Prodrug-Loaded Mesoporous Silica Nanoparticles for Stimuli-Responsive Chemotherapy

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ABSTRACT SUMMARY
An enzymatically triggerable prodrug of a potent anticancer agent, doxorubicin, was grafted via click-chemistry onto the surface of functionalized mesoporous silica nanoparticles. The release and activation of the prodrug was evaluated in the presence of β-glucuronidase, an enzyme which is present in necrotic areas of tumor lesions.

INTRODUCTION
Doxorubicin (DOX) is a very potent antitumor drug associated with cardiotoxicity. Therefore, prodrug therapies have been proposed to increase its therapeutic index.1 DOX-propGA3 is a synthetic derivative of doxorubicin, which is much less toxic, and after hydrolysis of the propargylic ester is activated or converted to doxorubicin by the enzyme β-glucuronidase. This enzyme is present in the lysosomes of cells, and is only released into the extracellular space in necrotic areas of tumors. DOX-propGA3 is linked to DOX (written as X in Scheme 1b), and has a spacer that can be self-eliminated, a glucuronide group, and a propargyl moiety in which the alkyne can be reacted using click-chemistry.2

Scheme 1. Structure of (a) DOX and (b) DOX-propGA3.

This enzymatically triggerable prodrug of DOX was grafted by click-chemistry onto the surface of functionalized mesoporous silica nanoparticles, aimed to transport the prodrug to the places where it would be selectively activated.

EXPERIMENTAL METHODS
Mesoporous silica nanospheres (size 100-150 nm) were synthesized by hydrolysis and polycondensation of a silica precursor, tetraethylorthosilicate, in the presence of a structure-directing agent, cetyltrimethylammonium bromide (CTAB), in basic medium. After removing CTAB by solvent extraction, the nanoparticles were functionalized in a two-step process with 3-chloropropyltrimethoxysilane and substitution with sodium azide (Scheme 2). The resulting nanoparticles were characterized by thermogravimetric analysis (TGA), infrared (IR) spectroscopy, X-ray diffraction (XRD), N2 adsorption porosimetry, dynamic light scattering (DLS) and transmission electron microscopy (TEM).

Scheme 2. Two-step synthesis of azide-functionalized mesoporous silica nanoparticles.

Covalent attachment of the alkyne-terminated prodrug was performed by click-coupling to the azide groups on the silica nanoparticles. In vitro release assays were
performed in the presence/absence of β-glucuronidase. Cellular viability was assessed with a neuroblastoma cell line, NB1691, by flow cytometry after 3 days incubation.

RESULTS AND DISCUSSION
Azide modification of the mesoporous silica nanoparticles (hydrodynamic size 194 nm, according to DLS) was confirmed by TGA and IR analysis. The effect of this surface functionalization and the following prodrug attachment on the porous structure of the inorganic matrix was monitored by low-angle XRD. The progressive intensity decrease of the characteristic reflections of the hexagonal mesoporous array indicates a pore filling effect. This result is in accordance with the observed reduction in surface area and pore volume. After reacting with the prodrug in the presence of Cu(I) as catalyst, homogeneous particles around 200 nm diameter (PDI 0.29) were obtained (Figure 1).

Figure 1. TEM image (a) and hydrodynamic size distribution (b) of prodrug-loaded mesoporous silica nanoparticles.

Before the action of β-glucuronidase, DOX-propGA3 has to be hydrolyzed to give an intermediate, DOX-GA3, which is a good substrate for the enzyme. The glucuronide group in DOX-GA3 is then cleaved by β-glucuronidase, finally releasing DOX. More than 40% of DOX-GA3 is formed after 6 days, but that amount decreases to less than 10% when β-glucuronidase is added in the medium (Figure 2). In that case, DOX-GA3 is almost quantitatively converted to DOX. The first hydrolysis of the prodrug is the limiting step in the formation of DOX.

DOX-propGA3 becomes very similar in toxicity to DOX when the enzyme is added. The prodrug-loaded material reaches a comparable level (concentration required for 50% viability inhibition, EC50, 0.27 µg/mL), which implies that the hydrolysis is not limiting the conversion of the prodrug in the cytotoxicity assay, probably due to the presence of esterases in the culture.

CONCLUSION
A prodrug of doxorubicin has been covalently attached to surface-functionalized mesoporous silica nanoparticles. Stimuli-responsive release and activation of the prodrug have been demonstrated in vitro, and confirmed by viability studies on neuroblastoma cells after the addition of β-glucuronidase.

REFERENCES

ACKNOWLEDGMENTS
This research was performed within the European Union FP7 under a Marie Curie Intra-European Fellowship for Career Development.