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Ascites interferes with the activity of lurbinectedin and trabectedin: Potential role of their binding to alpha 1-acid glycoprotein

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ABSTRACT

Trabectedin and its analogue lurbinectedin are effective drugs used in the treatment of ovarian cancer.

Since the presence of ascites is a frequent event in advanced ovarian cancer we asked the question whether ascites could modify the activity of these compounds against ovarian cancer cells.

The cytotoxicity induced by trabectedin or lurbinectedin against A2780, OVCAR-5 cell lines or primary culture of human ovarian cancer cells was compared by performing treatment in regular medium or in ascites taken from either nude mice or ovarian cancer patients.

Ascites completely abolished the activity of lurbinectedin at up to 10 nM (in regular medium corresponds to the IC90), strongly reduced that of trabectedin, inhibited the cellular uptake of lurbinectedin and, to a lesser extent, that of trabectedin. Since α1-acid glycoprotein (AGP) is present in ascites at relatively high concentrations, we tested if the binding of the drugs to this protein could be responsible for the reduction of their activity. Adding AGP to the medium at concentration range of those found in ascites, we reproduced the anticytotoxic effect of ascites. Erythromycin partially restored the activity of the drugs, presumably by displacing them from AGP. Equilibrium dialysis experiments showed that both drugs bind AGP, but the affinity of binding of lurbinectedin was much greater than that of trabectedin. KD values are 8 ± 1.7 and 87 ± 14 nM for lurbinectedin and trabectedin, respectively.

The studies intimate the possibility that AGP present in ascites might reduce the activity of lurbinectedin and to a lesser extent of trabectedin against ovarian cancer cells present in ascites. AGP plasma levels could influence the distribution of these drugs and thus they should be monitored in patients receiving these compounds.

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1. Introduction

Lurbinectedin (PM01183) is a trabectedin analogue under clinical development for the therapy of several malignancies including ovarian, breast and lung cancer [1–3]. The chemical structure of lurbinectedin is similar to that of trabectedin. It contains the same pentacyclic skeleton of the fused tetrahydroisoquinoline rings, but it differs by the presence of a tetrahydro beta-carboline that replaces the additional tetrahydroisoquinoline of trabectedin. (Fig. 1).

The mode of action of lurbinectedin is similar to that of trabectedin. Both drugs bind to the DNA minor groove forming adducts at the N2 position of guanines with similar effects on the DNA repair and transcription machineries [4,5]. Both drugs are more cytotoxic against cells that are deficient in Homologous Recombination Repair and less cytotoxic against cells deficient in Nucleotide Excision Repair [6–8]. Both drugs affect the regulation of transcription by displacing some oncogenic transcription factors from their target promoters [9,10] and at high concentrations they

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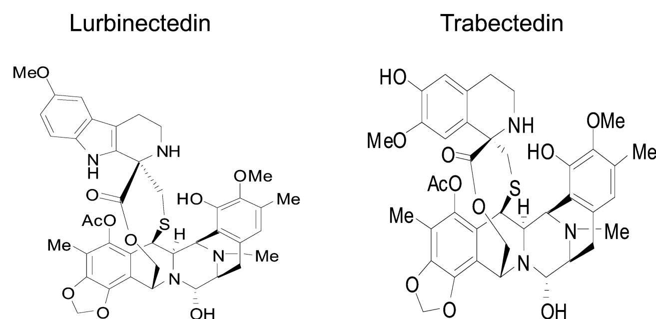

Fig. 1. Chemical structure of trabectedin and lurbinectedin.

cause degradation of RNA polymerase II [11]. The in vivo antitumor activity of both trabectedin or lurbinectedin seems to be due not only to their direct effect on cancer cells but also to their ability to reduce the number of tumor associated macrophages and to inhibit the production of several pro-tumoral inflammatory and angiogenic factors [12–14]. The clinical pharmacokinetic parameters of lurbinectedin differ from those of trabectedin. In particular the apparent volume of distribution of lurbinectedin is 4 times lower than that of trabectedin [15–19].

We previously reported that in Igrov-1 ovarian cancer cells the uptake and cytotoxicity of trabectedin was reduced by increasing the amount of foetal bovine serum (FBS) but not by human serum or by human serum albumin (HSA) suggesting that some proteins different from albumin, present in FBS, tightly bind to the drug reducing the amount of free drug available to enter the cells and to exert its action [20]. This observation prompted us to investigate whether a protein rich-pathological fluid - present in the peritoneal cavity of many ovarian cancer patients such as ascites- [21] influenced the cytotoxicity of trabectedin or lurbinectedin. The results show a dramatic inhibitory effect of ascitic fluid on the cytotoxicity of lurbinectedin and to a lesser extent of trabectedin and provide evidence that α1-acid glycoprotein (AGP) is likely to be implicated in this effect.

2. Materials and methods

2.1. Ascites samples

2.1.1. Patients

Ascites was collected in the operating theatre during the laparotomy carried out as primary treatment of epithelial ovarian cancer or for relapsed disease from patients hospitalized at the S Gerardo Hospital in Monza or at Istituto Tumori, Milan. Samples of ascites, obtained from 32 ovarian cancer patients, were collected in heparinized bottles and the cells were separated by centrifugation. Then the supernatant was stored at −20 °C before use (range of storage time at −20 °C 1–6 months).

The study was approved by the Ethics Committees of the S. Gerardo Hospital and Istituto Tumori with patients' written consent. All procedures were carried out in accordance with the ethical standards of the Declaration of Helsinki.

2.1.2. Animal model

Ascites was collected after sacrifice from 5 female Ncr-*nu/nu* mice orthotopically transplanted with HOC8 ovarian cancer cells derived from a malignant pleural effusion removed from a previously untreated patient [22].

Procedures involving animals and their care were conducted in conformity with the following laws, regulations, and policies governing the care and use of laboratory animals: Italian Governing Law (D.lgs 26/2014; Authorization n.19/2008-A issued March 6, 2008 by Ministry of Health); Mario Negri Institutional Regulations and Policies providing internal authorization for persons conducting animal experiments (Quality Management System Certificate – UNI EN ISO 9001:2008 – Reg. N° 6121); the NIH Guide for the Care and Use of Laboratory Animals (2011 edition) and EU directives and guidelines (EEC Council Directive 2010/63/UE) and in line with Guidelines for the welfare and use of animals in cancer research [23]. Animal experiments have been reviewed and approved by the IRFMN Animal Care and Use Committee (IACUC) that includes members "ad hoc" for ethical issues. Animals were housed in the Institute's Animal Care Facilities, which meet international standards; they are regularly checked by a certified veterinarian who is responsible for health monitoring, animal welfare supervision, experimental protocols and procedures revision.

2.2. Drugs

Trabectedin and lurbinectedin (purity: 100%) were kindly provided by Pharma Mar, S.A. (Colmenar Viejo, Spain), stocked in DMSO (Sigma Aldrich, St Louis, MO, USA) at a concentration of 1 mM, stored at −20 °C and were diluted in RPMI-1640 medium just before use. AGP, erythromycin, carboplatin and paclitaxel were purchased from Sigma Aldrich and were diluted in RPMI-1640 medium (Thermo Scientific, Waltham, MA, USA) just before use.

2.3. Cells

2.3.1. Cell lines

Human ovarian carcinoma A2780 and OVCAR-5 cells were grown in RPMI-1640, 10% FBS and 2 mM L-glutamine (Thermo Scientific) in T 25 cm^2 flasks (Eppendorf, Hamburg, Germany) and maintained at 37 °C in a humidified atmosphere at 5% CO2.

2.3.2. Human ovarian cancer primary cultures

The ascitic fluids were collected in heparinized bottles and the cells were separated by centrifugation. A first gradient with 100% of Ficoll-Hypaque (d = 1.077; Sigma Aldrich, St Louis, MO, USA) was performed (600 g for 20 min) to remove RBC contamination and debris. In cases of gross lymphocyte and granulocyte contamination, a second discontinuous gradient (75% Ficoll- Hypaque, layered on 100% Ficoll-Hypaque) was performed. After these steps, in one case, tumor cells were freed of macrophages by adhesion on plastic culture dishes. Final cell suspension was seeded at 70,000 cells cm^2 in 6-well multiwell tissue culture plates (Eppendorf).

2.4. Proliferation assay

The evaluation of the cytotoxicity induced by trabectedin or lurbinectedin on cells in the different experimental conditions was assessed by WST-1 cell proliferation assay (Roche, Basel, Switzerland) [24]. The cells were seeded at the concentrations of 5000 cells/ml in 96 –wells tissue culture plates; at 48 h after seeding the cells, in exponentially growing phase, were treated with trabectedin or lurbinectedin for 24 h in medium plus 10% FBS, regular medium, or in 100% ascites obtained from xenografts or from patients affected by ovarian cancer. In the cases of ascites obtained from human patients, in each experiment the ascitic fluid was obtained only from one patient. After 24 h the drug-containing medium was removed, the cells were washed with PBS and fresh medium plus 10% FBS, 2 mM L-glutamine and 100 U/ml penicillin/streptomycin was added for 72 h after drug-washout, when the effect of treatment was evaluated.

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