Journal of Controlled Release 149 (2011) 196–205

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

A LDL-masked liposomal-doxorubicin reverses drug resistance in human cancer cells

Joanna Kopecka [a,1], Ivana Campia [a,1], Paolo Olivero [b], Gianpiero Pescarmona [a,c], Dario Ghigo [a,c], Amalia Bosia [a,c], Chiara Riganti [a,c,*]

[a] Department of Genetics, Biology and Biochemistry, University of Turin, via Santena 5/bis, 10126 Turin, Italy
[b] Department of Experimental Physics, University of Turin and National Institute of Nuclear Physics, via P. Giuria 1, 10125, Turin, Italy
[c] Research Center on Experimental Medicine (Ce.R.M.S.), University of Turin, via Santena 5/bis, 10126 Turin, Italy

ARTICLE INFO

Article history:
Received 24 June 2010
Accepted 5 October 2010
Available online 12 October 2010

Keywords:
P-glycoprotein
Doxorubicin
Liposomes
LDL receptor
Simvastatin

ABSTRACT

Doxorubicin is one of the most employed anticancer drugs, but its efficacy is limited by the onset of adverse effects such as drug resistance, due to the drug efflux via P-glycoprotein (Pgp). Several factors are associated to a high Pgp activity, including the amount of cholesterol in plasma membrane, which is essential to maintain the pump function.

In this work we started from the following observations: 1) the drug-resistant colon cancer HT29-dx cells had a higher content of cholesterol in plasma membrane than drug-sensitive HT29 cells and a higher activity of Pgp, which was decreased by the cholesterol-lowering agent β-methyl-cyclodextrin; 2) HT29-dx cells showed a higher synthesis of endogenous cholesterol and a higher expression of the low-density lipoprotein receptor (LDLR); 3) the anti-cholesterolemic drug simvastatin reduced the cholesterol synthesis, increased the synthesis of LDLR and lowered the Pgp activity in resistant cells.

In order to circumvent drug resistance we designed a new liposomal doxorubicin, conjugated with a recombinant LDLR-binding peptide from human apoB100: this LDL-masked doxorubicin ("apo-Lipodox") was efficiently internalized by a LDLR-driven endocytosis and induced cytotoxic effects in HT29-dx cells, reversing their drug resistance. Its efficacy was further increased by simvastatin, which up-regulates the LDLR levels and contemporarily reduces the Pgp activity, thus increasing the liposomes uptake and limiting the drug efflux. We propose that the association of liposomal doxorubicin and statins may be a future promising strategy to reverse drug-resistance in human cancer cells.

1. Introduction

Chemotherapy is the most powerful therapeutic tool against advanced and disseminated cancers, but its efficacy is often limited by the onset of adverse effects and resistance to anticancer drugs. A multiple cross-resistance towards different chemotherapeutic agents, known as multidrug resistance (MDR), occurs in tumors at the diagnosis or during relapses and metastatic spreading [1].

Anthracyclines, in particular doxorubicin, represent the first-line therapeutic option in many hematological and solid tumors, despite the limitations due to their cardiotoxicity [2]. The resistance to doxorubicin is another problem to deal with during the therapy: being a substrate of different ATP-binding cassette (ABC) membrane pumps, such as P-glycoprotein (Pgp) and multidrug resistance-related proteins (MRPs), doxorubicin is actively extruded from cancer cells which overexpress these transporters, reducing its intracellular accumulation and then its antineoplastic effects [1]. Different inhibitors of Pgp have been included in clinical trials to enhance the efficacy of doxorubicin, but their poor specificity and high toxicity have withdrawn these compounds from the clinical practice [3]. Nitric oxide (NO) donors or NO synthase inducers, like statins, which elicit the nitration of critical tyrosines of Pgp, decrease the pump activity in *in vitro* models and we have proposed them as potential tools to overcome MDR [4,5]. Statins exert pleiotropic effects on mammalian tissues and are primarily used as anticholesterolemic drugs, because they are strong inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the rate-limiting enzyme of cholesterol synthesis. Under this perspective they may look particularly appealing as

Abbreviations: LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; Pgp, P-glycoprotein; MDR, multidrug resistance; ABC, ATP binding cassette; NO, nitric oxide; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; Lipodox, liposomal doxorubicin; apo-Lipodox, liposomal doxorubicin conjugated with LDLR-binding peptide from human apoB100; β-MCD, β-methyl-cyclodextrin; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; BSA, bovine serum albumin; TEM, transmission electron microscope; SEM, scanning electron microscopy; DAPI, 4′,6-Diamidino-2-phenylindole dihydrochloride; FITC, fluorescein isothiocyanate.

* Corresponding author. Department of Genetics, Biology and Biochemistry, University of Turin, via Santena 5/bis, 10126 Turin, Italy. Tel.: +39 0116705851; fax: +39 0116705845.
E-mail addresses: joanna.kopecka@unito.it (J. Kopecka), ivana.campia@unito.it (I. Campia), olivero@to.infn.it (P. Olivero), gianpiero.pescarmona@unito.it (G. Pescarmona), dario.ghigo@unito.it (D. Ghigo), amalia.bosia@unito.it (A. Bosia), chiara.riganti@unito.it (C. Riganti).

[1] These authors contributed equally to this work.

0168-3659/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2010.10.003