Uptake of new somatostatin analogue loaded recombinant human serum albumin nanoparticles in pancreatic carcinoma cells


1Institute of Pharmaceutical Technology, Biocenter Niederursel, Goethe University, Max-von-Laue-Str. 9, 60338 Frankfurt/Main, Germany
2Department of Medicine 1, University Hospital Frankfurt, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany
3Sheba Medical Center, Advanced Technology Center, Tel Hashomer 52621, Israel
4Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv 69978, Israel

i.rosenberger@em.uni-frankfurt.de

ABSTRACT SUMMARY

In the present study nanoparticles were prepared using recombinant human serum albumin (rHSA). A new somatostatin analogue (SSTA) was covalently attached to the nanoparticle surface to enhance the active targeting of the nanoparticles to pancreatic tumor tissue. The size distribution and the surface charge of the resulting drug delivery system were determined and the cellular uptake of the nanoparticles was confirmed by confocal laser scanning microscopy (CLSM).

INTRODUCTION

Pancreatic cancer is one of the ten leading causes of cancer-related deaths in Germany in 2008. The overall 5-year survival rate for male and female patients is around 7%. [1] As such, the main challenge in diagnosis and treatment of pancreatic carcinoma is a highly specific targeting to pancreatic cancer cells. For that duty somatostatin and its analogues are the most promising tools. [2]

As a receptor located on the cell membrane, the somatostatin receptor (SSTR) with its five subtypes plays an important role in the signal transduction of cell growth inhibition. [3]

One of the main challenges in cancer treatment is to avoid toxic or at least unrequested side effects of anticancer drugs or diagnostic tools in cancer therapy. Therefore nanoparticles show the most promising skills.

Nanoparticles are colloidal polymeric drug delivery systems with a size range of 10-1000 nm [4]. As a non-toxic and well known protein, human serum albumin (HSA) as natural product and rHSA show the best properties for the preparation of nanoparticles.

Balthasar et al. showed in 2005 that a very promising way to generate drug-loaded nanoparticles is the covalent attachment to the nanoparticle surface. [5]

EXPERIMENTAL METHODS

rHSA nanoparticles were prepared by a desolvation technique as described previously. [6] To enhance the contrast of the rHSA nanoparticles rhodamine123 was entrapped as a fluorescence contrast agent for CLSM into the nanoparticle core. Therefore rHSA was preincubated with rhodamine123. Afterwards the desolvation process took place as described by Weber et al. [6] The size distribution, polydispersity index, and surface charge were determined by dynamic light scattering (DLS) using a Zetasizer Malvern Nano ZS (Malvern Instruments Ltd., Malvern, UK).

The SSTA was attached to the surface of the rhodamine123-labeled rHSA (Rho123 rHSA) nanoparticles using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDC) as crosslinking moiety: 10 mg Rho123 rHSA nanoparticles were redispensed in buffer, incubated with an excess of EDC and 200 µg of SSTA for 1 hour at room temperature. Afterwards, the Rho123 rHSA-SSTA nanoparticles were purified three times by centrifugation and redispersion in ultrapurificated water. The amount of unbound SSTA in the supernatant was determined using a FLUOstar Galaxy (BMG Labtechnologies GmbH, Germany).
In *in vitro* experiments the cellular uptake of these nanoparticles was shown by CLSM.

**RESULTS AND DISCUSSION**

In order to assure covalent attachment of proteins as targeting units or of drugs on the surface of nanoparticles, the surface characteristics and size distribution play an important role. Therefore Rho123 rHSA nanoparticles were compared to the non-labeled rHSA nanoparticles to ensure a comparable binding capacity of the SSTA. The rhodamine123 entrapment was determined by using a U-3000-Spectrophotometer (Hitachi, U.S.A.) and the entrapment level was quantified as 69.9% ± 2.4%. The characteristics are shown in Figure 1:

![Figure 1: size, polydispersity index, and zeta potential of rHSA nanoparticles, rhodamine123-labeled nanoparticles and rhodamine123-labeled rHSA-SSTA nanoparticles](image)

The non-labeled rHSA and the Rho123 rHSA nanoparticles show similar characteristics regarding surface charge and size distribution.

SSTA was covalently bound to the surface of Rho123 rHSA nanoparticles to achieve an active drug targeting to pancreatic tumor cells. The residues of the protein were determined in the supernatant. The amount of bound SSTA is 93.7% ± 0.1%.

With these promising results the *in vitro* experiments were performed.

The cellular uptake of the Rho123 rHSA-SSTA nanoparticles by PANC-1 cells after 24 h incubation was determined by using CLSM:

![Picture 1. Cellular uptake of Rho123 rHSA-SSTA nanoparticles in PANC-1 cells (24 h incubation)](image)

After 24 h of incubation a high uptake of the modified nanoparticles can be observed.

**CONCLUSION**

The present study shows that SSTA as a very promising targeting moiety can be introduced to the surface of rHSA nanoparticles. The *in vitro* tests show higher amounts of targeted rHSA nanoparticles in PANC-1 cells than the untargeted rHSA nanoparticles. An unspecific uptake of the targeted nanoparticles can be excluded and the achieved rHSA-SSTA nanoparticles are well-suited for *in vivo* investigations.

**REFERENCES**


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