptor

In another approach targeting was attempted by attaching nucleic acid aptamers (3) onto the surface of drug-carrying PLGA nanoparticles. Aptamers are antibody like molecules made from nucleic acids which are directed against a ligand (Fig.3). The aptamers were prepared via the SELEX process (4), specifically against the mannose receptor (MR), which is significantly over-expressed during the activation of the macrophages in the presence of *M.tb. A*ptamer binding affinity towards the mannose receptor protein was tested on the Biacore biosensor.

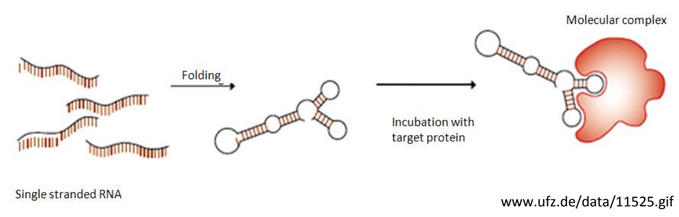

Fig.3 Formation of aptamers against a targeting ligand

The aptamers obtained from the SELEX process were then conjugated to the nanoparticles. Succinamide coupling was used to attach PEG to the PLGA nanoparticle. Aptamers were then derivatised to form an NH_2 group to which they were attached to the PEG.

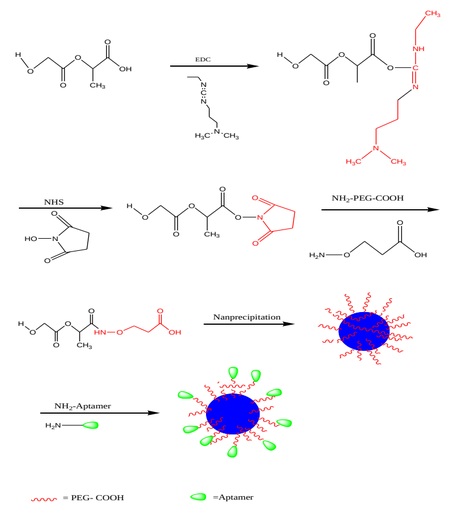

Fig.4 Conjugation of aptamers to nanoparticles.

Discussion and conclusion

MA as targeting ligand derives from its cholesterol attracting property. MA NP was taken up into macrophages *in vitro,* possibly localizing in the cytoplasm. *In vitro* drug testing via BACTEC indicated that the INH was released from the NP, but that the MA does not show advantage in the early mycobacterial replication.

Aptamers against the mannose receptor protein were made and coupled onto the surface of the PLGA nanoparticles. In vitro drug testing and confocal imaging are underway.

Acknowledgements

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