Pharmacological Reports 69 (2017) 696–701

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original article

The NK-1 receptor antagonist L-732,138 induces apoptosis in human gastrointestinal cancer cell lines

Miguel Muñoz[a,*], Marisa Rosso[a], Rafael Coveñas[b]

[a] Virgen del Rocío University Hospital, Research Laboratory on Neuropeptides (IBIS), Sevilla, Spain
[b] Institute of Neurosciences of Castilla y León (INCYL), Laboratory of Neuroanatomy of the Peptidergic Systems (Lab. 14), University of Salamanca, Salamanca, Spain

ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form 24 January 2017
Accepted 2 February 2017
Available online 12 February 2017

Keywords:
Gastric carcinoma
Colon carcinoma
Cell proliferation inhibition
NK-1 receptor
Apoptosis

ABSTRACT

Background: Gastric and colon cancer cells express the neurokinin-1 receptor (NK-1R) and the peptide substance P (SP), after binding to this receptor, elicits the proliferation of gastrointestinal cancer cells and an antiapoptotic effect. In these cells, NK-1R antagonists (L-733,060: a piperidine derivative; aprepitant: a morpholine derivative) block, after binding to the NK-1R, the action of SP and exert an antiproliferative action, both antagonists promote apoptosis and the death of cancer cells. However, it is currently unknown whether tryptophan derivative NK-1R antagonists (e.g., L-732,138) exert an antiproliferative effect against gastrointestinal cancer cells. L-732,138, L-733,060 and aprepitant being structurally unrelated compounds show a high specificity for the NK-1R.
Methods: To determine the number of viable cells, a Coulter counter was performed. For evaluation of tumor cell viability, an MTS colorimetric method was conducted. For apoptosis, a DAPI stain was carried out.
Results: L-732,138 blocked, in a concentration-dependent manner, the proliferation of gastrointestinal cancer cells (IC_{50}: 75.28 and IC_{100}: 127.4 for human SW-403 colon carcinoma cell line; IC_{50}: 76.8 and IC_{100}: 157.2 for 23132-87 gastric carcinoma cell line. Level of significance: $p \leq 0.01$). The antitumor effect elicited by L-732,138 was *via* the NK-1R and, in addition, 72.1% and 59.3% apoptotic cells (chromatin condensation and nuclear fragmentation) were respectively found in gastric and colon cancer cell lines when L-732,138 (at IC_{100} concentration) was administered.
Conclusion: It seems that the NK-1R is an emerging drug target for the treatment of gastrointestinal cancer and that the tryptophan derivative NK-1R antagonist L-732,138 must be considered as an anticancer drug in gastrointestinal cancer.

Introduction

Around 990,000 people/year are detected worldwide with gastrointestinal cancer (GIC), of which about 738,000 die [1,2]. For localized gastric cancer, surgery is a curative strategy, but unfortunately it is the advanced stages of the disease that are most frequently diagnosed. The prognosis for gastric cancer is not particularly positive (overall 5-year survival rates) [3]. In the USA, colorectal cancer is the third most common cancer [4]. It is known that around 90% of all cancer deaths are due to metastases development and not to primary tumors [5].

Substance P (SP), *via* the neurokinin-1 receptor (NK-1R), controls numerous pathophysiological actions related to cancer, e.g., proliferation and migration of cancer cells [6]. However, NK-1R antagonists block the action of SP and inhibit these functions [6]. SP is found throughout the whole body and in human cancer cell lines/samples the NK-1R is overexpressed [6,7]. Human GIC cells and tissues express the NK-1R [8,9]. The administration of high doses of SP promotes gastric carcinogenesis provoked by N-methyl-N'-nitro-N-nitrosoguanidine [10] and SP promotes the proliferation, adhesion and the migration and invasion of MKN45 gastric cancer cells [11]. In human gastric cancer, SP-containing nerves and NK-1 receptors (NK-1Rs) have been observed, and it seems that the number of SP-positive nerves is related to the progression of gastric cancer [11]. It has also been demonstrated that SP exerts an antiapoptotic effect; which, in a concentration-dependent manner, elicits the proliferation of human GIC cells, and

* Corresponding author
E-mail address: mmunoz@cica.es (M. Muñoz).

http://dx.doi.org/10.1016/j.pharep.2017.02.002
1734-1140/

that NK-1R antagonists (L-733,060, a piperidine derivative and aprepitant, a morpholine derivative) block, after binding to the NK-1R, the action of SP and inhibit the proliferation of human GIC cell lines (both antagonists induce apoptosis and death of cancer cells) [6,8,12,13]. However, it is currently unknown whether tryptophan derivative NK-1R antagonists (e.g., L-732,138) exert an antiproliferative effect against GIC cells.

Thus, in order to know more about the antiproliferative action of the NK-1R antagonists, the purpose of this study is: 1) to show the antitumor effect of the tryptophan derivative NK-1R antagonist L-732,138 against human SW-403 colon and 23132/87 gastric adenocarcinoma cell lines; 2) to demonstrate that the antitumor action is exerted *via* the NK-1R and 3) to show that L-732,138 induces apoptosis in both human gastrointestinal cancer cell lines.

Material and methods

Cell culture

Human SW-403 colon carcinoma and 23132-87 gastric carcinoma cell lines (Deutsche Sammlung von Mikroorganismen und Zellkulturen) were used in this study. As previously reported [8], cells were incubated at 37° C in a humidified atmosphere (5% CO_2/95% air).

Drug treatments

The NK-1R receptor L-732,138 (N-Acetyl-L-tryptophan 3, 5-bis (trifluoromethyl) benzyl ester) was dissolved in water containing dimethylsulphoxide (DMSO, 0.2%). To determine the IC_{50}, concentrations from 20 μM to 100 μM of L-732,138 were tested. SP, acetate salt, was dissolved in water and several concentrations of the undecapeptide (5 nM, 10 nM, 50 nM, 100 nM) were evaluated. 1 h before addition of the NK-1R antagonist, SP was added to the cultured cells (the SP nM concentration added was that showing the highest mitogenic action for each cancer cell line).

Proliferation assays

We used a tetrazolium compound 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) to evaluate the proliferation of cells and a Coulter counter to count the number of cells. The plate included blank wells (no cells), control wells (10^4 cells/0.1 ml), control wells with L-732,138 or with DMSO, and control wells with the NK-1R antagonist and the most mitogenic concentration of SP. Plates were inoculated with the NK-1R antagonist (20–100 μM) and incubated for the first doubling time (specific for each cancer cell line studied). Different concentrations of SP were added to the control wells. 90 min before reading the samples (on a multiscanner microplate reader at 492 nm), the MTS reagent (20 μl) was administered to each well. The experimental conditions, mentioned above, were tested in duplicate. Experiments were carried out at least three times.

Statistical analyses

The study was conducted using SPPS statistical software for Microsoft Windows, release 19.0 (Professional Statistic, Chicago, IL, USA). Data were expressed as means ± SD. Using the Levene test, variance homogeneity was evaluated. In the case that variances were homogeneous, the one-way ANOVA test was applied with

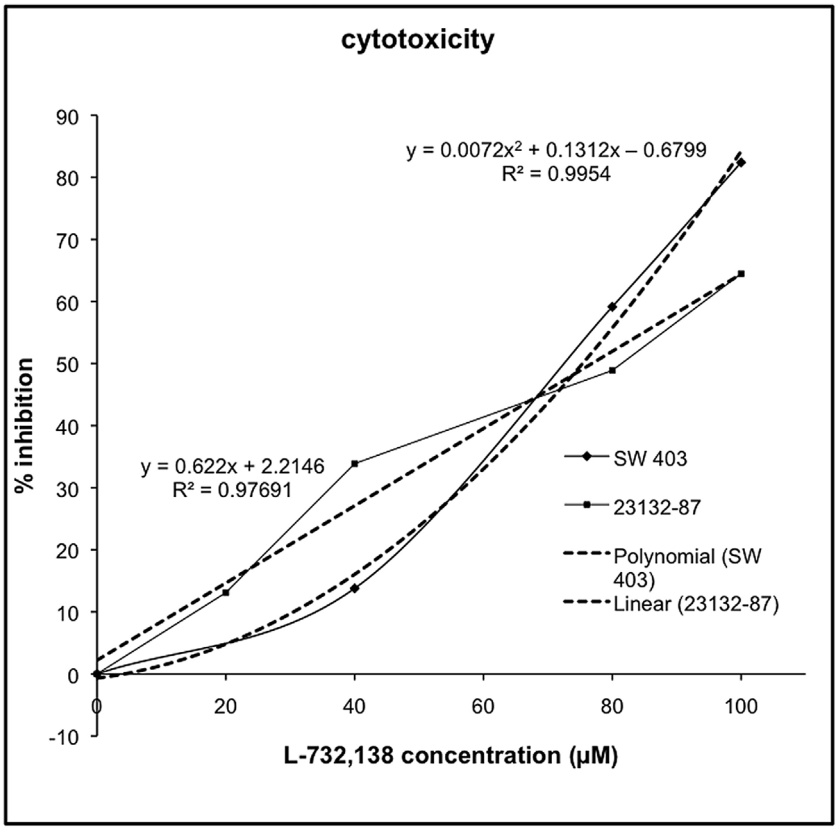

Fig. 1. Percentage of growth inhibition of human 23132-87 gastric and SW-403 colon carcinoma cell lines at 50 h and 48 h respectively in *in vitro* cultures following the addition of increasing concentrations (20–100 μM) of L-732,138. The percentage of inhibition or the first doubling time of incubation is plotted on a linear graph. Level of significance: * $p \leq 0.01$. The interpolation line is indicated, as well as the equation used to obtain the IC50. The experiment was repeated three times in duplicate.