European Journal of Pharmacology 791 (2016) 137–146

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Full length article

Anti-cancer activity and potential mechanism of a novel aspirin derivative

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ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form
27 July 2016
Accepted 28 July 2016
Available online 23 August 2016

Keywords:
Ca-Asp
Aspirin-derivative
Anticancer effect
Mechanism

Chemical compounds studied in this article:
Hydroxyapatite (PubChem CID: 14781)
Aspirin (PubChem CID:2244)
cyclooxygenase-2 (PubChem CID: 73416861)
Caspase-3 (PubChem CID: 9840223)
Cyclin D (PubChem CID: 171581456)

ABSTRACT

Aspirin has been used in the treatment and chemoprevention of many malignant cancers. The mechanism of its anti-cancer activity mainly involves the inhibition of cyclooxygenase-2 (COX-2). However, the application of aspirin is limited by the serious gastric mucosal damage that accompanies its usage. We have previously reported the preparation of a novel aspirin derivative that we named Ca-Asp, and showed that it causes less damage to gastric mucosa of rat and inhibits the expression of COX-2 to higher degree than Asp. However, the anti-cancer effect and mechanism of Ca-Asp was not demonstrated. In this study, the anti-cancer effect of Ca-Asp was investigated and compared with those of Asp and Hydroxyapatite (Hap) at the cell level. The results showed that treatment of SGC-7901 cells (human gastric cancer cell line) with 200–400 μg/ml Ca-Asp resulted in significant reduction in cell viability, compared to treatment with either Asp or Hap, and at a higher concentration (500 μg/ml). Subsequent investigation into the possible underlying mechanism showed that Ca-Asp induced apoptosis and caused cell cycle arrest at the G1 phase. Ca-Asp also up-regulated the levels of caspase-3 and p53, but down regulated the level of cyclin D1, NF-κB, COX-2 and PGE_2. Furthermore, simultaneous treatment of SGC-7901 cells with Ca-Asp and exogenous PGE_2 reduced the anti-proliferative effect of Ca-Asp on the cells. Taken together, the results suggested that Ca-Asp might act as a potential anti-cancer drug, and that its suppression of PGE_2 production might constitute an important part of its anti-cancer activity.

1. Introduction

Recent epidemiology and laboratory studies have indicated that taking aspirin (Asp) on a regular basis can reduce the risk of many cancers, such as colorectal (Tougeron et al., 2014), breast (Maity et al., 2015) and stomach cancers (Wang et al., 2015). Despite recent report that the anti-cancer activity of Asp is due to its inhibition of platelet (Dovizio et al., 2014), earlier studies have shown that the anti-cancer activity of Asp may be due to its inhibition of cyclooxygenase-2 (COX-2) expression in the tissues or cells (Kastrati et al., 2015; Rao et al., 2006). COX-2 has been considered as a potential target protein in cancer treatment because it has been found to be over expressed in many tumor cells (Bhat et al., 2014; Buskens et al., 2003), especially in gastrointestinal tumor cells (Ristimaki et al., 1997). Patients who had received a low-dose of Asp daily for more than 5 years have reported positive results for the anti-cancer treatment (Jonsson et al., 2013). Asp given at a high dose in vitro can even inhibit COX-2 expression (Dovizio et al., 2012; Hanif et al., 1996; Yao et al., 2009). However, high dose or prolonged usage of Asp has been limited by the serious stomach damage caused by Asp (Wolfe et al., 1999). Damage to the stomach is the direct result of injury to the gastric mucosa, a consequence of the inhibitory action of Asp against cyclooxygenase-1 (COX-1) and COX-2, with the inhibition toward COX-1 being 100 folds higher than that toward COX-2 (Kandimalla et al., 2013). Inhibition of COX-1 will block the synthesis of Prostaglandin E_2 (PGE_2), which acts as an important protective agent for the gastric mucosa (Tarnawski et al., 2013).

In our previous study, we have successfully designed and synthesized a novel Asp derivative that we named Ca-Asp (Fig. 1) and demonstrated that Ca-Asp causes less damage to rat gastric mucosa compared to Asp (Zhen et al., 2014). In that particular study, we have also indicated that Ca-Asp exerts stronger inhibition on the expression of COX-2 than COX-1. Thus, we hypothesized that Ca-Asp may have a better effect on the inhibition of cancer than Asp. In this study, the ability of Ca-Asp to inhibit the

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http://dx.doi.org/10.1016/j.ejphar.2016.07.050
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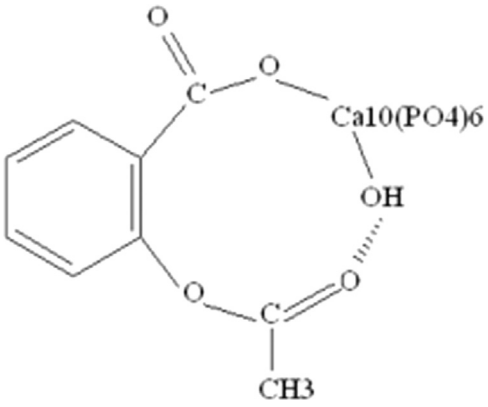

Fig. 1. Structure of Ca-Asp.

growth of human gastric cancer cell was investigated and the potential underlying mechanism was also explored.

2. Materials and methods

2.1. Drugs and chemicals

Asp was purchased from Aladdin Chemistry Co. Ltd. Hydroxyapatite (Hap) (50-nm particles) was purchased from Nanjing Emperor Nano Material. Primary Antibody against COX-2 (#4842), Cyclin D1 (#2922), Caspase-3 (#9664) and NF-κB p65 (#6956) were purchased from Cell Signal Technology (USA). Anti-p53 antibody was purchased from Santa Cruz (sc-6243) (USA). PGE_2 was purchased from Cayman Chemicals (USA). PGE_2 Enzyme Immunoassay Kit, Nuclear and Cytoplasmic Protein Extraction Kit (P0027) and Cell Cycle and Apoptosis Analysis Kit (C1052) were purchased from Beyotime Institute of Biotechnology (China). Trizol reagent was purchased from Invitrogen Technology Co., USA. High-Capacity cDNA Reverse Transcription Kit was purchased from Applied Biosystems, USA. SYBR Green PCR Master Mix Kit was purchased from Applied Biosystems, USA. Human gastric cancer cell line (SGC-7901) was purchased from the cell line bank of Chinese Academy of Sciences. Human gastric mucosa cell (GES-1) was purchased from Shanghai Baili Biology Co Ltd. Ca-Asp was prepared by the method described in our previous study (Zhen et al., 2014).

2.2. Effects of Ca-Asp, Asp or Hap on cell viability

GES-1 and SGC-7901 cells were treated with Asp, Hap or Ca-Asp at different concentrations 100–500 μg/ml) and for different time periods (6, 12 and 24 h). After that, cell viability was measured by MTT assay performed as previously described (Liu et al., 2014).

2.3. Cell Proliferation assay

The effects of Asp, Hap and Ca-Asp on the proliferation of SGC-7901 cells were determined by flow cytometry as previously described (Zhu et al., 2015) using 6-well plate culture. The cells were treated with 500 μg/ml Asp or Hap, or with 200–400 μg/ml Ca-Asp with or without exogenous 1 μM PGE_2 and incubated for 12 h followed by flow cytometry analysis.

2.4. Cell Cycle Analysis

SGC-7901 cells treated with each of the three drugs (Asp, Hap and Ca-Asp) were also subjected to cell-cycle analysis, performed by flow cytometry as previously described (Zhu et al., 2015). Before analysis, the cells were treated with 500 μg/ml Asp or Hap, or with 200–400 μg/ml Ca-Asp.

2.5. Western blot

GES-1 and SGC-7901 cells were seeded in separate 6-well plates and cultured for 24 h at 37 °C in a 5% CO_2 incubator. The culture medium was removed and replaced with 2 ml of fresh medium or fresh medium containing Asp, Hap or Ca-Asp and incubated for another 12 h. After that, the cells were harvested and subjected to western blot analysis as described previously (Zhen et al., 2014), using antibody directed against caspase-3, Cyclin D1, p53 or COX-2. In the case of NF-κB expression analysis, the harvested cells were subjected to nucleoprotein extraction using a Nuclear and Cytoplasmic Protein Extraction Kit, and the resulting nucleoproteins were probed with an antibody directed against NF-κBp65 via western blot. Antibody directed against β-actin was used as a loading control.

2.6. Determination of COX-2 mRNA level

Isolation of total RNA from the cells was performed with the Tripure Reagent (Invitrogen, USA) according to the manufacturer's protocol. The RNA (0.5 μg) was then reverse transcribed to obtain the corresponding *c*DNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). The level of COX-2 mRNA was quantified by *q*RT–PCR using COX-2-specific primers (forward primer, 5′-CAACTCCATCCTCCTGGAACA-3′; reverse primer, 5′-TATTTCATCTCTCTGCTCTGGTCAA-3′). *q*RT–PCR was performed for 40 cycles in a 7300 FAST Real-Time PCR System (Applied Biosystems, USA) using the SYBR Green PCR Master Mix Kit, with GAPHD as an internal control using the GAPHD-specific primers 5′-GCTTCGGCACATATACTAAAAT-3′ (forward) and 5′-CGCTTCACGAATTTGCGTGTCAT-3′ (reverse). The experiment was conducted three times.

2.7. Determination of PGE_2 level

SGC-7901 cells were seeded in separate 6-well plates and cultured for 24 h at 37 °C in a 5% CO_2 incubator. The culture medium was removed and replaced with 2 ml of fresh medium or fresh medium containing Asp, Hap or Ca-Asp with or without exogenous 1 μM PGE_2 and incubated for another 12 h. The culture medium was then removed and replaced with 2 ml of fresh medium and incubated for another 6 h. The level of PGE_2 in the culture supernatant was then determined by immunoassay using a PGE_2 Enzyme Immunoassay Kit.

2.8. Statistical analysis

All assays were performed at least three times and all data were expressed as means ± S.D. obtained from triplicate determinations. Significant differences between groups were analyzed using one-way ANOVA, SPSS (version 17) software. Statistical significance was considered at the $P < 0.05$ or $P < 0.01$ level.

3. Results

3.1. Effects of Asp and Ca-Asp on cell viability

GES-1 cell showed little or no reduction in cell viability when treated with Hap or Ca-Asp, and for all three durations of the treatment (Fig. 2A). However, at the highest concentration (500 μg/ml) of Ca-Asp used, some but significant loss in cell viability was