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Differential Cytotoxic Effects of Jaspine B in Various Cancer Cells

Jihoon Lee[1], Kwangik Choi[1], Mihwa Kwon[1], Dongjoo Lee[2], Min-Koo Choi[3] and Im-Sook Song[1]*

[1]*College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Korea*
[2]*College of Pharmacy, Ajou University, Suwon 16499, Korea*
[3]*College of Pharmacy, Dankook University, Cheon-an 31116, Korea*

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Jaspine B is an anhydrophytosphingosine that is isolated from a marine sponge. Because of its structural similarity to sphingosine, it shows anti-cancer effects in human carcinomas. Therefore, this study aims to investigate its anti-proliferative effect on various cancer cells and to correlate its association with the intracellular accumulation of Jaspine B in relevant cancer cells. The anti-proliferative effect of Jaspine B in various cancer cells was determined by a cell viability test, and the intracellular concentration of Jaspine B in relevant cancer cells was determined using mass spectrometry coupled with liquid chromatography. The correlation coefficient and p value between the cytotoxicity and the cell accumulation of Jaspine B were determined using SPSS 16.1. The cytotoxicity of Jaspine B varied depending on the type of cancer cell when compared the EC_{50} values of Jaspine B. Breast and melanoma cancer cells were susceptible to Jaspine B, whereas renal carcinoma cells were resistant. The intracellular concentrations of Jaspine B had a reciprocal correlation with the EC_{50} values in the same cells ($r = 0.838$). The results suggested that the anti-proliferative effect of Jaspine B was associated with the cellular accumulation of this compound. However, Jaspine B was not a substrate for P-glycoprotein and breast cancer resistance protein, as major efflux pumps caused multidrug resistance. The maintenance of a high intracellular concentration is crucial for the cytotoxic effect of Jaspine B; however, efflux pumps may not be a controlling factor for Jaspine B-related resistance in cancer cells.

Key words : Cancer cells, cytotoxicity, efflux pump, intracellular accumulation, Jaspine B

Introduction

Cancer is a constant and major burden to humanity as current cancer chemotherapy and surgery are not completely successful [5]. In recent years, various herbal medicines have emerged as potential alternatives to current anticancer drugs. The safety of many herbs has been demonstrated by their long and continuous history of dietary use, making some natural compounds ideal candidates for chemotherapy. One of the most abundant source for natural compound is the sea, which has been biomined since the 1950s [5].

Cytarabine was the first marine-derived anticancer drug approved by the FDA in 1998 for the treatment of acute melanogenous leukemia. It was derived from spongothymidine, which was isolated from the sponge *Cryototethia crypta* [19]. Yondelis, isolated from *Ecteinascidia turbinate*, was approved for clinical use in metastatic soft tissue carcinoma and ovarian cancer in 2009 in Europe [5]. Another approved marine-based drug is Halaven, an analogue of halichondrin B, extracted from the sea sponge *Halichondria okadai* [11], and was approved for the treatment of metastatic breast cancer in 2010 from FDA. Besides these three marine anti-cancer agents currently being marketed, several marine products and their derivatives such as aplidin, bryostatin-1, and zalypsis are undergoing phase II and III clinical trials [5].

Jaspine B (pachastrissamine) is an anhydrophytosphingosine, which is extracted from Marine sponge, *Pachastrissa spp.* [10]. Jaspine B has a similar structure to sphingosine, and shows effective anti-cancer effects in several human carcinomas. One of the possible reasons was that sphingolipid metabolism is important for the regulation of cell proliferation, survival and death [15]. Phytosphingosine, the metabolic product of sphingolipid metabolism, is the most widely distributed natural sphingoid found in plants and animals and it has been implicated as a key regulator of several parameters related to the metastatic potential of tumor cells [27]. The cytotoxicity of Jaspine B was evaluated using an A549 lung tumor cell line with an IC_{50} value of 0.34 μM. IC_{50} values of Jaspine B against P388, A549, HT29,

***Corresponding author**
Tel : +82-53-950-8575, Fax : +82-53-950-8557
E-mail : isssong@knu.ac.kr

and MEL28 cell lines were about 30 nM when live cells were visually counted against control wells [10]. When the toxicity of Jaspine B was assessed by MTT assay, treatment with 16.7 μM Jaspine B resulted in A375 melanoma cell viability of 60% [27]. The purpose of this study, therefore, is to evaluate the anti-proliferating effect of Jaspine B in various cancer cells of different origin by using an MTT assay and to compare the cytotoxic effects of Jaspine B across various cancer cell types.

Another issue relevant to the toxicity and resistance of anti-cancer drugs is the cellular accumulation of these drugs. In fact, defective uptake of anticancer drugs such as vinblastine, adriamycin, and cisplatin etc. has been one of the most consistently identified characteristics of cells associated with drug resistance both in vitro and in vivo [3, 6, 8, 16]. Hence, this study also investigated the accumulation of Jaspine B in various cancer cells and its association with cytotoxicity in relevant cancer cells.

Materials and Methods

Materials

Jaspine B was synthesized by Dr. D. Lee (Ajou University, Suwon, Korea) with a purity of over 99.0% and confirmed by NMR and MS results (Fig. 1) [12].

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), cyclosporin A (CsA), and Hank's balanced salt solution (HBSS) were purchased from Sigma (St. Louis, MO, USA). Fumitremorgin C (FTC) was purchased from Merck Millipore (Darmstadt, Germany). Fetal bovine serum and Dulbecco's Modified Eagle's medium (DMEM), RPMI 1640 medium, Medium 199, penicillin-streptomycin, and Trypsin-EDTA were purchased from Hyclone Laboratories (Logan, UT, USA). All other reagents were of reagent grade.

Cells

MDA-MB-435 and 786-O cells were purchased from ATCC (Rockville, MD, USA). LLC-PK1-mock and LLC-PK1-P-gp cells were obtained from Corning (Corning, NY, USA).

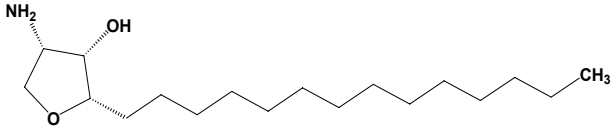

Fig. 1. Structure of Jaspine B.

LLC-PK1-BCRP cells were obtained from Dr. A.H. Schinkel, Netherlands Cancer Institute, Amsterdam, The Netherlands). SK-OV3, MDA-MB-231, and MCF-7 cells were obtained from the Korean Cell Line Bank (Seoul, Korea).

SK-OV3, 786-O, MDA-MB-231 were grown in tissue culture flasks in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. MDA-MB-435 and MCF-7 were grown in tissue culture flasks in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. LLC-PK1 cells were grown in tissue culture flasks in Medium 199 supplemented with 10% fetal bovine serum, 50 μg/ml gentamycin, and 50 μg/ml hygromycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2/95% air. For cell uptake study, the cells were grown and seeded in 24 well plates at a density of 5×10^5 cells/well. For cytotoxicity assay, the cells were grown and seeded in 96 well plates at a density of 1×10^4 cells/well.

Cytotoxicity of Jaspine B on various cancer cells

The cytotoxicity of Jaspine B was assessed in HepG2, 786-O, MCF-7, SK-OV3, MDA-MB-231, MDA-MB-435, LLC-PK1-mock, LLC-PK1-P-gp cells, which were grown for 24 hr in 96 well plates (10^4 cells/well) and incubated for 48 hr in the absence or presence of varying concentrations of Jaspine B (0.1, 0.5, 1, 5, 10, 50, 100 μM). After 48 hr, the incubation medium was aspirated and 200 μl of the culture medium containing 0.5 mg/ml MTT was added to each well, and the plate was incubated for 4 hr. The medium was removed and the formed purple formazan product was dissolved in 120 μl of DMSO. The cellular content was then determined by measuring the absorbance at 590 nm, and the viability of the treated cells was expressed as a percentage of live cells compared with the control group after background correction of the Jaspine B concentration. Relevant data were fitted to an inhibitory effect model [i.e., $v = E_{max}(1 - \frac{[I]}{EC_{50}+[I]})$] [28]. The EC_{50} value reflects the concentration of inhibitor (Jaspine B) to show half-maximal inhibition and [I] represents the concentration of Jaspine B.

Uptake of Jaspine B in various cancer cells

Uptake of Jaspine B was measured in HepG2, 786-O, MCF-7, SK-OV3, MDA-MB-231, MDA-MB-435, LLC-PK1-P-gp, and LLC-PK1-mock cells, which were grown for 24 hr in 24-well plates (5×10^5 cells/well). The cells were incubated with an HBSS medium containing Jaspine B (20 μM) at 37

°C for 10 min and 1 hr. After the incubation, the cells were washed twice with ice-cold HBSS. Jaspine B within the cells was extracted by adding 200 µl of 80% acetonitrile containing 0.5 ng/ml berberine as an internal standard (IS) and vigorous mixing for 10 min followed by sonication for 5 min and centrifugation at 14,000 g for 5 min at 4°C. After centrifugation, an aliquot (5 µl) was injected into an LC-MS/MS system.

Involvement of efflux pumps on Jaspine B transport

LLC-PK1-mock, LLC-PK1-P-gp, and LLC-PK1-BCRP cells were seeded on collagen-coated 24 transwell membranes at a density of 5×10^5 cells/ml and were maintained at 37℃ in a humidified atmosphere with 5% CO_2/95% air for 5 days. The culture medium was replaced every 2 days. The culture medium consisted of Medium 199 supplemented with 8% fetal bovine serum, 50 mg/ml hygromycin, and 100 mg/ml gentamycin. The integrity of cell monolayers was evaluated prior to transport experiments by measuring the transepithelial electrical resistance (TEER) values. TEER values of plated cells ranged from 600-1,000 W·cm^2. On the day of transport experiments, the growth media were discarded and the attached cells were washed with HBSS and preincubated for 20 min in HBSS at 37°C. To measure apical to basal (A to B) transport of Jaspine B, 0.5 ml of HBSS media containing Jaspine B (100 µM) was added to the apical side and 1.5 ml of HBSS media without Jaspine B was added to the basal side of the insert. The insert was transferred to a well containing fresh transport medium at 15, 30, 45, and 60 min. Aliquots (0.4 ml) from the basal side were transferred to clean tubes and samples were stored at -80°C until analysis.

To measure basal to apical (B to A) transport of Jaspine B, 1.5 ml of transport media containing Jaspine B (100 µM) was added to the basal side and 0.5 ml of fresh transport media was added to the apical side of the insert. Aliquots (0.4 ml) from the apical side were transferred to clean tubes and the transport medium in the apical side was replaced with 0.4 ml of fresh transport media at 15, 30, 45, and 60 min. Samples were stored at -80°C until analysis.

For the analysis of Jaspine B, Jaspine B in the thawed 50 µl samples was extracted by 200 µl of 80% acetonitrile containing 0.5 ng/ml berberine (IS) and vigorous mixing for 10 min followed by sonication for 5 min and centrifugation at 14,000 g for 5 min at 4°C. After centrifugation, an aliquot (5 µl) was injected into an LC-MS/MS system.

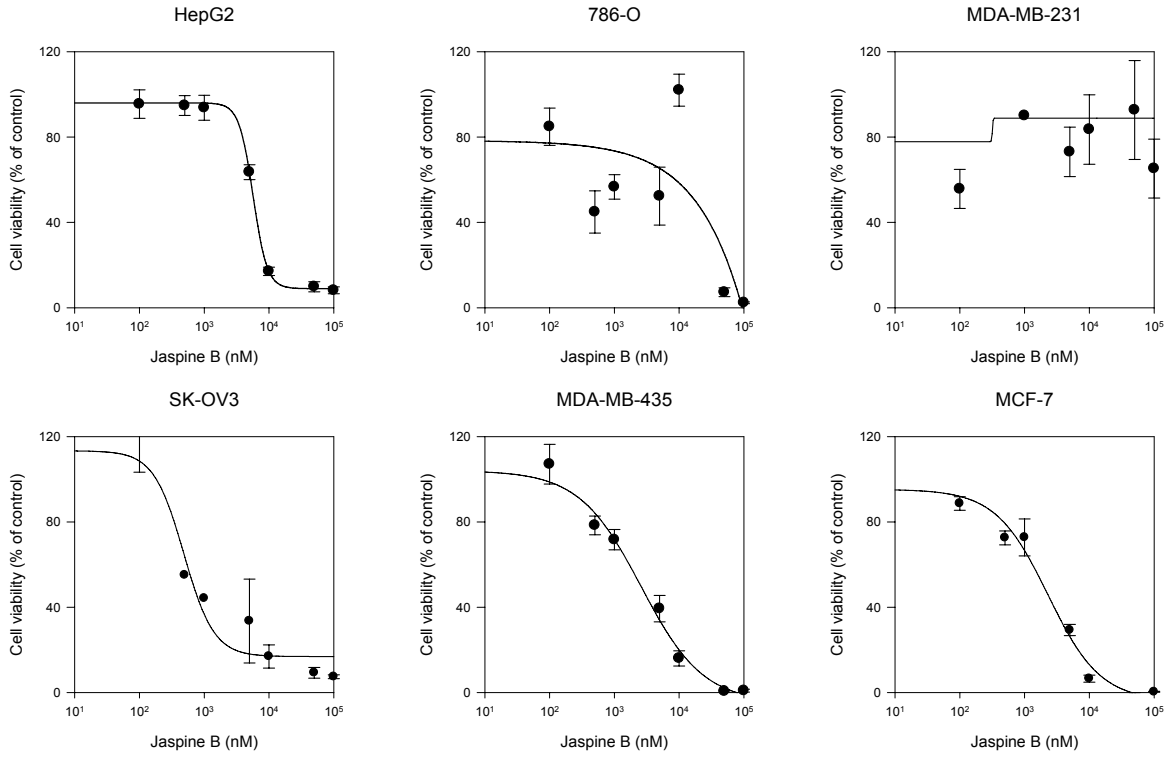

Fig. 2. Cell viability of cancer cells to Jaspine B was determined using a MTT assay after treatment of various concentrations of Jaspine B (100-100,000 nM) for 48 hr. Each data point represents the mean ± SD of three independent experiments. Data were fitted to an inhibitory effect E_{max} model and the EC_{50} value was calculated.

Analysis of Jaspine B

The concentrations of Jaspine B were analyzed using an Agilent 6430 Triple Quad LC/MS-MS system (Agilent, Wilmington, DE, USA) coupled with an Agilent Infinity 1290 series HPLC system. The separation was performed on a Synergi Polar RP column (2.0 mm i.d.×150 mm, 4 μm, Phenomenex) using a mobile phase that consisted of acetonitrile and DDW (85:15, v/v) with 0.1% formic acid at a flow rate of 0.2 ml/min. Mass spectra were recorded by electrospray ionization with a positive mode. Quantification was carried out using selected reaction monitoring (SRM) at m/z 300.3 → 270.2 for Jaspine B and m/z 336.1 → 320.0 for berberine. In this study, the lower limit of quantitation (LLOQ) was determined to be 80 nM and standard curve range was 80-1,500 nM. Intra- and inter-day precision and accuracy were found to be within the acceptance criteria for assay validation guidelines.

Data analysis

Linear regression analysis was performed to assess the linear relation between the cytotoxicity and cellular accumulation of Jaspine B. Correlation coefficient and p value were used to determine the relationship. Analyses were performed using SPSS software version 16.1. For all statistical procedures, p values of <0.05 were deemed statistically significant.

The permeation coefficient (P_{app}) was calculated by dividing the slope (transport rate) of the regression line of the mean permeated amounts and incubation times by the initial concentration of Jaspine B and membrane area. Efflux ratio (ER) was calculated by dividing the permeation coefficient (P_{app}) from B to A direction by the permeation coefficient (P_{app}) from A to B direction. Net efflux ratio (NER) was the ratio of ER in LLC-PK1 overexpressing P-gp or BCRP to ER in LLC-PK1-mock cells.

Results

Anti-proliferative effect of Jaspine B

The cytotoxicity of Jaspine B in various cancer cells of different origin was evaluated. For example, HepG2 cells are hapatoma cells, 786-O cells are renal carcinoma cells, SKOV3 cells are ovarian cancer cells, and MCF-7 cells are breast cancer cells. As the molecular mechanism of Jaspine B was reported in melanoma cells [27], two different melanoma cells such as MDA-MD-231 and MDA-MB-435 were selected.

The cytotoxicity of Jaspine B in various cancer cells was determined using an MTT assay at a concentration range of 0.1-100 μM (Fig. 2). The estimated EC_{50} values are shown in Table 1. EC_{50} values varied within a range of 2.31 μM to over 100 μM. Interestingly, the EC_{50} values of Jaspine B for 786-O renal carcinoma cells and MDA-MD-231 melanoma cells were relatively high while MCF-7 breast cancer cells were susceptible to Jaspine B in this system, suggesting that the cytotoxic effect of Japine B differed depending on the cell characteristics.

Cellular uptake of Jaspine B

To determine whether the cellular concentrations of Jaspine B are associated with its cytotoxic effect, the concentration of Jaspine B in the cancer cells was accessed. First, to determine the cell penetration rate of Jaspine B, the initial uptake of Jaspine B into cells was measured for 10 min based on the preliminary study in which cellular uptake of Jaspine B increased linearly up to 20 min and became saturated within 1 hr (data not shown). As shown in Table 2, the uptake rate of Jaspine B was the highest in HepG2 cells while it was the lowest in MDA-MD-231 cells. The uptake rate of these cells varied and a 36.4-fold difference was observed between HepG2 cells and MDA-MB-231 cells. To investigate the correlation between EC_{50} and initial uptake rate, linear regression analysis was performed using SPSS. As shown in Fig. 3A, the correlation between EC_{50} and the initial uptake rate of Jaspine B was not statistically significant. The steady state cellular concentration of Jaspine B in the cancer cells was also measured by measuring the intracellular drug concentration after 1 hr of incubation. The correlation co-

Table 1. Cytotoxicity of Jaspine B in various cancer cells

Cell line	Description	EC_{50} (μM)
HepG2	Hepatoma cancer	5.69
786-O	Renal carcinoma	29.40
MDA-MB-231	Melanoma	>100
MDA-MB-435	Melanoma	2.60
SK-OV3	Ovarian cancer	4.78
MCF-7	Breast cancer	2.31

Table 2. Cytotoxicity of Jaspine B in kidney derived cells

Cell line	Description	EC_{50} (μM)
786-O	Renal carcinoma	29.40
LLC-PK1-mock	Kidney epithelium	10.86
LLC-PK1-P-gp	Kidney epithelium overexpressing P-gp	10.24

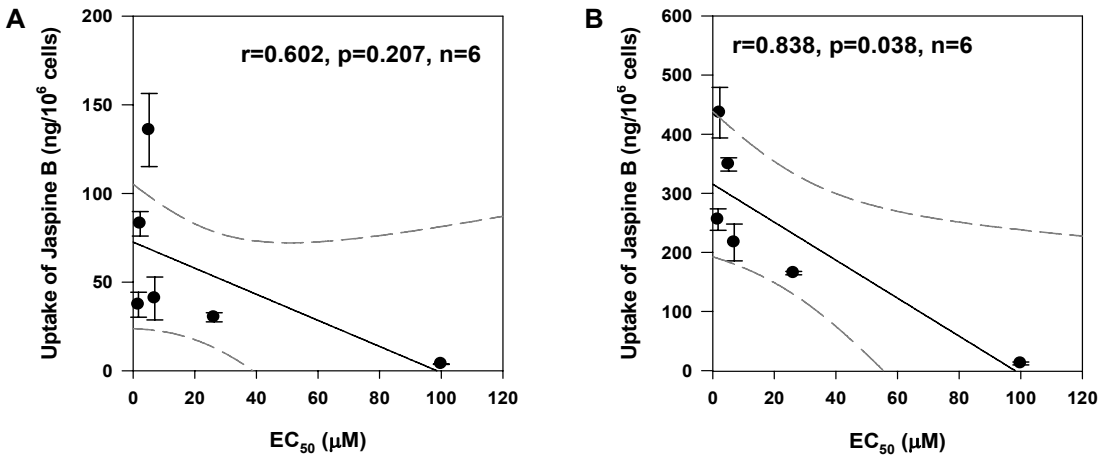

Fig. 3. Correlation between EC_{50} of Jaspine B and its cellular accumulation in various cancer cells. Uptake of Jaspine B was measured for (A) 10 min or (B) 1 hr after the addition of Jaspine B 20 μM into cells. Each data point represents the mean ± SD of three independent experiments. Lines were generated from the linear regression analysis and dotted lines represent the 90% confidence internal from the geometric mean value.

efficient was 0.838(p<0.05) (Fig. 3B). These results suggested that steady state concentration of Jaspine B was associated with its cytotoxicity and therefore, maintenance of an effective cellular concentration of this compound would be critical for the efficacy of Jaspine B.

Involvement of efflux pumps in Jaspine B transport

As the overexpression of P-gp in MDA-MB-231 and 786-O cells has been reported to be related to resistance to anti-cancer drugs and P-gp was highly expressed in 786-O cells but not in MCF-7 cells [1, 21], the effect of P-gp on the efflux of Jaspine B, as well as its ability to modulate the intracellular concentration of Jaspine B was investigated.

The B to A transport rate of Jaspine B was not significantly different from the A to B transport rate and it was not inhibited by the treatment with CsA, a P-gp inhibitor in LLC-PK1-P-gp cells [4], which was evidenced by the efflux ratio (ER) of around 1.0 (Table 3). Moreover, the B to A transport of Jaspine B was not enhanced in LLC-PK1-P-gp cells compared with that in LLC-PK1-mock cells, which was also evidenced by the net efflux ratio (NER) of around 1.0 (Table 3). These results suggested that P-gp was not involved in the transport process of Jaspne B. Similar results were observed for LLC-PK1 cells overexpressing BCRP. The B to A

Table 3. Transport properties of Jaspine B in LLC-PK1 cells overexpressing P-gp and BCRP

Treatment	LLC-PK1-P-gp			LLC-PK1-mock			NER[d]
	Pa_{pp} (10^{-6} cm/s)		ER[c]	P_{app} (10^{-6} cm/s)		ER[c]	
	A to B[a]	B to A[b]		A to B	B to A		
Control	0.33±0.08	0.30±0.03	0.9	0.39±0.07	0.28±0.05	0.7	1.3
CsA[e] 25 μM	0.35±0.05	0.30±0.04	0.8	0.41±0.08	0.28±0.03	0.7	1.2
Treatment	LLC-PK1-BCRP			LLC-PK1-mock			NER[d]
	Pa_{pp} (10^{-6} cm/s)		ER[c]	P_{app} (10^{-6} cm/s)		ER[c]	
	A to B	B to A		A to B	B to A		
Control	0.71±0.04	0.70±0.11	1.0	0.49±0.10	0.47±0.05	1.0	1.0
FTC[f] 10 μM	0.66±0.12	0.58±0.06	0.9	0.57±0.07	0.49±0.12	0.9	1.0

[a], A to B indicates apical to basal.
[b], B to A indicates basal to apical.
[c], ER indicates efflux ratio calculated by the equation ($\frac{\text{Papp of B to A direction}}{\text{Papp of A to B direction}}$).
[d], NER indicates net efflux ratio calculated by the equation ($\frac{\text{ER in LLC-PK1-P-gp or-BCRP}}{\text{ER in LLC-PK1-mock}}$).
[e], CsA indicates cyclosporine A.
[f], FTC indicates fumitremorgin C.

transport rate of Jaspine B was not significantly different from the A to B transport rate and it was not inhibited by the treatment with FTC, a BCRP inhibitor in LLC-PK1-BCRP cells [17]. ER of Jaspine B in LLC-PK1-BCRP cells was similar to that in LLC-PK1-mock cells and therefore, NER of Jaspine B in LLC-PK1-BCRP was around 1.0. These results also suggested that Jaspine B was not a substrate for BCRP.

For further comparison, the concentration of this compound in kidney derived cancer cells (i.e., 786-O) and P-gp overexpressing cells (i.e., LLC-PK1-P-gp) was measured. The accumulation of Jaspine B in LLC-PK1-P-gp cells was not different from that in LLC-PK1-mock and 786-O cells, suggesting that the cellular penetration of Jaspine B was not affected by the expression of P-gp (Fig. 4). However, the accumulation of this compound after 1 hr in 786-O cells was significantly lower than that in LLC-PK1-mock and LLC-PK1-P-gp cells (Fig. 4). Consistently, the EC_{50} values in the LLC-PK1-mock and LLC-PK1-P-gp cells were lower than that of 786-O cells (Table 2).

Discussion

Sphingolipid metabolites such as ceramide, sphingosine, and sphingosie-1-phosphate have emerged as critical signaling molecules in cancer progression [22]. Ceramide and sphingosine mediate cell proliferation/differentiation cycles

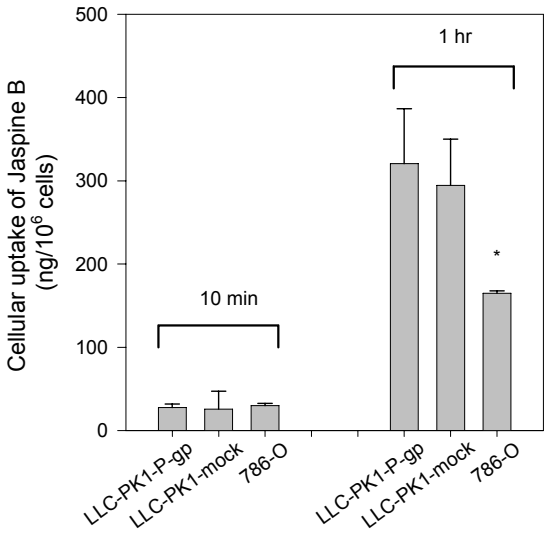

Fig. 4. The uptake of Jaspine B in LLC-PK1-P-gp, LLC-PK1-mock, and 786-O cells was measured for 10 min or 1 hr after the addition of Jaspine B 20 μM into cells. Each bar represents the mean ± SD of three independent experiments. *: Statistically different by t-test ($p<0.05$), compared with the LLC-PK1-mock cell group

and induce apoptosis, whereas sphingosine-1-phosphate promotes cells growth, proliferation, and survival. Therefore, the balance between ceramide/sphingosine and sphingosine-1-phosphate is important for cancer treatment and sphingosine kinase is responsible for the conversion of sphingosine to sphingosine-1-phosphate [22, 24].

Several studies addressing the effectiveness of sphingosine kinase inhibition for cancer therapy have been reported [9, 13, 14, 18]. Jaspine B also possesses a sphingosine moiety and is known to show anti-proliferative activity. Owing to its structural similarity with sphingosine, Jaspine B inactivated sphingosine kinase and resulting in enhanced ceramide levels as well as apoptotic signals [20]. In addition to this, Jaspine B inhibited the phosphorylation of Forkhead box O3 (FOXO3), a tumor suppressor and a component of the sphingosine-induced cell death machinery [25], and induced apoptosis [27] in melanoma cells. However, elevated expression of sphingosine kinase has been reported in multiple types of cancer. The mRNA levels of sphingosine kinase were significantly increased in breast, colon, lung, ovary, uterus, and kidney cancer patients, as well as in acute leukemia patients [2, 7, 23].

The principal objective of this study was to evaluating the sensitivity of Jaspine B on various cancer types in which overexpression of sphingosine kinase was reported. For this, hepatoma (HepG2), renal carcinoma (786-O), ovarian cancer (SKOV3), breast cancer (MCF-7), and melanoma (MDA-MB-231 and MDA-MB-435) cells were selected. In spite of the overexpression of sphingosine kinase, the sensitivity of Jaspine B varied over 12.7-fold and MBA-MD-231 cells were resistant to it. It was hypothesized that the differential sensitivity of different cancer cells to Jaspine B could be due to the differences in the cellular accumulation of Jaspine B as efflux pumps such as P-glycoprotein and BCRP have often been obstacles to cancer chemotherapy by decreasing the cellular accumulation of structurally diverse cancer drugs in various cancers [1, 21]. Indeed, the cellular accumulation could be a factor responsible for the differential cytotoxic effects of Jaspine B, which was demonstrated by the significant reciprocal correlation between EC_{50} values and the intracellular levels of Jaspine B in different cancer cells.

To differentiate cell surface bound Jaspine B from that inside cells, ice-cold drug solution was added to the cells, was removed immediately from the cells, and the cells were washed twice with ice-cold HBSS medium. This process resulted in almost negligible binding of Jaspine B to cell sur-

face (less than 10% of the total uptake). Therefore, the uptake amount represented the intracellular level of Jaspine B rather than that surface bound. Considering the mechanism of Jaspine B, Jaspine B inside the cells could act as a blockade of the phosphorylation of FOXO3 and an inhibition of the sphingosine-induced cell death machinery [25] and resulted the cytotoxic effect on various cancer cells.

Interestingly, MCF-7 cells are most susceptible to Jaspine B while 786-O and MDA-MB-231 cells were resistant to Jaspine B. In these cells, P-gp was reported to be highly expressed in 786-O cells but not in MCF-7 cells [1, 21]. Then, the possibility of Jaspine B being a substrate for efflux pumps such as P-gp and BCRP was investigated. Transport characteristics of Jaspine B in the P-gp and BCRP overexpressed cells did not significantly differ from those in mock cells, suggesting that efflux pumps such as P-gp and BCRP do not modulate the intracellular Jaspine B concentration.

Importantly, cytotoxic effect of Jaspine B in MDA-MB-231 and MDA-MB-435 cells were quite different although they are all melanoma cancer cells (Fig. 2 and Table 1). Molecular difference in FOXO3 between MDA-MB-231 and MDA-MB-435 cells would be a reason for the resistance or the response, respectively, to Jaspin B. FOXO3-mediated apoptotic mechanisms were detected in MDA-MB-435 cells while MDA-MB-231 cells were resistant to AZD6244, a FOXO3a inhibitor [26]. Since FOXO3 was reported to the underlying mechanism of Jaspine B-mediated anti-proliferative effect, the presence of FOXO3-mediated pathway in the cell system would be a crucial factor for the response of Jaspine B. Moreover, the difference in the ratio between ceramide and sphingosine/ sphingosine-1-phosphate and the difference in the sphingosine kinase activity could also provide the mechanistic understanding to the different cytotoxic effect of Jaspine B to MDA-MB-231 and MDA-MB-435 cells.

In conclusion, the cytotoxic effect of Jaspine B can be achieved when effective intracellular concentration of Jaspine B is maintained. Additionally cancer cells that failed to achieve effective drug concentrations (in case of 786-O and MDA-MB-231 cells) will be resistant to this compound. Further studies using P-gp and BCRP overexpression system are warranted that efflux pumps such as P-gp and BCRP does not seem to play a critical role in Jaspine B resistance.

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초록 : 다양한 암세포주에서 Jaspine B의 함암활성 비교

이지훈[1] · 최광익[1] · 권미화[1] · 이동주[2] · 최민구[3] · 송임숙[1]*
([1]경북대학교 약학대학, [2]아주대학교 약학대학, [3]단국대학교 약학대학)

Jaspine B는 석회해면류에서 추출된 sphingosine유도체로 인간 암세포에서의 항암활성이 보고되었다. 그러므로 본 연구는 다양한 인간 암세포주에서 항암활성을 비교하고, 암세포주에서의 Jaspine B의 농도를 측정하여 항암활성과의 연관성을 확인하고자 하였다. 항암활성은 MTT 방법을 이용하여 측정하였고, EC_{50} 값으로 표현하였다. 암세포주내 Jaspine B의 농도는 LC-MS/MS를 이용하여 분석하였다. 항암활성은 세포주마다 다양하게 나타났는데, 유방암과 흑색종 세포주에서 항암활성이 높게 나타났으며(EC_{50} 각각 2.3 μM과 2.6 μM), 신장암세포주에서는 EC_{50} 값이 29.4 μM이었다. 암세포주에서의 EC_{50} 값은 동일한 세포에서의 Jaspine B 농도와 높은 상관성을 나타내었으며(r=0.838), 암세포내 약물농도를 조절하는 것으로 잘 알려진 P-glycoprotein과 breast cancer resistance protein 등의 배출수송계와는 관련이 없음을 확인하였다. 이상의 결과는 세포내 약물농도를 높게 유지하는 것이 항암활성에 매우 중요하며, 세포내 약물농도가 암세포주에 따라 다른 약효를 보이는 원인으로 사료된다.