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Colloidal stability of nano-sized particles in the peritoneal fluid: Towards optimizing drug delivery systems for intraperitoneal therapy

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ABSTRACT

Intraperitoneal (IP) administration of nano-sized delivery vehicles containing small interfering RNA (siRNA) has recently gained attention as an alternative route for the efficient treatment of peritoneal carcinomatosis. The colloidal stability of nanomatter following IP administration has, however, not been thoroughly investigated yet. Here, enabled by advanced microscopy methods such as single particle tracking and fluorescence correlation spectroscopy, we follow the aggregation and cargo release of nano-scaled systems directly in peritoneal fluids from healthy mice and ascites fluid from a patient diagnosed with peritoneal carcinomatosis. The colloidal stability in the peritoneal fluids was systematically studied as a function of the charge (positive or negative) and poly(ethylene glycol) (PEG) degree of liposomes and polystyrene nanoparticles, and compared to human serum. Our data demonstrate strong aggregation of cationic and anionic nanoparticles in the peritoneal fluids, while only slight aggregation was observed for the PEGylated ones. PEGylated liposomes, however, lead to a fast and premature release of siRNA cargo in the peritoneal fluids. Based on our observations, we reflect on how to tailor improved delivery systems for IP therapy.

1. Introduction

Peritoneal metastases are one of the major causes of death in patients diagnosed with ovarian cancer [1]. Also in colorectal cancer, cancer cells often migrate to the abdomen, where they spread and form peritoneal carcinomatosis [2]. The often late stage of discovery of peritoneal metastases, which can spread over the entire surface of the peritoneum (~2 m^2), makes the treatment very difficult. This fact is well-demonstrated from clinical trials indicating the low median survival of patients diagnosed with peritoneal carcinomatosis [3].

Current treatment of peritoneal carcinomatosis involves removing the majority of peritoneal metastases (cytoreductive surgery) followed by intravenous (IV) administration of chemotherapeutic agents such as oxaliplatin in combination with 5-fluorouracil or leucovorin [4,5] to kill remaining tumor cells. Also platinum-based (i.e. oxaliplatin, cisplatin) chemotherapeutics in combination with paclitaxel [6,7] are used. Unfortunately, the majority of the patients develop disease recurrence [8,9]. Therefore, more efficient post-surgical strategies to kill the remaining tumor cells are needed [10]. In this context, intraperitoneal (IP) administration of chemotherapeutics has been shown to be superior over the intravenous route [11,12], particularly due to the ability to maintain high concentrations of cytotoxic agents in the peritoneal cavity [13]. Also, promising data have resulted from clinical trials evaluating hyperthermic intraperitoneal chemoperfusion (HIPEC) immediately after cytoreductive surgery [14,15]. HIPEC involves flushing the peritoneal cavity with chemotherapeutic agents at an elevated temperature of 41–42 °C. It is hypothesized that HIPEC is more efficacious compared to conventional IP therapy since it not only takes advantage of the hyperthermic effect, but also enables distribution of the drug in all parts of the peritoneal cavity [16]. Nevertheless, the efficacy of HIPEC is still controversial as several studies claim that no synergistic effect exists between the anti-cancer agent and the hyperthermia [15,17].

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One strategy to improve the anticancer effect upon cytoreductive surgery is to use specialized drug delivery systems (DDSs) with the ability to reside in the peritoneal cavity for a prolonged period of time. Interestingly, recent in vivo data suggest that the IP administration of DDSs that release chemotherapeutics results in an enhanced body distribution in general, and on the intratumoral level in particular [18]. Also the delivery of small interfering RNA (siRNA) for the treatment of ovarian cancer and peritoneal carcinomatosis has recently attracted considerable attention [19]. siRNAs are small (20–21 nucleotides), double-stranded RNA molecules that can downregulate specific protein production. siRNA has the benefit that it can target genes which are specific for tumor cells, leaving healthy, non-tumor tissue unaffected. Interestingly, carriers for combinatorial therapy of (specific) siRNA and conventional (non-specific) anti-cancer drugs (e.g. paclitaxel (PTX) or doxorubicin (DOX)) have been reported to result in some benefits compared to each one alone [20].

In the past few years, different DDSs were evaluated for IP administration [21,22], Among them are targeted nanocarriers [23], nanoparticles (NPs) for IP gene delivery [24], micelles [25], microparticle [26,27] and hydrogels for sustained release in the peritoneal cavity [28–30]. For nanosized drug carriers, the state of aggregation and the release profile following IP administration may play a crucial role in their delivery performance. Indeed, the colloidal stability of nanocarriers influences, for example, the internalization of the cargo into cancer cells, and thus may alter the expected anti-tumor efficacy. Following administration, nano-carriers tend to bind/interact with various components that are present in biofluids [31], including proteins and enzymes forming the so called "protein corona" [32,33]. For instance, recent reports suggest that the targeting capability of ligands conjugated to nanomaterials is lost by the adsorption of a protein corona to their surface [34,35]. Increasing our knowledge of the relation between the physicochemical properties of delivery systems and their obtained therapeutic effect is crucial. Since the route of administration plays a major role in whether or not certain carriers will work, each carrier should be optimized for the in vivo situation where it is intended to be used, e.g. the IP fluid in the case of IP delivery. Although several studies have addressed the colloidal stability of NPs in biofluids like blood, plasma and serum [36,37], the physicochemical behavior of delivery vehicles in terms of aggregation and release of cargo in peritoneal fluids has not yet been investigated.

The main objective of this study is to provide insight into the requirements for IP delivery systems in terms of charge and PEGylation degree, to be colloidally stable and to have an optimal release profile in the peritoneal fluid. Herein, for the first time, we study the aggregation of polystyrene (PS) NPs and liposomal formulations in peritoneal fluid from healthy mice (transsudate) and ascites fluid (exudate) from a patient diagnosed with peritoneal carcinomatosis. Additionally, we study the release profile of liposomal formulations carrying siRNA in the peritoneal fluids. For this purpose, we utilize state-of-the-art fluorescence techniques that were previously developed in our laboratory, namely single particle tracking (SPT) and fluorescence correlation spectroscopy (FCS) to respectively gain information on the aggregation of NPs and the release of siRNA in undiluted biofluids [36,38]. The results are compared to measurements of the same NPs dispersed in human serum.

2. Materials and methods

2.1. Materials

(2,3-Dioleoyloxy-propyl)-trimethylammonium-chloride (DOTAP) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were purchased from Corden Pharma LLC (Liestal, Switzerland).

1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-N-(methoxy (polyethyleneglycol)-2000) (DSPE-PEG) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Chloroform, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), N-hydroxysulfosuccinimide (sulfo-NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), dimethylethylenediamine (DMEDA) and sodium chloride (NaCl) were purchased from Sigma Aldrich (Bornem, Belgium). Yellow-green fluorescent (λ_{ex} = 505 nm, λ_{em} = 515 nm) carboxylated PS FluoSpheres (0.1 μm in size) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate (DID) (λ_{ex} = 644 nm, λ_{em} = 665 nm) were purchased from Invitrogen (Merelbeke, Belgium). Methoxy-polyethylene glycol-amine (mPEGa) 2 kDa was purchased from Creative PEGWorks (Winston Salem, USA). Alexa Fluor-488 negative control siRNA was purchased from Eurogentec (Seraing, Belgium).

2.2. Animals

Mice, heterozygous for Foxn1 (nu/+) were purchased from Charles River (Sulzfeld, Germany) and maintained by the animal core facility. Animals were kept at 22 °C in a humidified atmosphere with food and water ad libitum.

2.3. Collection of biofluids

To collect samples containing mouse IP fluid, a lavage of the peritoneal cavity was performed. To this end, mice were killed by an overdose of the inhalation anesthetic isoflurane followed by cervical dislocation. The abdominal wall was opened immediately and the peritoneal cavity was washed with 1 ml of water. The lavage was taken and stored frozen until use. The procedure was approved and carried out in compliance with the guidelines for animal experiments of Leipzig University.

Human serum was obtained from a healthy donor. Briefly, blood was collected at the Ghent University Hospital into Venosafe™ 6 ml tubes containing gel and clotting activator (Terumo Europe™, Leuven, Belgium). Then the tubes were centrifuged for 10 min at 4000*g* and 20 °C. The supernatant (serum) was transferred into microvette® 500 Z-Gel (SARSTEDT, Numbrecht, Germany) and centrifuged for 5 min at 10,000*g* and 20 °C. The serum was portioned into 50 μl aliquots (to avoid freezing–thawing cycles) in sterile polypropylene tubes and stored at −20 °C until use. Human ascites fluid was obtained from a patient diagnosed with peritoneal carcinomatosis at the medical oncology department, Ghent University Hospital. The experiments with the ascites fluid were approved by the ethics committee of the Ghent University hospital (# 2013/589).

2.4. Protein analysis and capillary electrophoresis

Total protein in human serum, human ascites fluid and mice peritoneal fluid was assayed using a pyrogallol red/molybdate method on a Cobas 8000 analyzer (Roche, Mannheim, Germany) [39].

Human serum and human ascites fluid protein electrophoresis was performed using a Capillarys 2™ CE system (Sebia, Paris, France), which is routinely employed in clinical laboratories [40,41]. Prior to the hydrodynamic injection (4"), 40 μl of serum is automatically diluted 5× in the running buffer (pH 10). Then, 7 kV is applied in the eight silica-fused capillaries (effective length 15.5 cm; internal diameter 25 μm; optical cell 100 μm)for 4′ at 35.5 °C (Peltier device). Proteins are detected at the cathode (deuterium lamp; 200 nm) as five fractions (γ-globulins, β-globulins, α2-globulins, α1-globulins and albumin) that are automatically quantified as percentages of the total signal.

For mice peritoneal fluid (characterized by low protein concentrations), agarose gel electrophoresis was carried out, followed by a

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