RESEARCH COMMUNICATION

Reversion of Multidrug Resistance by SKI-II in SGC7901/DDP Cells and Exploration of Underlying Mechanisms

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Abstract

In order to investigate whether SKI-II could reverse drug resistance and its possible mechanisms, we treated SGC7901/DDP cells with SKI-II or SKI-II in combination with DDP. Then cell growth, apoptosis, micro-morphological changes, and expression of SphK1, P-gp, NF-κB, Bcl-2 and Bax were assessed by MTT assay, flow cytometry, electron microscopy, immunocytochemistry and Western blot assay respectively. SGC7901/DDP cells were insensitive to cisplatin 2.5mg/L, but when pretreated with SKI-II, their proliferation was inhibited by cisplatin 2.5mg/L significantly, the inhibition rate increasing with time and dose. The apoptosis rate was also significantly elevated. Expression of SphK1 and P-gp was decreased significantly, Pearson correlation analysis showing significant correlation between the two (r=0.595, P<0.01). Expression of NF-κB and Bcl-2 was decreased significantly,while that of Bax was increased, compared to the control group. There were significant correlations between SphK1 and NF-κB(r=0.723, P<0.01), NF-κB and Bcl-2(r=0.768, P<0.01). All these data indicated that SKI-II could reverse drug resistance of SGC7901/DDP to cisplatin by down-regulating expression of P-gp and up-regulating apoptosis through down-regulation of SphK1. The increased apoptotic sensitivity of SGC7901/DDP to cisplatin was due to the decreasing proportion of Bcl-2/Bax via down-regulating NF-κB.

Keywords: SGC7901/DDP cells - resistance - SKI-II - apoptosis - SphK1

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Introduction

The chemotherapy drug cisplatin [cis-diamminedichloroplatinum (II)] is widely used in the treatment of small-cell and non-small-cell lung cancer, esophageal cancer, testicular cancer, ovarian cancer, and bladder cancer, among others. However, the efficacy of cisplatin is often limited because some tumors are intrinsically resistant to anti-tumor drugs, while in others resistance is selected for during the course of therapy (Köberle et al., 2010).

Sphingolipid ceramide and S1P are critical signal transduction molecules regulating cell death and survival. Ceramide mediates a wide array of stress signals such as anticancer treatments leading to apoptosis, whereas S1P exerts prosurvival capabilities by antagonizing ceramide effects (Modrak et al., 2006). So a ceramide/S1P rheostat has been hypothesized to determine the fate of cell, such that the relative cellular concentrations of ceramide and S1P determine whether a cell proliferates or undergoes apoptosis (Andrieu-Abadie and Levade, 2002; Reynolds et al., 2004; Bieberich et al., 2008). At the same time, previous work using *Dictyostelium discoideum* as a model for studying drug resistance showed that mutants lacking sphingosine-1-phosphate (S1P) lyase, the enzyme that degrades S1P, had increased resistance to cisplatin, whereas mutants overexpressing the enzyme were more sensitive to the drug (Andrieu-Abadie and Levade, 2002). That means modulating the levels of sphingolipids in cells can be a powerful way to increase the sensitivity of tumor cells to cisplatin.

A key regulator of this balance is sphingosine kinase-1 (SphK1), which phosphorylates sphingosine (the catabolite of ceramide) to generate S1P, because it reduces proapoptotic ceramide levels by driving the sphingolipid metabolism toward antiapoptotic S1P. SphK1 mRNA content was found elevated in cancers, such as lung cancer, glioblastoma and colon carcinoma (Johnson et al., 2005; Van Brocklyn et al., 2005; Kawamori et al., 2006). On the contrary, down-regulation of SphK1 could arrest cell cycle and induce apoptosis (Pchejetski et al., 2005; Bonhoure et al., 2006; Cuvillier et al., 2007). Moreover, it has been shown that SphK1 impairs the efficacy of chemotherapy in prostate adenocarcinoma cells (Pchejetski et al., 2005). Anti-SphK1 therapies [anti-SphK1-based small interfering RNA (siRNA) or pharmaco-logic inhibition methods] have proven their efficacy to kill some cancer cell lines whether or not they are sensitive to conventional chemotherapy or radiotherapy (Pchejetski et al., 2008; Guillermet-Guibert et al, 2009). So targeting the sphingolipid metabolism pathway for improving tumor chemosensitivity has recently emerged as a promising strategy. One of the

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most attractive sites of intervention in this pathway is the conversion of sphingosine to S1P by the enzyme SphK1.

Despite the high level of concern regarding the role of sphingolipid-derived signalling, very few inhibitors of the enzymes of this pathway have yet been established. To date, pharmacological studies have used sphingosine analogues, especially N,N-dimethylsphingosine (DMS) and DHS (DL-threo-dihydrosphingosine), but these analogues can't be used widely due to their adverse effect of serious hemolysis and inhibiting protein kinase C which were proven by experiment in vivo. Recently, a few natural product inhibitors (compounds I–V) of SphK were isolated. It has been reported that compound II is the most selective SphK inhibitor among the natural product inhibitors of SphK and therefore may be the most attractive candidate for additional medicinal chemistry efforts. 4-[4-(4-chloro-phenyl)-thiazol-2-ylamino]-phenol (SKI-II) is orally bioavailable, and it can be detected in the blood for at least 8 h, and showed a significant inhibition of tumor growth in mice. These compounds are the first examples of nonlipid selective inhibitors of SphK in vivo antitumor activity and provide leads for further development of inhibitors of this important molecular target (French et al., 2003; 2006) .

It is still unclear whether SphK1 can be a new target of reversion of multidrug resistance in cisplatin-resistant gastric cancer. The present study was therefore undertaken to investigate whether a dysregulation of the SphK1 by SKI-II in Cisplatin-resistant SGC7901/DDP gastric cancer cells is involved in their resistance toward cisplatin.

Materials and Methods

Cell line and culture

Human cisplatin-resistant gastric cancer cell line SGC7901/DDP was purchased from Nanjing KeyGen Biotech. Co. Ltd. Cells were cultured in RPMI 1640 supplemented with 10% FCS, 1% glutamine, 500ng/ml cisplatin and 1% antibiotics. Cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. After 24h in RPMI 1640 medium, when the cells adhered to the glass coverslips in the culture plates or at the bottom of the culture plates, the cells were treated with various concentration of SKI-II or cisplatin. When the cells treated with SKI-II in combination with cisplatin, cells were pretreated with SKI-II for 2 hours.

Reagents

AnnexinV-FITC Apoptosis Detection Kit (KGA107) was purchased from Nanjing KeyGen Biotech. Co. Ltd. SKI-II (S5696), MTT Detection Kit were purchased from Sigma-Aldrich Co. .Antibodies against SphK1 (sc-48825), NF-κB (sc-20112), Bcl-2(sc-783), Bax(sc-70406) and P-gp (sc-53510) were purchased from Santa Cruz Biotechnology, Inc. Cisplatin was purchased from Shandong Qilu pharmaceutical factory. Other drugs were all reagent grade.

MTT assay

Cell survival and viability in vitro were evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5 -diphenyltetrazolium bromide (MTT) assay. SGC7901/DDP cells were plated on 96-well plates. Then cells were treated with cisplatin (2.5mg/L), SKI-II (1.25, 2.5, 5, 10, 20, 40umol/L) and cisplatin(2.5mg/L) combined with SKI-II(1.25, 2, 5, 5, 10umol/L) for 24 h, 48 h, 72h respectively. Then 20μl of 5mg/ml MTT solution was added to each well and cells were further incubated at 37°C for 4h. Then we removed the media, added 150μl DMSO to each well. At last the plate was put into the incubator for 5min to dissolve air bubbles and transferred to plate reader and measure absorbance at wavelength of 490nm. The negative control had medium without serum and cells, and was used as the zero point. The inhibitory rate (IR) of cells was calculated according to the equation as follows: IR (%) = [1-(A490nm absorbance in treated group/A490nm absorbance in control group)] ×100%.

Morphological observation

After treatment with cisplatin(2.5mg/L), SKI-II(1.25,10umol/L) and cisplatin(2.5mg/L) combined with SKI-II(1.25,10umol/L), cytologic morphological changes were observed under an Olympus optical microscope. Cells were subcultured on coverslips in 6-well culture plates. After 48h the coverslips were taken out and observed after staining with H&E.

Detection of apoptosis

SGC7901/DDP cells were incubated with cisplatin(2.5mg/L), SKI-II(1.25,10umol/L) and cisplatin(2.5mg/L) combined with SKI-II(1.25,10umol/L) for 48h. Cells were harvested using 0.25% trypsin (without EDTA), washed with PBS for two times, then we counted and adjusted the cells to 1×10^6/ml. After treated with Annexin V-FITC and propidium iodide for 15minutes, the apoptosis rates were detected using flow cytometry.

Electron microscope analysis

After 48h when the SGC-7901 cells had been treated with cisplatin(2.5mg/L), SKI-II(1.25,10umol/L) and cisplatin(2.5mg/L) combined with SKI-II(1.25, 10umol/L), the cells were collected and fixed with 4% glutaraldehyde and immersed in Epon 821. The cells were transferred into ultrathin sections (60nm) and stained with uranylacetate and lead citrate. Cell morphology was observed by transmission electron microscopy.

Immunohistochemical analysis

Immunohistochemical analysis was done to study altered protein expressions. SGC7901/DDP cells treated with cisplatin (2.5mg/L), SKI-II (1.25, 10umol/L) and cisplatin(2.5mg/L) combined with SKI-II (1.25, 10umol/L) were cultured on coverslips in a 6-well plate. After 48 h the glass coverslips on which the cells mounted were collected and fixed with 10% neutral formalin. Streptavidin/peroxidase-Plus kits were used for the immunostaining SphK1, NF-κB, Bcl-2, Bax, and P-gp. In brief, after washing with PBS, endogenous peroxidase activity was eliminated by preincubation in 3% H2O2 for 10 min. Primary antibodies were used with the following dilutions: SphK1(1:100), NF-κB(1:100), Bcl-2(1:75), Bax(1:75), and P-gp(1:100). After incubation overnight at

Table 1. Inhibitory Effects of SKI-II Alone and in Combination with Cisplatin on the Proliferation of SGC7901/DDP Cells (X ±S, n=12)

Group	24h		48h		72h	
	OD	IR (%)	OD	IR (%)	OD	IR (%)
control	0.586±0.053	0	0.610±0.032	0	0.624±0.035	0
cisplatin 2.5mg/L	0.568±0.044	2.917±2.261	0.593±0.035	2.833±1.601	0.605±0.032	3.050±1.739
SKI-II 1.25umol/L	0.565±0.031	3.217±5.853	0.583±0.025	4.350±3.536	0.597±0.046	4.383±3.728
SKI-II 2.5umol/L	0.555±0.057	5.242±5.664	0.575±0.042	5.783±4.428 *	0.584±0.044*	6.375±4.957 *
SKI-II 5umol/L	0.531±0.053*	9.175±7.383*	0.554±0.049*	9.300±6.549 *	0.542±0.043*&	12.983±6.565*&
SKI-II 10umol/L	0.497±0.034*&	14.750±8.367*&	0.509±0.043*&	16.633±5.302*&	0.500±0.044*&	19.467±9.439*&
SKI-II 20umol/L	0.453±0.040*&	22.525±5.445*&	0.465±0.050*&	23.825±7.635*&	0.451±0.056*&	27.675±7.829*&
SKI-II 40umol/L	0.414±0.028*&	28.633±9.124*&	0.423±0.039*&	30.558±7.819*&	0.406±0.045*&	34.658±9.310*&
cisplatin 2.5+SKI-II1.25	0.515±0.014*#	11.592±7.465*#	0.526±0.032*#	13.633±6.743*	0.542±0.036*#	12.767±7.964*#
cisplatin 2.5+SKI-II2.	0.507±0.034*#	12.992±7.455*	0.517±0.030*#	15.333±4.524*	0.502±0.028*#	19.308±5.308*#
cisplatin 2.5+SKI-II5	0.455±0.037*#	22.067±6.126*#	0.471±0.031*#	22.8333±2.151*	0.463±0.027*#	25.683±3.962*#
cisplatin 2.5+SKI-II10	0.412±0.031*#	29.050±9.919*#	0.425±0.035*#	30.250±5.891*	0.410±0.041*#	34.200±7.079*#

*vs control, p<0.05; &SKI-II 10 vs SKI-II 5, SKI-II 20 vs SKI-II 10, SKI-II 40 vs SKI-II 20 , p<0.05; #cisplatin2.5+SKI-II10 vs cisplatin2.5 and SKI-II10, cisplatin2.5+SKI-II 5 vs cisplatin2.5 and SKI-II5, cisplatin2.5+SKI-II2.5 vs cisplatin2.5 and SKI-II2.5, cisplatin2.5+SKI-II1.25 vs cisplatin2.5 and SKI-II1.25, p<0.05, SGC7901/DDP cells were treated with SKI-II alone or in combination with cisplatin for 24, 48, and 72 h respectively. Then the inhibitory rate (IR) of cells was calculated. Data are expressed as Mean ± SD (n=12)

4°C, the cells were washed with PBS and then incubated with a secondary antibody for 30 min at room temperature. DAB was used as the chromogen and the cells were counterstained with hematoxylin. Negative controls were established by replacing the primary antibody with PBS and known immunostaining-positive slides were used as positive controls. Clear staining of the brown-color cytoplasm (SphK1, NF-κB, Bcl-2, Bax, and P-gp) was the criterion for a positive reaction. The positive number of cells in each high-power field was counted and the percentage of positive cells relative to the total tumor cells calculated as follows: PR= (number of positive cells/total number) ×100%.

All the immunostained slides were assessed by 2 pathologists.

Western-blot analysis

SGC7901/DDP cells were collected after treatment with cisplatin(2.5mg/L), SKI-II(10umol/L) and cisplatin(2.5mg/L) combined with SKI-II(10umol/L). Aliquots of cell lysates containing equal protein were separated on SDS-polyacrylamide gels for 2-3 h, and then transferred to nitrocellulose filters followed by incubation with SphK1(dilution 1:1000), NF-κB(dilution 1:500), Bcl-2(dilution 1:500), Bax(dilution 1:500), and P-gp (dilution 1:500) antibody overnight at 4 °C. Then the filters were washed with PBS-T followed by the addition of horseradish peroxidase-linked goat or anti-mouse goat anti-rabbit IgG (dilution 1:2000) for 2 h at room temperature and NBT/BCIP visualization of the bands.

Statistical analysis

A statistical package SPSS16.0 was used for statistical analysis. Mean ± SD, One-way ANOVA and Pearson correlation analysis were used to analyze the correlation between groups. Statistical significance was determined at P<0.05 level.

Results

SKI-II can inhibit the proliferation of SGC7901/DDP cells and increase sensitivity to cisplatin

After 24h, 48h,72h treatment, the inhibition rate of SKI-II was significantly higher than that of the negative control group, the lowest concentration of SKI-II being able to affect cell growth was 5umol/L (P<0.05). The inhibitory effect of SKI-II on the proliferation of SGC7901/DDP cells is in a concentration-dependent manner (P<0.05). Correspondingly, the inhibition was enhanced with time gradually extending after 48h and 72h treatment respectively.

There was no significant difference between cisplatin 2.5mg/L group, and negative control group. There was no significant difference between SKI-II1.25umol/L group and negative control group and the inhibition rate of SKI-II1.25umol/L group is below 5%. But when the cells were pretreated with SKI-II1.25umol/L, the proliferation of SGC7901/DDP cells was inhibited by cisplatin 2.5mg/L significantly, compared to negative control group, cisplatin 2.5mg/L group and SKI-II1.25umol/L group respectively (P<0.05),which means SKI-II1.25umol/L

Table 2. Apoptosis Rates of SGC7901/DDP cells treated with SKI-II and in Combination with Cisplatin (X ±S, n=12)

group	apoptosis rate(%)
control	11.930±1.973
cisplatin 2.5mg/L	12.225±1.902
SKI-II1.25μmol/L	12.325±2.416
SKI-II1.25+cisplatin2.5mg/L	17.125±1.993*&
SKIII10μmol/L	21.418±1.189*
S10μmol/L+ cisplatin2.5mg/L	24.795±2.823*&

*vs control, p<0.05; &vs SKI-II1.25, p<0.05; &vs SKIII 10μmol/L, p<0.05

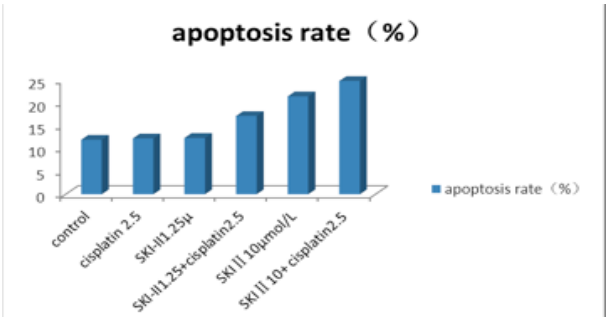

Figure 2. Apoptosis Rates of SGC7901/DDP Cells Treated with SKI-II and in Combination with Cisplatin. We incubated SGC7901/DDP cells with cisplatin, SKI-II, cisplatin combined with SKI-II for 48h. After treated with Annexin V-FITC and Propidium Iodide, the apoptosis rates were detected using Flow cytometry

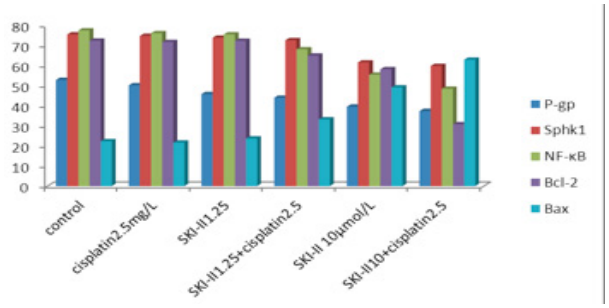

Figure 3. Expression of SphK1, P-gp, NF-κB, Bcl-2 and Bax in SGC7901/DDP Treated with SKI-II and in Combination with Cisplatin Detected by Immunocytochemical Staining

reversed the sensitivity of cisplatin to SGC7901/DDP cells. The inhibitory effect was enhanced in combination groups with the increasing concentration of SKI-II, there were significant differences between combination groups (P<0.05) (Table 1).

Morphological changes of SGC7901/DDP with SKI-II

H&E stained cells in the negative control group and DDP group showed normal structures, but with increasing levels of SKI-II, and in combination with DDP, the SGC7901/DDP cells became sparse. Some dead cells showed apoptotic morphologic changes, such as nuclear condension, fragmentation and chromatin crescents. Under electron microscopy cells at SKI-II in combination with DDP had more apoptotic characteristics including chromatin condensation, nuclear fragmentation, chromatin crescents and apoptotic bodies (Figure 1).

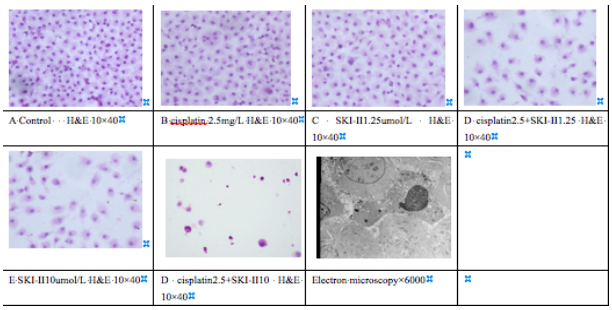

Figure 1. Morphological changes of SGC7901/DDP treatd by cisplatin 2.5mg/L, SKI-II10umol/L and cisplatin2.5 in combination with SKI-II10. Cells were treated with SKI-II alone or in combination with cisplatin for 48h. Morphological changes were detected by Olympus optical microscope and electron microscope analysis

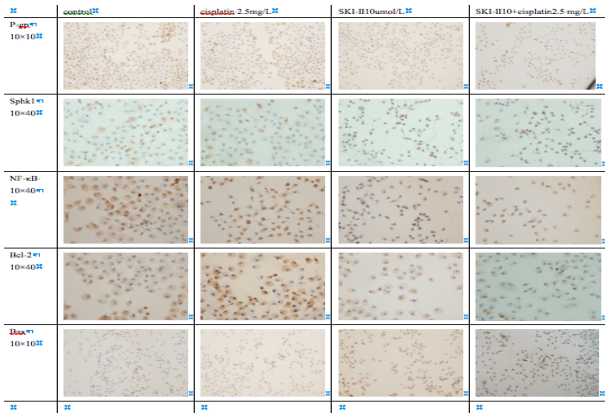

Figure 4. Expression of SphK1, P-gp, NF-κB, Bcl-2 and Bax in SGC7901/DDP Treated with SKI-II and in Combination with Cisplatin Detected by Immunocytochemical Staining (SP method). Cells were treated with 2.5mg/L cisplatin, 10umol/L SKI-II and 2.5mg/L cisplatin combined with 10umol/L SKI-II for 48h. Expression of SphK1, P-gp, NF-κB, Bcl-2 and Bax were performed using the Streptavidin/Peroxidase-Plus Kits. The changes were photographed at 100× or 400× magnification

SKI-II increased the apoptosis sensitivity of SGC7901/DDP to cisplatin

Cisplatin 2.5mg/L and SKI-II1.25μmol/L did not induce apoptosis of SGC7901/DDP cells alone (P>0.05). When SGC7901/DDP cells were pretreated with SKI-II1.25μmol/L, cisplatin2.5mg/L can induce the apoptosis rate significantly higher compared to negative control group, cisplatin2.5mg/L group and SKI-II1.25μmol/L group alone (P<0.05). The effect of inducing apoptosis was enhanced in the group of SKI-II 10μmol/L and SKI-II 10μmol/L in combination with Cisplatin2.5mg/L. There is significant difference between combination group and SKI-II or cisplatin group alone (P<0.05) (Table 2, Figure 2). SKI-II and in combination with cisplatin down regulated the expressions of SphK1, P-gp, NF-κB, Bcl-2 and up regulated the expression of Bax in SGC7901/DDP cells.

In immunocytochemical staining, Positive staining was located in the cytoplasm. Table 3 and Figure 3 show the expressions of SphK1, P-gp, NF-κB, Bcl-2 and Bax proteins before and after treatment of SKI-II and cisplatin. Immunocytochemical staining (Figure 4) and Western-blot

Table 3. Expression of SphK1, P-gp, NF-κB, Bcl-2 and Bax in SGC7901/DDP Treated with SKI-II and in Combination with Cisplatin Detected by Immunocytochemical Staining

group	P-gp	Sphk1	NF-κB	Bcl-2	Bax	Bcl-2/ Bax
control	52.90±3.77	75.50±8.72	77.53±7.20	72.43±6.85	22.35±3.82	3.34±0.86
cisplatin2.5mg/L	50.18±1.97	74.80±8.33	76.15±2.24	71.77±4.32	21.63±3.18	3.38±0.60
SKI-II1.25	45.83±2.76*	73.90±4.32	75.55±2.43	72.35±2.48	23.70±3.68	3.10±0.44
SKI-II1.25 +cisplatin2.5mg/L	43.97±4.48*	72.65±3.09	68.15±3.72*&	64.98±2.04*&	33.25±2.00*&	1.95±0.08*&
SKI-II 10μmol/L	39.55±5.82*	61.60±2.31*&	55.48±4.15*&	58.20±1.79*&	49.18.±0.84*&	1.18±0.02*&
cisplatin2.5mg/L +SKI-II 10μmol/L	37.40±4.54*	59.85±8.55*	48.5±3.86*&&	30.80±2.18*&&	62.95±2.30*&&	0.49±1.21*&&

(X ±S,%, n=4*); *vs control, p<0.05; &vs SKI-II1.25, p<0.05; &vs SKI-II 10μmol/L, p<0.05

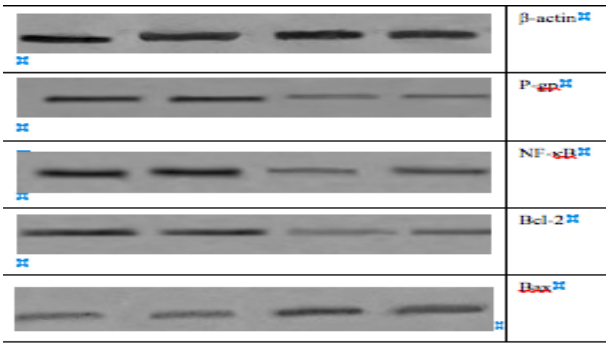

Figure 5. Expression of SphK1, P-gp, NF-κB, Bcl-2 and Bax in SGC7901/DDP Treated with SKIII and in Combination with Cisplatin Detected by Western-blot Analysis. From the left to right, the band refer to control group, cisplatin 2.5mg/L group, SKI-II10umol/L group and SKI-II10umol/L in combination with cisplatin 2.5mg/L group. Cells were treated with 2.5mg/L cisplatin, 10umol/L SKI-II and 2.5mg/L cisplatin combined with 10umol/L SKI-II for 48h. Western-blot analysis was used to detect the expression of SphK1, P-gp, NF-κB, Bcl-2 and Bax. Immunocomplexes were visualized using DAB. β- actin protein was used as a loading control

analysis (Figure 5) indicated that SphK1, P-gp, NF-κB and Bcl-2 had high expression levels in SGC7901/DDP cells, but Bax expression level was low. The expression of Sphk1 was decreased treated by SKI-II 10μmol/L, SKI-II 10μmol/L in combination with cisplatin 2.5mg/L, whereas cisplatin 2.5mg/L alone showed no significant differences compared with compared group, which means cisplatin has no effect on SphK1. The expression of P-gp was decreased treated by SKI-II1.25μmol/L, SKI-II1.25μmol/L in combination with cisplatin2.5mg/L showed no significant differences compared with SKI-II1.25μmol/L, which means cisplatin has no effect on P-gp. Single treatment of cisplatin 2.5mg/L and SKI-II1.25μmol/L did not change the expression levels of NF-κB, Bcl-2 and Bax significantly compared to negative control group. When SGC7901/DDP cells were pretreated with SKI-II1.25μmol/L then, the expression of NF-κB and Bcl-2 decreased by different degrees, and the expression of Bax increased. Additionally, Pearson correlation analysis showed that there were significant correlations between SphK1 and P-gp (r=0.595, P<0.01), SphK1 and NF-κB (r=0.723, P<0.01), NF-κB and Bcl-2 (r=0.768, P<0.01).

Discussion

The chemosensitivity or chemoresistance of cancer cells toward anticancer drugs is closely related to the rates of entry and extrusion of drugs into and out of cells through transporter molecules, and with the signal transduction cascade leading to apoptosis in response to anticancer drugs (Huang and Sadee, 2006). The chemoresistance mediated by ABC transporters, such as MDR or MRP transporters, is mainly due to decreased cellular accumulation of anticancer drugs caused by extrusion of the drugs out of the cells, and involves diverse mechanisms including up-regulation of the drug efflux pumps, MDR-associated protein (MRP-1) and P-glycoprotein (P-gp). P-gp is an anti-apoptotic membrane glycoprotein encoded by the MDR-1 gene, which could reduce the accumulation of chemotherapeutic drug in cells. P-gp could transport lots of hydrophobic lipophilic drugs such as colchicine, doxorubicin, vincristine out of cells using the energy released by ATP hydrolysis actively, decreased the intracellular drug concentration, redistributed the drugs, this all could lead to drug resistance (Veldman, 2004; Szulc, 2006; Bielawska, 2008). In a recent study, overexpression of SphK1 in RBE-4 cerebral endothelial cells was shown to enhance the expression of P-gp at the mRNA and protein levels. Furthermore, as demonstrated in these brain tumor-derived endothelial cells, S1P also stimulated the transport activity of P-gp via activation of S1P1 and S1P3 receptors (Pilorget et al., 2007).

Sphingolipids have recently emerged as potent second messenger molecules controlling cellular responses to various prosurvival or stress stimuli. Ceramide, sphingosine, and sphingosine-1-phosphate (S1P) are interconvertible lipids that mostly compose the sphingolipid metabolism. Ceramide and sphingosine levels are up-regulated on cell treatment with different cytokines, anticancer drugs, and other stress-causing agonists and in turn mediate cell growth arrest and apoptosis via the regulation of various signaling pathways and subsequent caspase activation. On the contrary, S1P, a further metabolite of ceramide, is a growth promoter and survival factor. At the same time increased resistance to cisplatin was due to an elevation of S1P and it was predicted that lowering levels of S1P should increase sensitivity to the drug. So inhibiting the expression of SphK1 was expected to be a successful treatment of gastric cancer (Cuvillier, 2007; 2008; Herr and Chun, 2007).

In our experiment, SGC7901/DDP cells were insensitive to cisplatin 2.5mg/L. There was no significant difference between SKI-II1.25umol/L group and negative control group and the inhibition rate of SKI-II1.25umol/L

group is below 5%. But when the cells were pretreated with SKI-II 1.25umol/L, cisplatin 2.5mg/L can induce the apoptosis and inhibit the proliferation of SGC7901/DDP compared to negative control group, cisplatin2.5mg/L group and SKI-II1.25μmol/L group alone (P<0.05). More apoptotic body in the cells can be seen by electron microscope analysis in combination groups. The expression of P-gp was decreased after treated wthi SKI-II 1.25μmol/L, and the effect was enhanced with concentration extending. SKI-II in combination with cisplatin 2.5mg/L showed no significant differences compared with SKI-II alone, which means cisplatin has no effect on P-gp. The expression of Sphk1 was decreased after treated with SKI-II 10μmol/L, SKI-II 10μmol/L in combination with cisplatin 2.5mg/L, which showed no significant differences compared with SKI-II 10μmol/L, which means cisplatin has no effect on SphK1. In additiona, Pearson correlation analysis showed that there was significant correlation between SphK1 and P-gp (r=0.595, P<0.01). So we could conclue that SKI-II could reverse drug resistance of SGC7901/DDP to cisplatin by down-regulating expressions of P-gp via down- regulating SphK1.

It was reported by a study that in prostate cancer, inhibition of the oncogenic SphK1/S1P pathway is a key element in chemotherapy-induced apoptosis (Pchejetski D et al., 2008). S1P acts as second messenger intracellularly as well as a ligand for membrane-bound G-protein-coupled receptors, and it plays an important role in regulating central cellular processes, such as cell growth, cell apoptosiss, differentiation and motility (Köberle et al., 2010; Modra., 2006; Bieberich E, 2008; Reynolds CP., 2004) by up-regulating several antiapoptotic pathways including phosphatidy-linositol-kinase or NF-κB.

NF-κB was first extracted from B lymphocytes, and it was involved in the process of immune stress, inflammation, cell proliferation and apoptosis (Esteban V et al., 2004). Research shows that NF-κB was activated in many cancers, including gastric cancer (Lee SY et al., 2008). NF-κB can regulate the expression of Bcl-2, which can bind with Bax to inhibit apoptosis via inhibiting the release of cytochrome C and preventing cytoplasmic cytochrome C from activating Caspase protease (Fan et al., 2005; Lin et al., 2005;). Bax does not directly induce cell death, but it can significantly speed up the death signal which induced apoptosis and its overexpression can antagonize the protective effect of Bcl-2, leading to apoptosis (Misao et al., 1996; Tsujimoto et al., 2002). The balance between Bcl-2 and Bax within a cell determines its relative resistance or sensitivity to apoptosis (Xie et al., 2001).

In our experiments, when cisplatin2.5mg/L was in combination with SKI-II, the expressions of NF-κB and Bcl-2 decreased significantly, and the expression of Bax increased, compared to the control group and SKI-II group alone. Pearson correlation analysis showed that there were significant correlations between SphK1 and NF-κB(r=0.723, P<0.01), NF-κB and Bcl-2(r=0.768, P<0.01). So we supposed SKI-II increased apoptotic sensitivity of SGC7901/DDP to cisplatin by decreasing the proportion of Bcl-2/Bax, which was regulated by NF-κB.

However, these are all experimental data in vitro, experiments in vivo as well as specific cell factor pathway of SKI-II reversing drug resistance remains unclear, so further research is needed.

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