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Original research

Absorption improvement of sepantronium bromide (YM155) by aminoalkyl methacrylate copolymers in *in situ* intestinal tracts of mice

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ABSTRACT

Sepantronium bromide (YM155) is a small molecule suppressant of survivin with time-dependent antitumor activity. In Phase II trials, the dosage form is a parenteral solution for continuous intravenous administration for 7 days. Although an oral YM155 formulation is required to improve patient convenience, oral bioavailability is low due to poor membrane permeability. Plasma concentrations of YM155 were significantly increased in intestinal loops pre-washed with phosphate buffered saline ($p < 0.05$). In addition, plasma concentrations of YM155 significantly decreased in the presence of mucin, which is a typical anionic substance in the intestinal tract ($p < 0.05$). Freeze-dried powder of aminoalkyl methacrylate copolymer (Eudragit® E) with HCl (E-FD) improved YM155 absorption in mice. In addition, E-FD suppressed the interaction between YM155 and mucin. These results suggest that E-FD improves YM155 absorption by reducing the interaction between YM155 and mucin. E-FD is therefore a promising candidate as an absorption-improving agent for the development of oral formulations of YM155.

1. Introduction

Sepantronium bromide (YM155) is a small molecular suppressant of survivin, which is a member of the apoptosis protein family (Fig. 1). YM155 exhibits potent antitumor activity against a broad spectrum of human cancer cell lines and various human-derived tumor xenograft mouse models [1–6]. The membrane permeability of YM155 is low as it is a cationic compound with high water solubility [7] and time-dependent antitumor activity [1,5]. The dosage form in clinical studies is therefore a parenteral solution for continuous intravenous administration for 7 days [8–11]. A more convenient dosage form is therefore required for clinical use. In this regard, an oral formulation is less painful than parenteral routes and easier for ambulant patients, making it one of the most convenient dosage forms to achieve good compliance. We therefore investigated the oral absorption of YM155 to develop an oral formulation and improve patient adherence. Based on the $AUC_{0-\infty}$ after intravenous administration, the absolute bioavailability after oral dosing was calculated as 0.8% in mice (see Section 3.1). Improved oral bioavailability of YM155 is therefore required for use at the bedside.

There are various approaches for improving the oral absorption of high water-soluble and low-permeable compounds (the Biopharmaceutical Classification System [BCS] class III compounds). These approaches include the utilization of absorption enhancers that increase cell-membrane fluidity and/or open tight junctions [12–18] and enhancement of membrane-permeability by forming a permeable complex via non-covalent and/or electrostatic interactions between drugs and excipients [19–22]. In addition, low intestinal absorption of a proportion of cationic compounds can be attributed to their electrostatic interaction with bile acids in the intestinal tracts [23,24] and mucin in the mucus layer [25]. Intestinal absorption of a cationic drug is reportedly improved by Eudragit® E spray-dried with HCl via the inhibition of electrostatic interactions between the drug and bile acids [26]. Eudragit® E is an aminoalkyl methacrylate copolymer that is normally only soluble in acidic conditions. In contrast, the spray-dried powder of Eudragit® E with HCl and the freeze-dried powder of Eudragit® E with

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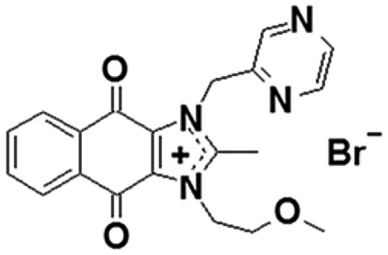

Fig. 1. Chemical structure of sepantronium bromide (YM155).

HCl (E-FD) are novel preparations that can even dissolve in neutral pH [26,27].

Here, we investigated the cause of low intestinal absorption of YM155 and approaches for improved intestinal absorption of YM155 using an *in situ* loop method.

2. Materials and methods

2.1. Materials

Sepantronium bromide (YM155) and YM-208,794, an internal standard (I.S.), were obtained from Astellas Pharma Inc (Tokyo, Japan), Eudragit® EPO (Eudragit® E) from Evonik Japan Co., Ltd. (Tokyo, Japan) and Carbopol® 971P NF (CBP) from CBC Co., Ltd. (Tokyo, Japan). Sodium dextran sulfate (Dex-sul), sodium lauryl sulfate (SLS) and mucin (bovine submaxillary glands, type I-S) were purchased from Sigma–Aldrich Co. LLC. (Tokyo, Japan). Methyl cellulose, SM-4000 (MC) was obtained from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Sodium dioctyl sulfosuccinate (DOSS) was purchased from Nihon Cytec Industries Inc. (Tokyo, Japan). All other reagents and excipients used in this study were of commercially available grade.

2.2. Preparation of freeze-dried Eudragit® E with HCl

One hundred and fifty grams of Eudragit® E and 300 ml of 1 mol/l HCl were added to 600 ml of distilled water, and the mixture was stirred until completely dissolved (final pH = 0.6). The solution was then freeze-dried to obtain powder of Eudragit® E with HCl (E-FD).

2.3. Pharmacokinetic studies and in situ intestinal loop method

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc., Yaizu Pharmaceutical Research Center (YPRC). Astellas Pharma Inc., which was awarded Accreditation Status by the AAALAC International in 2010. Seven-week-old male mice (BALB/c, body weight; 18–25 g) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Mice were allowed free access to water but were fasted for 15 h before being subjected to experiments. YM155 or phenol red (PR) solution was administered to fasted mice via an oral gastric tube at a dose of 30 and 20 mg/kg, respectively. A dose of 30 mg/kg for YM155 and 20 mg/kg for PR was set to successfully obtain plasma concentration profiles for both YM155 and PR. PR is the hydrophilic compound with low absorption and an established low absorption marker via paracellular route [28]. In intravenous administration, YM155 was dissolved in saline (1 mg/ml) and administered to the caudal vein at 3 mg/kg. Anesthesia was

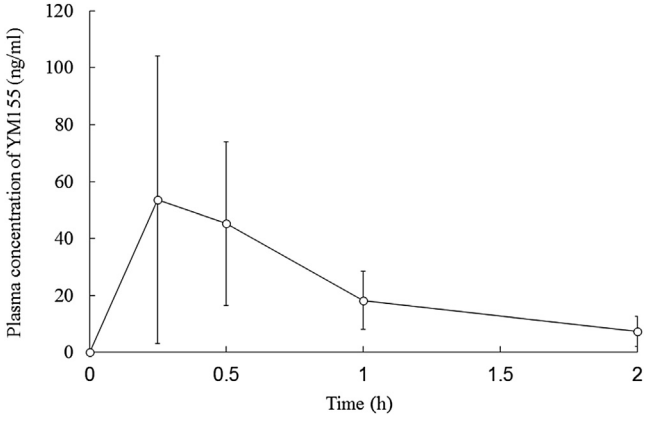

Fig. 2. Plasma sepantronium bromide (YM155) concentrations after oral administration of YM155 solution at a dose of 30 mg/kg. Each point with vertical bar represents mean with S.D. from 6 animals.

Table 1
Pharmacokinetic parameters of sepantronium bromide (YM155) after oral administration to mice.

Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-2h} (ng·h/ml)
30	53.6	0.25	47.7

Each parameter was calculated from the mean plasma concentration profile. Mean plasma concentration at each sampling point was determined from 6 animals.

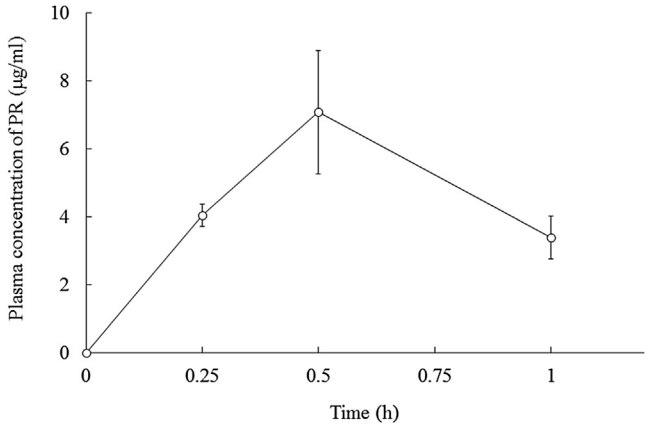

Fig. 3. Plasma phenol red (PR) concentrations after oral administration of PR solution at a dose of 20 mg/kg. Each point with vertical bar represents mean with S.D. from 6 animals.

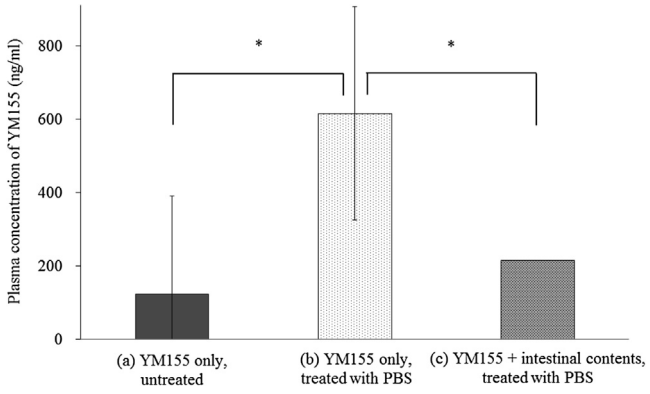

Fig. 4. Plasma sepantronium bromide (YM155) concentrations at 1 h after injection of YM155 solution (10 mg/kg) into *in situ* intestinal loops of mice. (a) YM155 solution was injected into *in situ* intestinal loops. (b) YM155 solution was injected into *in situ* intestinal loops treated with phosphate buffered saline (PBS) to wash intestinal contents. (c) YM155 solution with recovered fluid from mice intestine was injected into *in situ* intestinal loops treated with PBS. Each column with vertical bar represents mean with S.D. from 5 to 6 animals. *Significant difference ($p < 0.05$).