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SN-38-loaded nanofiber matrices for local control of pediatric solid tumors after subtotal resection surgery

Carles Monterrubio [a, b], Guillem Pascual-Pasto [a, b], Francisco Cano [c], Monica Vila-Ubach [a, b], Alejandro Manzanares [a, d], Paula Schaiquevich [e], Jose A. Tornero [c, f], Alejandro Sosnik [g, h], Jaume Mora [a, b], Angel M. Carcaboso [a, b, *]

[a] Preclinical Therapeutics and Drug Delivery Research Program, Developmental Tumor Biology Laboratory, Fundació Sant Joan de Déu, Santa Rosa 39-57, Esplugues de Llobregat, 08950, Barcelona, Spain
[b] Department of Pediatric Hematology and Oncology, Hospital Sant Joan de Déu Barcelona, Passeig Sant Joan de Déu 2, Esplugues de Llobregat, 08950, Barcelona, Spain
[c] Institut de Investigació Tèxtil i Cooperació Industrial de Terrassa (INTEXTER), Universitat Politecnica de Catalunya, Colom 15, Terrassa, 08222, Barcelona, Spain
[d] Department of Pediatric Surgery, Hospital Sant Joan de Déu Barcelona, Passeig Sant Joan de Déu 2, Esplugues de Llobregat, 08950, Barcelona, Spain
[e] CONICET-Clinical Pharmacokinetics Unit, Hospital de Pediatría JP Garrahan, Combate de los Pozos 1881, 1245, Buenos Aires, Argentina
[f] Cebiotex Biomedical Nanofibers, Parc Cientific de Barcelona, Baldiri i Reixac 4, 08028, Barcelona, Spain
[g] Department of Materials Science and Engineering, Technion-Israel Institute of Technology, Technion City, Haifa, Israel
[h] Russell Berrie Nanotechnology Institute (RBNI), Technion-Israel Institute of Technology, Technion City, Haifa, Israel

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ABSTRACT

In addition to surgery, local tumor control in pediatric oncology requires new treatments as an alternative to radiotherapy. SN-38 is an anticancer drug with proved activity against several pediatric solid tumors including neuroblastoma, rhabdomyosarcoma and Ewing sarcoma. Taking advantage of the extremely low aqueous solubility of SN-38, we have developed a novel drug delivery system (DDS) consisting of matrices made of poly(lactic acid) electrospun polymer nanofibers loaded with SN-38 microcrystals for local release in difficult-to-treat pediatric solid tumors. To model the clinical scenario, we conducted extensive preclinical experiments to characterize the biodistribution of the released SN-38 using microdialysis sampling *in vivo*. We observed that the drug achieves high concentrations in the virtual space of the surgical bed and penetrates a maximum distance of 2 mm within the tumor bulk. Subsequently, we developed a model of subtotal tumor resection in clinically relevant pediatric patient-derived xenografts and used such models to provide evidence of the activity of the SN-38 DDS to inhibit tumor regrowth. We propose that this novel DDS could represent a potential future strategy to avoid harmful radiation therapy as a primary tumor control together with surgery.

1. Introduction

Treatment of most malignant solid tumors in children relies on a combination of local control (surgery and radiation therapy; RT) and systemic chemotherapy [1]. Local tumor recurrence after resection surgery and RT remains a challenge. Despite local control of high-risk neuroblastoma, local tumor recurrences are developed in 10% of newly diagnosed patients and 50% of patients with locally persistent re-resected disease [2,3]. Incidence of local recurrence after first complete remission in other pediatric malignancies such as Ewing sarcoma and primary localized rhabdomyosarcoma is 25% and 22%, respectively [4,5]. The relevance of an adequate local control is underscored by the worse outcome observed in patients that develop local failure after initial complete remission [6].

The intensification of RT to improve local control after resection surgery is limited by unacceptable toxicity, especially in young children [7], and the increased risk of second malignancies [8]. In this context, new technology platforms are urgently called for to overcome the drawbacks associated with RT after tumor resection in children [9].

* Corresponding author. Address: Fundació Sant Joan de Déu, Santa Rosa 39-57, Esplugues de Llobregat, 08950, Barcelona, Spain.
E-mail address: amontero@fsjd.org (A.M. Carcaboso).

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Polymeric drug delivery systems (DDSs) for the localized delivery of anticancer drugs emerged as one of the most promising approaches to treat resectable solid tumors [10]. Advantages of localized delivery comprise reduced systemic exposure to highly toxic agents and achievement of high local concentration of potent anticancer agents that are not suitable for systemic administration due to poor aqueous solubility [10,11]. However, the lack of comprehensive preclinical studies aiming to understand the pharmacokinetics of localized DDS in cancer still represents a significant hurdle towards a robust bench-to-bedside translation. One of the fundamental questions that remain unanswered is whether a substance locally released in the proximity of a solid tumor penetrates into the bulk of the malignant tissue or, conversely, the penetration is restricted to the tumor margins in direct contact with the DDS. To elucidate this, complex imaging techniques [12], radiation [13], or computer simulation [14] are usually required. In a previous study, we demonstrated the potential use of microdialysis to gain insight into these complex mechanisms *in vivo* [15].

SN-38 (10-hydroxy-campthothecin) in its lactone (active) form is a poorly soluble molecule that has shown potent preclinical activity against several pediatric solid tumors [16,17]. Irinotecan, the marketed soluble prodrug of SN-38, undergoes extensive conversion (>70%) to SN-38 in nude mice [18], though it has demonstrated low clinical efficacy, likely due to only partial conversion (less than 10%) into the active derivative upon systemic administration in patients [19]. In addition, SN-38 is rapidly hydrolyzed to an inactive carboxylate form in plasma. The encapsulation of SN-38 into polymeric nanocarriers protected it from biodegradation and prolonged the half-life of the active form [20]. In this framework, SN-38 emerges as an optimal model anticancer drug to investigate the development of a novel DDS for application in the localized chemotherapy of pediatric solid tumors.

Electrospun polymer nanofiber matrices appear as one of the most versatile, reproducible and scalable nano-DDS [21]. They allow to adjust their size and shape to fill the space left by tumor resection, and provide a large surface area and porosity that facilitate the efficient release of the active cargo from the DDS to the tumor tissue [21]. Moreover, their monolithic nature eases manipulation, implantation and retention in the action body site, and prevents the characteristic migration of nanoparticles and microparticles.

Following this rationale, the present work reports for the first time on the development of a novel nanofiber DDS loaded with SN-38 microcrystals for the localized chemotherapy of pediatric solid tumors and the comprehensive characterization of the release rate, the *in vivo* localized biodistribution, the systemic pharmacokinetics and the antitumor activity in pediatric solid tumor models. A unique feature of the study is the use of microdialysis probes inserted in the tissue targeted by the localized release of SN-38 to quantify local drug levels at different depths in the tumor bulk or in the virtual space of the resection bed [15]. To our knowledge, such sampling technique has not yet been employed to monitor localized drug delivery in tumors.

2. Materials and methods

2.1. Reagents

SN-38 was obtained from Seqchem (Pangbourne, UK). Poly(-lactic acid) (PLA) was from Velox, GmbH (Hamburg, Germany). Pluronic® F68 block polymer was a gift from BASF (Ludwigshafen, Germany). Irinotecan was purchased from Hospira (Lake City, IL, USA). 2-Hydroxypropyl-beta-cyclodextrin (HPBCD; molecular weight of 1400 g/mol) and dimethyl sulfoxide (DMSO) were from Sigma–Aldrich (St. Louis, MO, USA). Methanol was from Merck (Darmstad, Germany). RPMI high glucose medium and supplements (fetal bovine serum, glutamine, penicillin and streptomycin) were from Life Technologies (Grand Island, NY, USA).

2.2. Preparation of PLA nanofiber matrices loaded with SN-38 microcrystals

SN-38 microcrystal suspensions were prepared by pH-dependent crystallization the day before the preparation of the nanofiber matrices. The crystallization method takes advantage of the pH-dependent reversible equilibrium between SN-38 carboxylate (water soluble and predominant at basic or neutral pH) and SN-38 lactone (insoluble in water and predominant at acidic pH). To form the microcrystals, one volume (100 μL) of SN-38 solubilized in basic pH (4 mg/mL in NaOH 0.1 N) was mixed with 9 volumes (900 μL) of pH 5.0 acetate buffer containing 2% Pluronic® F68. The mixture resulted in a final pH value of 5.5 and it was stored at 4 °C for 24 h with hourly agitation during the first 6 h to favor the slow precipitation of the SN-38 lactone microcrystals. The size of the crystals at 24 h was measured by dynamic light scattering (DLS) with a ZetaSizer Nano ZS (Malvern Instruments, Malvern, UK).

SN-38 microcrystal-loaded nanofiber matrices were prepared by electrospinning. PLA (10% in dichloromethane) was loaded in a 2 mL syringe and pumped at a constant rate of 0.5 mL/h at a 10 kV voltage. The PLA solution was spun for 20 min on a rotating rod wrapped with vegetal paper, to build a first layer of SN-38-free nanofibers that would prevent the direct release of the intact drug microcrystals to the physiologic medium. During the following 45 min, the SN-38 microcrystal suspension (loaded in a syringe) was pumped (90 μL/min) simultaneously from the opposite part of the rotating rod, and sprayed with a pneumatic nozzle. The theoretical load of SN-38 in the matrix was 18 $\mu g/cm^2$. Finally, after loading the complete suspension of the drug microcrystals, the PLA solution was spun for extra 20 min to generate another free-drug layer that isolates the cargo. Finally, the matrix was dried under vacuum for 24 h, at room temperature. The products (SN-38-loaded nanofiber matrices cut into 0.25, 0.5 or 1 cm^2 sheets) were characterized by scanning electron microscopy (SEM; Phenom G1, Eindhoven, The Netherlands), fluorescence microscopy (Leica DM 5000 B, Wetzlar, Germany), and differential scanning calorimetry (DSC 2 STAR^e system simultaneous thermal analyzer with STAR^e Software V13, Mettler-Toledo, Schwerzenbach, Switzerland) equipped with intra-cooler Huber TC100 under dry N_2 atmosphere and In as standard. The amount of SN-38 loaded in the matrices was analyzed by extraction of the drug with methanol and injection of the extract in a high-performance liquid chromatography (HPLC) system with fluorescence detector, as previously described [15].

2.3. SN-38 release

Several *in vitro* and *in vivo* experiments characterized the release profile of SN-38 from the matrices upon dissolution of internal SN-38 microcrystals in physiologic conditions.

In vitro, SN-38 matrices containing 5 μg SN-38 in 0.25 cm^2 (n = 24) were placed in glass vials with 5 mL of pre-warmed PBS (pH 7.4) and incubated at 37 °C away from light. At time points 0.25, 2, 4, 8, 24, 48, 72 and 96 h, three matrices were removed from the vials for drug analysis by HPLC. The removed matrices were vigorously vortexed in 5 mL methanol to extract the unreleased SN-38 for analysis. To favor sink conditions, the release medium of the remaining matrices was completely replaced with fresh pre-warmed PBS at all sampling times.

We repeated the *in vitro* release experiment described above though in the presence of the solubilizer HPBCD (10% w/v in PBS). The sampling times in these experiments were 0.25, 0.5, 0.75, 1, 2,