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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

İNSAN LÖSEMİ HÜCRE HATLARINDA S-ALLİL-L-SİSTEİN'İN APOPTOZ VE OTOFAJİ ÜZERİNE OLASI ETKİLERİNİN ARAŞTIRILMASI

INVESTIGATION OF THE POSSIBLE EFFECT OF S-ALLYL-L-CYSTEINE ON APOPTOSIS AND AUTOPHAGY IN HUMAN LEUKEMIA CELL LINE

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ÖZET

AMAÇ: S-Allil-L-sistein (SAC), sarımsağın biyolojik olarak aktif bir organosülfür bileşenidir ve çeşitli farmakolojik etkilere sahiptir. SAC anti-kanser aktivite göstermektedir, ancak mekanizması belirlenememiştir. Bu çalışma, SAC'nin iki insan lösemi hücre dizisi üzerindeki olası apoptotik ve otofajik etkilerini araştırmaya odaklanmıştır: akut promiyelositik lösemi (HL-60) ve kronik miyeloid lösemi (K562).

GEREÇ VE YÖNTEM: Hücre sitotoksisitesi MTT testi ile değerlendirildi. Bax, Bcl-2, kaspaz 3, mTOR, AKT ve PI3K gen ekspresyon miktarları, kantitatif gerçek zamanlı ters transkripsiyon polimeraz zincir reaksiyonu (qRT-PCR) yoluyla tanımlandı. HL-60 ve K562 hücreleri, sırasıyla üç farklı dozda (5 mM, 10 mM ve 20 mM) (3,75 mM, 7,5 mM ve 15 mM) SAC ile inkübe edildi.

BULGULAR: SAC, sırasıyla yaklaşık 11.525 mM ve 10.025 mM IC50 değerleri ile HL-60 ve K562 hücreleri üzerinde sitotoksik etkiye neden olmuştur. HL-60 hücrelerinde, Bax ekspresyon seviyelerinde 5 mM ve 10 mM SAC dozlarında artış tespit edildi ($p = 0.027$, $p = 0.000$). 10 mM SAC ile tedavi, kontrol ve 5 mM SAC ile tedavi edilen hücrelere kıyasla HL-60 hücrelerinde kaspaz 3 ekspresyon seviyesini artırdı ($p = 0.020$, $p = 0.000$). K562 hücrelerinde SAC, tüm dozlarda mTOR, AKT ve PI3K ekspresyon seviyelerinde önemli bir düşüşe neden oldu ($p = 0.000$, $p = 0.000$, $p = 0.000$).

SONUÇ: Sonuç olarak, verilerimiz SAC'nin K562 hücrelerinde PI3K/AKT/mTOR sinyal yolunu down-regüle ederek otofajiyi indüklediğini göstermektedir. Ayrıca, artan Bax ve kaspaz 3 gen ekspresyon seviyeleri, SAC'nin HL-60 hücrelerinde apoptozu indüklemek için etkili bir aktif bileşen olabileceğini düşündürmektedir.

ANAHTAR KELİMELER: S-Allil-L-sistein, Bax, kaspaz 3, Bcl-2, mTOR

ABSTRACT

OBJECTIVE: S-Allyl-L-cysteine (SAC) is a biological active organosulfur component of garlic and has various pharmacological effects. SAC has displayed anti-cancer activity but the mechanism is unresolved. This study has focused on investigating the possible apoptotic and autophagic effects of SAC on two human leukemia cell lines: acute promyelocytic leukemia (HL-60) and chronic myeloid leukemia (K562).

MATERIAL AND METHODS: Cell cytotoxicity was evaluated via MTT test. Bax, Bcl-2, caspase 3, mTOR, AKT, and PI3K gene expression amounts were identified via Real time quantitative reverse transcription polymerase chain reaction (qRT-PCR). HL-60 and K562 cells were incubated with SAC at three diverse doses (5 mM, 10 mM, and 20 mM) (3,75 mM, 7,5 mM, and 15 mM), respectively.

RESULTS: SAC caused a cytotoxic effect on HL-60 and K562 cells with IC50 values of approximately 11.525 mM and 10.025 mM, respectively. In HL-60 cells, an increase in Bax expression levels was detected at doses of 5 mM and 10 mM SAC ($p=0.027$, $p=0.000$). Treatment with 10 mM SAC increased the expression level of caspase 3 in HL-60 cells as compared with the control and 5 mM SAC treated cells ($p=0.000$, $p=0.020$). In K562 cells, SAC induced a significant decrease in mTOR, AKT, and PI3K expression levels in at all doses ($p=0.000$, $p=0.000$, $p=0.000$).

CONCLUSIONS: In conclusion, our data indicates that SAC induces autophagy in K562 cells by downregulating the PI3K/AKT/mTOR signaling pathway. Furthermore, increased Bax and caspase 3 gene expression levels suggest that SAC may be an effective active ingredient with which to induce apoptosis in HL-60 cells.

KEYWORDS: S-Allyl-L-cysteine, Bax, caspase 3, Bcl-2, mTOR

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INTRODUCTION

Leukemia is a heterogeneous group of hematological disorders that affect cells originating from the bone marrow (1). The broad-spectrum cytotoxic agents against molecular therapies targeting specific signal transduction pathways and rapidly proliferating cells are the pre-treatment in leukemia (2). The failure of leukemia cure is often due to the progression of cellular drug resistance (3).

Chemotherapy is a significant therapeutic component for numerous cancers, and novel anti-cancer agents represent one of the broadest fields of pharmaceutical progress. Whereas, chemotherapeutic agents not only target the cancer cells but also affect normal cells (4). For this reason, researchers focus more on agents derived from natural products (5). However, searching of bioactive compounds for use as anticancer drugs has led to the identification of several natural chemotherapeutic drugs (6). In particular, large-scale epidemiological studies have suggested an association between garlic consumption and decreased rate of cancer.

Furthermore, more research presented that garlic-derived organosulfur compounds may be responsible for the reduced risk of cancer (7). S-Allyl-L-cysteine (SAC), a compound derived from garlic, has stable oral bioavailability and low toxicity and plays role like an antioxidant by different mechanisms (8). Increasing studies propose that SAC has anti-tumor, antioxidant anti-bacterial, anti-fungal, anti-hepatotoxic, and cardioprotective effects (9). Numerous studies presented that SAC has antitumor properties by inhibiting the metastasis and cellular proliferation and stimulates apoptotic cell death in many cancer models, containing hepatocellular, ovarian, and prostate carcinoma (10).

Natural products move in a manner as traditional chemotherapy agents do, either by inducing apoptosis or damaging the cell cycle (7).

Several studies have revealed that apoptosis has a fundamental significance in the pathogenesis of cancers. Induction of apoptotic cell death is one of the most significant targets to cure cancer (9). In human tumour cells, besides apoptosis, autophagy has been found to contribute to cell death (11). Autophagy is a catabolic pathway by which long-lived proteins and injured organelles are removed for recycling and important for maintaining cellular homeostasis.

Mammalian target of rapamycin (mTOR) is a kinase that modulates the autophagic progression (12). Several works have specified that the phosphatidylinositol-3-kinase(PI3K)/protein kinase B (AKT)/ mTOR pathway is intimately related to autophagy (13). The oncogenic activation of MTOR signalling stimulates certain processes necessary for the survival, proliferation and growth of the cancer cell (14). The mTOR complexes are stimulated by AKT and regulate some cell growth functions containing cell survival, inhibition of autophagy and protein synthesis (15). Since cancer cells constantly initiate the PI3K/AKT/mTOR, which adversely regulates autophagy, this pathway can be manipulated to stimulate autophagy in cancer cells (11). One study has presented that SAC could effectively prevent the induction of Akt/mTOR signalling pathways (16). However, there are very few clinical reports on the relationship among PI3K/AKT/mTOR signal pathway and leukemia. Therefore, in the current investigation, in vitro effects of SAC were tested by analysing the expression of genes related with apoptosis and PI3K/AKT/mTOR signal pathway in leukemia cell lines, HL-60, and K562.

MATERIALS AND METHODS

Cell culture

HL-60 and K562 cancer cell lines acquired through the İzmir Institute of Technology. The cells were cultivated in RPMI 1640 medium (Life Technologies) complemented with 1% penicillin-streptomycin (100 U/ml) (Life Technologies), 10% heat inactivated fetal bovine serum (Life Technologies) in a humidified atmosphere containing 5% CO_2 at 37ºC.

Preparation of SAC

SAC was obtained from LKT Laboratories, Inc (lot number 295231). SAC was prepared freshly in water.

Cell proliferation assays

The HL-60 and K62 cells were treated with 1, 2.5, 5, 7.5 or 10 mM of SAC, and were incubated

for 24 h. The cell viability of HL-60 and K62 cells were determined via a MTT (3- (4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay in 96-well plates as we previously described in detail (17). Absorbance was measured at 570 nm.

RNA isolation and cDNA synthesis

Prior to RNA isolation, HL-60 and K562 cells were grown for 24 h and then, cultured alone, with certain concentrations of SAC for 24 h. As a control, only DMSO was supplemented as vehicle. RNA isolation was carried out using a PureLinkTM Mini Kit (Life Technologies, Carlsbad, CA, USA) using manufacturer's instructions and estimated using a Nanodrop 2000 spectrophotometer (Thermo Scientific). cDNA synthesis was performed from total RNA using the High Capacity cDNA reverse Transcription Kit (Applied Biosystems, Foster City, CA) using the manufacturer's protocol and diluted with RNase-free water at 42ºC for 1 h.

Gene expression analysis by qRT-PCR

Gene expression analysis by qRT-PCR was examined by TaqMan gene expression assays to quantify Bax, Bcl-2, and caspase 3 as described by the manufacturer (Applied Biosystems, Foster City, CA) **(Table 1)**.

Table 1: IDs for TagMan® gene expression assay

Target gene	Assay ID
Bax	Hs00180269_m1 FAM-MGB
Bcl-2	Hs00608023_m1 FAM-MGB
Caspase 3	Hs00234387_m1 FAM-MGB
mTOR	Hs00234522_m1 (FAM-MGB)
PI3KCA	Hs00907957_m1 (FAM-MGB)
AKT1	Hs00178289_m1 (FAM-MGB)

The PCR mixture contained diluted cDNA, TaqMan Master Mix (Applied Biosystems) and gene-specific TaqMan Gene Expression Assay in a final volume. RT-qPCR was assayed using 7500 Fast RT-PCR (Applied Biosystems). Raw data were evaluated by the 7500 System Sequence Detection Software (Applied Biosystems).

Ethical Committe

This study does not require ethical approval because of the research project uses commercial cell lines samples.

Statistical Analysis

The SPSS 18.0 program was used for statistical analysis. Data were analyzed by one way analysis of variance (ANOVA) test followed by Tukey post hoc tests. Data are reported as mean, standard deviation (mean ± SD) and $p<0.05$ was accepted as significant.

RESULTS

In order to find out IC50 value of SAC in HL-60 and K562 cell lines, we performed an MTT assay. The cells were exposed to different concentrations of SAC for 24 hours **(Table 2, Table 3)**. The IC50 values of SAC in HL60 and K562 cell lines were found 11.525 mM and 10.025 mM, respectively **(Table 4)**. Therefore, it is important to point out that increasing SAC concentration has a cytotoxic effect on HL-60 and K562 cell lines.

Table 2: Effects of SAC on the viability of HL-60 cell line. Cells were treated 24 h in the presence of SAC

SAC (mM)	0	1	2.5	5	7.5	10
%Viability	100	88.5	78.5	63.6*	56.6**	51.6**,#
SD	0.085	0.232	0.188	0.294	0.173	0.166

Table 3: Effects of SAC on the viability of K562 line. Cells were treated 24 h in the presence of SAC

SAC (mM)	0	1	2.5	5	7.5	10
%Viability	100	77.3	72.7	62.1	52.5	47.5*
SD	0.094	0.177	0.154	0.196	0.289	0.231

Table 4: IC_{50} values of SAC on HL-60 and K562 cell lines

	HL-60 (IC_{50} mM)	K562 (IC_{50} mM)
SAC	11.525	10.025

In here, we have performed gene expression analysis under different concentration of the SAC (5 mM, 10 mM, 20 mM) for 24 hours so as to investigate whether apoptotic gene expressions change or not. Compare to the control, Bax expression level as a pro-apoptotic gene was increased in application of different SAC concentrations. However, only 5mM and 10 mM SAC application were shown significant increase in HL-60 cell line as compared with the control for Bax gene expression ($p=0.027$, $p=0.000$).

20 mM SAC application has significant decrease Bax gene expression compared to 10 mM SAC treated group ($p=0.000$). Also, an increase in the expression level of caspase 3 which is an another proapoptotic gene was observed in HL-60 cells under 10 mM SAC concentrati-

ons (p=0.000). Meanwhile, the changes between 5mM and 10mM SAC application in HL-60 cell line for caspase 3 gene expression were also significant when compared to control cell (p=0.020). On the other hand, the expression of Bcl-2 also showed a significant increase in HL-60 cells treated with 10 and 20 mM of SAC (p=0.000, p=0.000) **(Table 5)**.

Table 5: Effect of SAC complex on expression levels of Bax, Bcl-2, caspase 3, mTOR, PI3K and AKT genes in HL-60 cells

	Control	5 mM	10 mM	20 mM
Bax	1.0067±0.14	1.62±0.24*,###	2.6667±0.55**	1.4517±0.3###
Bcl-2	1.0100±0.14	2.2850±0.56	4.58±1.39***	3.8267±0.95***,#
Caspase 3	1.0283±0.25	1. 5483±0. 51##	2.51±0.82***	1.7967±0.28
mTOR	1.0697±0.41	2.8672±1.68**,	1.9252±0.66	0.5906±0.12#
PI3K	1.0011±0.05	1.9515±0.46###	3.6840±1.01***	2.7358±0.58
AKT	1.0069±0.12	1.2974±0.27###	2.2398±0.47***	1.1941±0.30###

Next, we have explored whether different concentrations of SAC administration in HL-60 cell line has an effect on mTOR signaling pathway or not. For this reason, we have performed gene expression analysis for mTOR and its upstream activators AKT and PI3K for 24 hours of SAC applications. Our results were demonstrated that mTOR gene expression level have significantly increased in 5mM SAC application in 24 hours when compared with control (p=0.000).

However, although the gene expression level of mTOR was decreased under 20mM SAC treated group as compare to the 5mM SAC treated group, this decreasing trend was not significant when compare with 10mM SAC treated group.

Meantime, although the gene expression level of PI3K was elevated in all three SAC concentrations, 10 mM and 20 mM SAC treated groups showed significant increase in PI3K gene expression when compare with control (p=0.000, p=0.001). The change in the PI3K gene expression levels between 5 mM and 10 mM SAC administrations, but not 20 mM, was also significant as compare to 10mM SAC group. However, only 10 mM SAC administration was shown significant increase in AKT gene expression level as compare to the control (p=0.000). Besides, the differences of the gene expression level of AKT between 5 mM and 20 mM SAC administration were significant when compare to 10 mM SAC (p=0.000, p=0.000) (Table 5). In this study, we have explored the effects of SAC on apoptotic gene expressions in K562. After cytotoxicity analysis, we have determined to use 3.75 mM, 7.5 mM, and 15 mM of SAC for qRT-PCR. Gene expression analysis revealed that Bax expression level was dramatically decreased under all SAC administration in K562 cell line. These reductions were significant as compare to control gene expression level. In addition to Bax gene expression, caspase 3 expression levels as a pro-apoptotic gene was also significantly decreased for all concentrations of SAC. Notably, while pro-apoptotic gene expressions were declined, we expected to see an increase in Bcl-2 as an anti-apoptotic gene expression for SAC administrations. However, the gene expression levels of Bcl-2 under different SAC concentrations were significantly diminished compare with the control gene expression. We conclude that SAC might prefer another cell death mechanism instead of inducing apoptotic cell death in the K562 cell line **(Table 6)**.

Table 6: Effect of SAC complex on expression levels of Bax, Bcl-2, caspase 3, mTOR, PI3K and AKT genes in K562 cells

	Control	3.75 mM	7.5 mM	15 mM
Bax	1.041±0.32	0.251±0.02*	0.115±0.07*	0.186±0.11*
Bcl-2	1.003±0.279	0.056±0.01*	0.0166±0.03*	0,0602±0.02*
Caspase 3	1.056±0.38	0.318±0.28*	0.2021±0.15*	0.3026±0.17*
mTOR	1.0182±0.07	0.4544±0.23*	0.2057±0.25*,###	0.2264±0.48*,##
PI3K	0.9370±0.14	0.2489±0.13*	0.998±0.035*,#	0.4243±0.31*,**
AKT	1.0188±0.21	0.2635±0.11*	0.0905±0.021*,#	0.0738±0.02*,#

Our previous results demonstrated that SAC administration may trigger different cell death mechanism in K562 cell line. Therefore, we asked the question about whether autophagy plays a role in SAC-induced cell death in K562 cell line or not. For this reason, we have explored the gene expression level of mTOR, and its upstream activators PI3K and AKT. The gene expression analysis revealed that mTOR expression levels were decreased under different SAC concentrations. These reductions in treated groups were statistically significant as compare to control group gene expression (p=0.000, p=0.000, p=0.000). Moreover, the decrease of mTOR gene expression level under 7.5 mM and 15 mM SAC administration was also significant compare with 3.75 mM administration, (p=0.001, p=0.002). Similarly, AKT gene expression level was gradually diminished by increasing SAC concentration and these reductions were statistically significant as compare to the control group (p=0.000, p=0.000, p=0.000). Both 7.5 mM and 15 mM SAC administrations in K562 cell line were shown significant reductions

compare with the 3.5mM SAC administration for AKT gene expression level (p=0.032, p=0.037). On the other hand, unlike mTOR and AKT gene expressions under 7.5 mM SAC administration, PI3K expression level was slightly increased (p=0.000). However, both 3.75 mM and 15 mM SAC applications were demonstrated significant reductions as compare to the control gene expression in K562 cell line (p=0.000, p=0.000). We concluded that reduction of mTOR gene expression levels under different SAC administrations may stimulate autophagy as a cell death mechanism in K562 cell line.

DISCUSSION

The high toxicity of anticancer drugs, accepted in first medical line treatment of cells and normal tissues, is an almost unsurpassable barrier for cancer cure. At the same time, substances derived from diverse various plants with elicit little or no side effects have been considered as a possible option or adjunctive therapy for cancer patients (18). SAC, a water-soluble organosulfur compound derived from garlic, has been revealed to have anticancer properties in various human malignancies (19). Whereas, the anti-cancer effect of SAC in the cure of leukemia stays unclear. In this study, we determined the effect of SAC on Bax, Bcl-2, caspase 3, mTOR, AKT, and PI3K gene expression levels in HL-60 and K562 cells.

SAC, which showed low toxicity and high anti-cancer activity, was explored in various cancer cell lines (7). Earlier investigations showed that SAC had both anticancer and antioxidant activity at a similar dose range in mM (8). Other studies indicating that SAC does not prevent breast cancer cell growth have used shorter treatment times (48 hours) and low SAC doses (up to 1 mM) (20). On the other hand, in different investigations, comparable doses of SAC did have antiproliferative effects on prostate, neuroblastoma, and melanoma carcinoma cells. For this reason, SAC can be influential like an antiproliferative compound in certain cell lines and/or under certain conditions (21). In this research we explored the anticancer potential of SAC against HL-60 and K562 cells. SAC reduced the viability of K562 and HL-60 cells in a dose-related manner and exhibited an IC50 of 11.525 mM and 10.025 mM, respectively. In general, cancer development results from abnormalities in apoptosis and aberrant cell proliferation (16). Stimulating apoptotic cell death is an effective approach to treat cancer. The apoptotic cell death can be initiated by extrinsic (death ligand) or intrinsic (mitochondrial) pathways (9). Apoptosis is a cell suicide process that kills cancer cells and causes minimal damage to nearby cells. Apoptotic cell death is controlled by the Bcl-2 family of proteins i.e. Mcl-1, Bcl-2 and Bcl-XL which are anti-apoptotic proteins. These proteins regulate apoptotic events. Other members of the Bcl-2 protein family are Bax, Bak, and Ki-67. Bax is a pro-apoptotic protein that is found in the cytosol and moves to the mitochondrial membrane upon stimulation of apoptosis (22).

The elevated ratio of Bax/Bcl-2 leads to impaired mitochondrial transmembrane permeabilization, which causes mitochondrial dysfunction and triggers apoptosis (Sun et al. 2013). This pathway includes involves the release of pro-apoptotic cytochrome c from the mitochondrial compartment into the cytosol, which triggers the caspase cascade (23). Both extrinsic and intrinsic pathways cause activation of caspase 3 in the apoptotic cell (24). For this reason, the activity of caspase 3 is regarded as a suitable measurement of cytotoxic sensitivity.

Numerous researchers have revealed that the anticancer effects of certain chemotherapeutic mediators play a role in inducing apoptosis, which has become the main mechanism for effective anticancer therapy (9). The mechanisms leading to the stimulation of apoptotic cell death by garlic derivatives have been defined in a number of cancer cell lines (25). Recent reports have demonstrated that levels of pro-apoptotic Bax expression are increased, in PC-3 cells following SAC administration, whereas levels of anti-apoptotic Bcl-2 expression are decreased. (24). In our study, Bax gene expression showed increased levels in 5 and 10 mM of SAC treated cells in HL-60 cells. However, treatment of cells with 10 mM of SAC induced expression of Bax gene, compared with the other treated groups. On the other hand, we found that 10 mM SAC induced caspase 3 expression level in HL-60 cells as compared with the control and 5 mM SAC

treated cells. Enhanced induction of Bax gene expression may initiate apoptosis from the mitochondria of the HL-60 cells upon SAC administration. This may be induce the mitochondrial release of cytochrome c, which stimulates the caspase cascade pathway. The increased caspase 3 activity by SAC treatment also supports the apoptosis hypothesis. For this reason, our findings suggest that SAC-induced apoptosis may be mediated by mitochondrial pathways in HL-60 cells. Contrary to expectations, SAC treatment also led to increased expression of Bcl-2. Taken together with other study, these outcomes suggest that the increase in Bcl-2 gene expression levels may be responsible for cellular senescence and cell cycle arrest at G1 phase mediated by p27Kip1 (26, 27). Whereas K562 cells revealed low gene expression of caspase 3, Bax and Bcl-2 in SAC treated cells when compared to control. In this study, we found that SAC could not induce apoptosis. On the other hand, it has been suggested that the absence of caspase 3 and Bax expression in K562 cells may induce resistance to apoptosis triggered by SAC. Bcl-2 was associated with better survival (28), but we also identified deterioration in Bcl-2 levels. SAC administration decreased Bcl-2 expression, and this finding suggests that K562 cells might develop resistance to apoptotic signals.

The mammalian target of rapamycin (mTOR) kinase has recently emerged as a promising therapeutic target. mTOR is a serine-threonine kinase that regulates cell proliferation and cell growth (29). Disturbance in the mTOR signalling pathway is one of the most common pathologic changes in human cancers (14). Upregulation of the mTOR pathway has been reported in many oncogenic disorders, including acute and chronic leukemias. mTOR activation is usually induced by PI3K/AKT (29). PI3K is an intracellular protein kinase that plays a role in cell proliferation and differentiation, apoptosis, glucose transport, and tumor development by activating Akt. When mTOR is mutated or overexpressed, normal cell growth becomes uncontrolled resulting in cellular transformation and tumor progression. The activation of PI3K can activate mTOR, leading to rapid proliferation of tumor cells, increasing cancer protein secretion, speeding up the cell cycle, and reducing the G1 phase process (30). Moreover, SAC significantly suppresses the phosphorylation of AKT, the mammalian target of rapamycin in tumour tissues (24). Here we show that SAC has important cytotoxic activity on human leukemia cell lines. Contrary to expectations, it was determined that the expression of genes involved in the PI3K/AKT/mTOR signalling pathway in HL-60 cells was increased by SAC administration.

This indicates that the effect of herbal active ingredients on cancer cells may be related to the dose. The increased apoptosis in HL-60 cells suggests that pharmacological inhibition of autophagy may increase apoptosis. PI3K, AKT and mTOR gene expression in K562 cells were down regulated in response to treatment with SAC. On the other hand, autophagy is a process that suppresses tumor formation, and induction of autophagic pathway can lead to cell death in apoptosis-resistant cancer (11). Relying on this information, the reduced apoptotic protein level identified in K562 cells displays that there might be resistance to apoptosis, and consequently the autophagic pathway is induced by SAC. In this study, the inhibition of mTOR by SAC was able to induce autophagy in K562 cells. Taken together, this data demonstrates the new and necessary role of mTOR, AKT, and PIK3 signalling in the cell death mechanism of SAC's K562 cell lines.

These observations demonstrate that new treatment approaches may be developed for human cancers targeting the PI3K/AKT/mTOR signalling pathway. Herein the cytotoxic activities, apoptotic, and autophagic effects of SAC were investigated. We have demonstrated the original exploration that SAC induced cell death in HL-60 cell line involved with the modulation of apoptotic genes expression levels. To reveal whether SAC triggers autophagy, we evaluated the expression of the PI3K, AKT, and mTOR genes, and observed that SAC down-regulated the expression of the PI3K, AKT, and mTOR genes in K562 cells. Although more potent investigations researchs are warranted, the present outcomes supply the basis for the use of SAC as a novel, hopeful target in the search to cure leukemia.

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