Screening of newly synthesized xanthones and potential P-glycoprotein modulation at intestinal barrier - in vitro and ex vivo studies

INTRODUCTION

P-glycoprotein (P-gp) is an efflux pump belonging to the ABC-binding cassette (ABC) transporter superfamily and has an ubiquitous and constitutive distribution throughout the body. Due to its wide distribution, namely to its polarized expression in barrier and excratory tissues, to its varied range of substrates and to its large efflux capacity, P-gp is vital in the pharmacokinetic processes of absorption and distribution of toxic substrates, reducing their intracellular accumulation and, consequently, their toxicity. This defense mechanism is particularly important at the intestinal level, significantly reducing the intestinal absorption of xenobiotics, limiting its access to the target organs, resulting in a decrease in their toxicity. Thus, P-gp can be faced as a potential antidotal pathway, when induced and/or activated.

AIM

The aim of the present study was to investigate, in a human colorectal adenocarcinoma cell line (SW480 cells), six newly synthesized xanthones derivatives (Xs), since xanthones are known to interact with P-gp through modulation mechanisms such as induction and/or activation. Additionally, for the most promising compounds, ex vivo studies were conducted for the same purpose.

MATERIALS AND METHODS

In vitro studies:

- The cytotoxicity of the tested xanthones (0 - 50 µM) was evaluated by the Neutral Red (NR) and MTT uptake assays, 24 h after exposure, to select a noncytotoxic concentration to be used in the subsequent studies.
- The effect of the tested xanthones on P-gp expression was evaluated by flow cytometry, using a P-gp monoclonal antibody (UC2) conjugated with Phycoerythrin (PE), 24 h after exposure.
- P-gp activity was measured through two different protocols, both using Rhodamine 123 (RHO, 3 µM) as a fluorescent P-gp substrate. In the first protocol, the accumulation of RHO 123 was evaluated in SW480 cells previously exposed to the tested Xs (20 µM) for 24 h, assessing to eventual alterations in P-gp activity due to the possible effects on P-gp expression caused by the xanthones. In the second protocol, the accumulation of RHO 123 (5 µM) was evaluated in the presence of the tested Xs (20 µM), allowing a direct detection of alterations in P-gp activity without affecting protein expression.

Ex vivo studies:

- The effect of X12 on P-gp activity was evaluated, ex vivo, at the distal portion of the ileum of adult Wistar-Han male rats. After gently washed in an ice-cold saline solution, the intestinal portions were exerted and the corresponding exerted intestinal sacs were placed in a chamber containing 5 mL Krebs-Hensealt (KH) buffer (40 mM glucose, pH 7.4), continuously aerated (95% O2 - 5% CO2) and at 37°C, with or without the addition of X12 (20 µM), in the presence or absence of Zosuquidar (10 µM), a known P-gp inhibitor. The serosal compartment was filled with 1 mL of KH buffer containing 300 µM RHO 123, which was used as P-gp substrate. Serosal to mucosal transport was evaluated by sampling aliquots of the buffer every 5 min for a 45-min period. RHO 123 concentration was determined spectrofluorometrically (measured at excitation/emission wavelengths of 485-528 nm, in a multi-well plate reader) in all samples taken from mucosal medium.

RESULTS

In vitro results

Xanthones cytotoxicity assays

Figure 1. Xanthones 1 – 9, µM, cytotoxicity in CAS cells with 72 h incubation. All compounds were tested at 100 µM. *p < 0.05, **p < 0.01.

Figure 2. Xanthones 1 – 9, 10 µM, cytotoxicity in CAS cells with 24 h incubation. All compounds were tested at 100 µM. *p < 0.05, **p < 0.01.

Figure 3. Xanthones 1 – 9, 10 µM, cytotoxicity in CAS cells with 24 h incubation. All compounds were tested at 100 µM. *p < 0.05, **p < 0.01.

In the MTT reduction assay, no significant cytotoxicity was detected for any of the tested concentrations (5 - 100 µM) and up to 24 hours of exposure to X2, X 5 and 16. For X1 and X12, no significant effects were observed for concentrations up to 20 µM, but a significant decrease was observed with a concentration of 50 µM.

Concerning the NR uptake assay, no significant effects were observed after 24 hours of exposure to X2, 6 and 12, and for all the tested concentrations. However, for X1, X 5 and 16, a small but significant reduction in the NR uptake was observed only for the highest tested concentration (50 µM).

The 20 µM concentration was selected as a noncytotoxic concentration to be used in the subsequent studies.

CONCLUSIONS

As previously reported for other xanthone derivatives, the newly synthesized xanthones demonstrated to interact with P-gp, in vitro.

Flow cytometry analysis of P-gp expression demonstrated that X6 and X12 (20 µM). DHAN, significantly decreased the P-gp expression compared X1 and X2, although to a lower extent, also significantly increased P-gp expression.

P-gp activity evaluated 24 h after exposure to the tested xanthones demonstrated that the increase in P-gp activity observed after pre-exposure to X1, X6 and X12 may result from an increase in the expression of this efflux pump. Although no increases in P-gp expression were observed, X5 and X12 were significantly increased P-gp activity.

P-gp activity was also evaluated with the tested compounds present only during the short RHO 123 incubation period, indicating that X5, X6 and X12 were the most effective P-gp activators.

Ex vivo studies demonstrated X12 ability to increase the efflux of RHO 123 in exerted intestinal sacs. This increase was blocked by Zosuquidar, a specific P-gp inhibitor, which proves the involvement of the protein in the efflux of the substrate.

In conclusion, the in vitro and ex vivo results confirmed the potential of these xanthones as P-gp inducers and activators, and, therefore, they can be faced as a potential therapeutic approaches in cases of intoxications with toxic substrates.