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Locally delivered nanoencapsulated tyrphostin (AGL-2043) reduces neointima formation in balloon-injured rat carotid and stented porcine coronary arteries

Shmuel Banai[a,1], Michael Chorny[b,1], S. David Gertz[c], Ilia Fishbein[b], Jianchuan Gao[b], Louise Perez[c], Galila Lazarovichi[b], Aviv Gazit[d], Alexander Levitzki[d], Gershon Golomb[b,*]

[a] *Heiden Department of Cardiology, Bikur Cholim Hospital,Jerusalem, Israel*
[b] *Department of Pharmaceutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Ein Kerem Box 12065, Jerusalem 91120, Israel*
[c] *Department of Anatomy and Cell Biology, Jerusalem, Israel*
[d] *Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel*

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Abstract

Local delivery of antiproliferative drugs encapsulated in biodegradable nanoparticles (NP) has shown promise as an experimental strategy for preventing restenosis development. A novel PDGFRβ-specific tyrphostin, AGL-2043, was formulated in polylactide-based nanoparticles and was administered intraluminally to the wall of balloon-injured rat carotid and stented pig coronary arteries. The disposition and elimination kinetics within the vessel wall, as well as the antirestenotic potential of the novel drug and delivery system, were evaluated. The efficacy and the local drug elimination kinetics were affected by the size of the NP and the drug-carrier binding mode. Despite similar arterial drug levels 90 min after delivery in rats, small NP were more efficacious in comparison to large NP (90 and 160 nm, respectively). AGL-2043 selectively inhibited vascular SMC in a dose-dependent manner. The antiproliferative effect of nanoencapsulated tyrphostin was considerably higher than that of surface-adsorbed drug. In the pig model, intramural delivery of AGL-2043 resulted in reduced in-stent neointima formation in the coronary arteries over control despite similar degrees of wall injury. The results of this study suggest that locally delivered tyrphostin AGL-2043 formulated in biodegradable NP may be applicable for antirestenotic therapy independent of stent design or type of injury.

Keywords: Controlled drug release; Drug delivery; In-stent restenosis; Intimal hyperplasia; Local delivery; Nanoparticles; Neointima; Protein tyrosine kinase blocker; Restenosis; Smooth muscle cell; Stent; Tyrphostin

1. Introduction

Platelet derived growth factor (PDGF), expressed by platelets, smooth muscle cells (SMC), endothelial cells, and macrophages, has been shown to play an important role in the pathogenesis of restenosis acting as both a mitogen and chemoattractant for SMC, as well as being involved in the transformation of SMC from their contractile to proliferative phenotype [1,2].

The tyrphostins are low-molecular weight, synthetic compounds whose basic structure can be modified to block specific receptors or intracellular protein tyrosine kinases (PTKs) [3]. Unlike larger receptor antibodies, the small size of the tyrphostins permits easier access to receptor sites within tissues such as the depths of the media. The profound, selective PTK inhibition of such compounds results from competitive interaction with the ATP binding domain as well as mixed competitive inhibition with substrate binding sub-sites.

Novel pharmacological strategies of systemic drug administration may result in revision of the viability of the systemic approach in the future [4,5], but it is generally accepted that an effective antirestenotic therapy should combine sufficiently high and sustained

*Corresponding author. Tel.: +972-2675-7504; fax: +972-2675-7503.
E-mail address: golomb@md.huji.ac.il (G. Golomb).
[1] Equal contribution to this work.

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drug levels at the injury site with minimal systemic and local toxicity. Drug-laden NP offer the advantage of high tissue uptake and protracted drug residence at the injury site [6–9]. Drug-eluting polymer-coated stents have recently shown promising results in clinical studies [10]. Nevertheless, drug delivery via polymeric biodegradable nanoparticles (NP) may provide a valuable alternative. Site-specific delivery of drug-impregnated NP may be accomplished with a variety of catheters [11], and it is neither restricted to stenting in general [12], nor to a particular stent design or type. It should be noted that 30–40% of critical lesions cannot be stented, largely because they occur at branch sites or in small arteries. Hence, other methods for prevention of restenosis beyond drug-eluting-stents strategy are necessary.

In a series of in vitro experiments we have shown that tyrphostin AG-1295, an inhibitor of PDGFβ receptor (PDGFRβ) PTK, selectively inhibits SMC proliferation in culture and attenuates outgrowth of SMC from porcine and human arterial explant tissue [8]. Moreover, in vivo studies in isolated balloon-injured rat carotid [6,7] and pig femoral arteries [8] have shown a reduction of neointima formation after intramural incubation with AG 1295 NP. In the present study, we utilized a novel, structurally related compound, AGL-2043, a more potent inhibitor of SMC [13]. This study was designed to determine whether local, intramural delivery of nanoencapsulated tyrphostin of the PDGF-receptor-kinase blocker family, via an oozing balloon, will inhibit neointima formation at the site of stent implantation in porcine coronary arteries. In this study we characterize arterial and cellular uptake as well as elimination kinetics, and identify optimally effective nanoparticulate formulation of AGL-2043 which may be of relevance to the human interventional setting.

2. Materials and methods

2.1. NP preparation and characterization

AGL-2043-loaded matrix-type NP were formulated using the modified nanoprecipitation method as previously described [14]. The method was adapted to produce NP of different sizes with the drug either encapsulated or adsorbed to the carrier surface. The reference formulation (large-sized NP) was prepared as follows: 200 mg of D,L-PLA poly(D,L-lactide) M_W 90,000–120,000 (Sigma, St. Louis, MO, USA) and 2 mg of 1,2-dimethyl-6-(2-thiophene) imidazolo [5,4-g] quinoxaline (AGL-2043, Oz Chemicals, Jerusalem, Israel) were dissolved in an organic phase consisting of dichloromethane and acetone (0.5 and 19.5 ml, respectively). An aqueous phase was prepared by dissolving 50 mg of surfactant, Pluronic F-68 (Sigma, St. Louis, MO, USA), in 40 ml of distilled water. The organic phase was rapidly poured into the aqueous solution under stirring on a magnetic plate leading to spontaneous formation of NP. The organic solvents were evaporated, and formulation volume adjusted to 10 ml under pressure gradually reduced from 180 to 12 mmHg using Rotavapor (Büchi, Switzerland) at 35°C. Finally, the formulation was filtered through 0.45 μm hydrophilic syringe filter (Sartorius, Germany). Small-sized NP were prepared similarly with inclusion of ethanol in the organic phase. Following complete dissolution of the polymer and the drug in 0.5 ml dichloromethane and 9.5 ml acetone, ethanol was added to a final volume of 20 ml.

The association of the tyrphostin with the NP surface was accomplished by dropwise addition of drug solution in acetone (0.5 ml) to the stirred suspension of preformed blank NP followed by acetone evaporation, volume adjustment, and filtration.

NP size was measured using photon correlation spectroscopy (ALV-GmBH, Langen Germany) at 25°C following NP dilution 1:400 with distilled water filtered using 0.22 μm filter.

The drug release rate from the NP was determined by the equilibrium dialysis technique as described elsewhere [7]. Fine powder of AGL-2043 (60 μm) mixed with blank NP was used as a control.

2.2. In vitro growth inhibition and cellular uptake

2.2.1. Porcine smooth muscle cells (SMC) and endothelial cells (EC)

Cells were isolated and cultured as described elsewhere [8]. Monolayer cell growth inhibition was performed on SMC or EC from passages 2–3. The medium (M199 with ECGS for EC and DMEM for SMC) was supplemented with serum and changed every other day. Cultures were treated with AGL-2043 (10 and 5 μM) dissolved in 0.1% dimethylsulfoxide (DMSO) on days 1 and 4. Cells were counted on day 6, and the vehicle (DMSO 0.1%) served as control.

2.2.2. Rat SMC

Cells were obtained from rat thoracic aortas and cultured as described previously [15]. AGL-2043 dissolved in DMSO was diluted in growth medium containing 15% FCS yielding drug concentrations of 0.5–50 μM and added to the cells on day 1. In control experiments, a corresponding amount of DMSO was added to the growth medium. The inhibitory effect was measured on day 4 by cell counting. In a separate experiment, the inhibitory effect of large and small AGL-2043-loaded NP, in suspension at drug concentration of 5 μM, was compared to that of free drug, and to NP-surface adsorbed AGL-2043, using blank NP and DMSO solution in growth medium (0.1%) as a reference (Fig. 1).