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Opium induces apoptosis in Jurkat cells via promotion of pro-apoptotic and inhibition of anti-apoptotic molecules

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ABSTRACT

Objective(s): The aim of this study was to determine the important molecules involved in apoptosis induction by opium in Jurkat cell line.
Materials and Methods: Jurkat cells were incubated 48 hrs with 2.86×10^{-5} g/ml concentration of opium and apoptosis as well as expression levels of related molecules were measured.
Results: Our results demonstrated that 50.3±0.2 percent of opium treated Jurkat cells were revealed apoptotic features. The levels of mRNA of several pro-apoptotic and anti-apoptotic molecules were increased and decreased, respectively, in the opium treated cells. The results also demonstrated that expression levels of BCL2, DFFA and NOL3 as anti-apoptotic molecules were increased in the opium treated cells.
Conclusion: It seems that opium induces apoptosis in Jurkat cells via both intrinsic and extrinsic pathways. Although opium induces apoptosis in the cells but increased expression of some anti-apoptotic molecules may be a normal resistance of the cell for death.

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Introduction

Apoptosis or programmed cell death is a normal process to remove candidate cells for death (1). Accordingly, this is a physiological process for progression and homeostasis of human systems such as immune and nervous systems (2). Previous investigations revealed that several endogenous and exogenous agents can induce apoptosis (3, 4). Alkaloids (morphine, heroine and codeine) are the important exogenous agents which induce apoptosis (5). There are more than 20 alkaloids (5) and more than 70 components (6) in opium, thus, its effect on the cells could be different from its derivatives. Our previous studies demonstrated that chronic opium treatment can induce brain and liver cell apoptosis in rats (7) and apoptosis in Jurkat cells (8). Additionally, we have reported that opium can decreased mean number of peripheral white blood cells in animal models (9). Moreover, it has been demonstrated that opium addicted individuals suffer from an attenuated immune responses and repeated infectious diseases (10, 11). Therefore, it appears that opium can induce apoptosis in immune cells via unknown pathway. Jurkat cells are lymphocyte cancer cell lines which are used in several malignancies and immune responses based studies (12). Due to the fact that the main molecules and pathways which are involved in the apoptosis induced by opium are yet to be identified and based on the fact that opium can induce apoptosis in Jurkat cells (8), hence, the main aim of this study was to evaluate expression of reference genes involved in the apoptosis pathways using Real-Time PCR Array technique.

Materials and Methods

Cell culture, opium treatment and cell survival analysis

Cell culture condition has been described in our previous study (8). Briefly, Jurkat cells were prepared from Pasture Institute of Iran and were cultured in RPMI1640 culture medium (Invitrogen Co. Germany) in standard condition. Opium dedication, its origin, components (using GC-mass spectrometry) and appropriate dilution for inducing apoptosis have also been described previously (8, 11). According to our previous study which demonstrated that, opium dilution at concentration of 2.86×10^{-5} and after 48 hr were the optimum concentration and time of culture

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for induction of apoptosis in Jurkat cells, respectively, hence Jurkat cells were treatment at the concentration and incubation period. The apoptotic changes were evaluated using an annexin-V and propidium iodide (PI) commercial kit (Invitrogen, USA) according to the manufacturer's guidelines. Briefly, the washed jurkat cells (~5 × 10^5 cells) were resuspended in 100 µl 1X annexin-binding buffer and then 5 µl annexin V and the 5 µl PI were added to each 100 µl of cell suspension. The cells mixed gently and then incubated in a dark place at room temperature for 10 min. After incubation period, apoptotic cells were quantified by the BD flow cytometry system.

RNA extraction, reverse transcription and quantitative real-time PCR

Total RNA was purified from opium treated and untreated Jurkat cells using RNX quality extraction kit (Cinnaclon Co, Tehran-Iran). The RNA quality was measured on 260/280 nm by spectrophotometer. RNA were convert to cDNA using a commercial cDNA synthesis kit and oligo(dT) primers from Parstous Company, Tehran-Iran, which is described in our previous study (13). Expression levels of ABL1, AKT1, APAF1, BAD, BAG1, BAG3, BAG4, BAK1, BAX, BCL10, BCL2, BCL2A1, BCL2L1, BCL2L10, BCL2L11, BCL2L2, BCLAF1, BFAR, BID, BIK, NAIP, BIRC2, BIRC3, XIAP, BIRC6, BIRC8, BNIP1, BNIP2, BNIP3, BNIP3L, BRAF, NOD1, CARD6, CARD8, CASP1, CASP10, CASP14, CASP2, CASP3, CASP4, CASP5, CASP6, CASP7, CASP8, CASP9, CD40, CD40LG, CFLAR, CIDEA, CIDEB, CRADD, DAPK1, DFFA, FADD, FAS, FASLG, GADD45A, HRK, IGF1R, LTA, LTBR, MCL1, NOL3, PYCARD, RIPK2, TNF, TNFRSF10A, TNFRSF10B, TNFRSF11B, TNFRSF1A, TNFRSF21, TNFRSF25, CD27, TNFRSF9, TNFSF10, CD70, TNFSF8, TP53, TP53BP2, TP73, TRADD, TRAF2, TRAF3 and TRAF4 were measured by quantitative Real-Time PCR Array technique using a commercial kit (SABiosciences company, USA). The full names of the genes are defined in the Table 1. The levels of mRNA of B2M, HPRT1, RPL13A, GAPDH, ACTB and HGDC have been evaluated as housekeeping genes and were used for normalization of the results. Bio-Rad CFX96 instrument have been used for running the PCR array plates. Raw data were analyzed using RT^2 Profiler PCR Array Data Analysis software version 3.5.

Results

The results showed that 50.3 ± 0.2 percent of Jurkat cells were shown apoptotic features after 48 hrs incubation with 2.86×10^{-5} g/ml opium concentration ($P< 0.001$) (Figure 1).

Our results demonstrated that expression levels of ABL1, BCL2, BNIP3, NOD1, CASP3, CASP4, CASP6, DFFA, LTBR, NOL3, TNFRSF1A and TP53BP2 were increased more than two folds, while, mRNA levels of BCLAF1, CASP9, RIPK2 and CD27 were decreased more than two folds (Table 1 and Figure 2). The expressions of other molecules were not changed after treatment with opium (Table 1 and Figure 1).

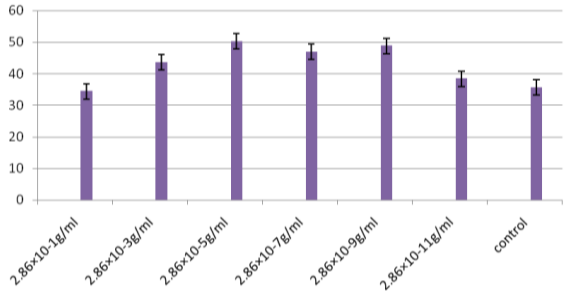
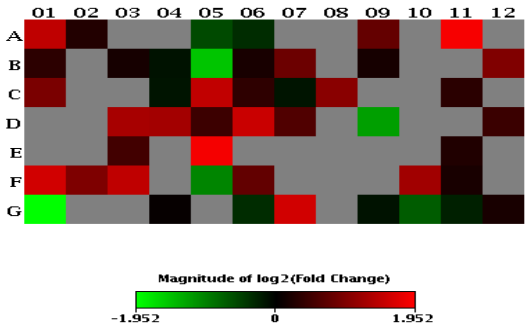


Figure 1. The percent of Jurkat cells apoptosis at different concentrations of opium after 48 hrs incubation. The Figure illustrates that 50.3 ± 0.2 percent of Jurkat cells illustrated apoptotic features in opium with 2.86× 10 -5 g/ml concentration ($P< 0.001$)



	1	2	3	4	5	6	7	8	9	10	11	12
A	ABL1	AKT1	APAF1	BAD	BAG1	BAG3	BAG4	BAK1	BAX	BCL10	BCL2	BCL2A1
B	BCL2L1	BCL2L10	BCL2L11	BCL2L2	BCLAF1	BFAR	BID	BIK	NAIP	BIRC2	BIRC3	XIAP
C	BIRC6	BIRC8	BNIP1	BNIP2	BNIP3	BNIP3L	BRAF	NOD1	CARD6	CARD8	CASP1	CASP10
D	CASP14	CASP2	CASP3	CASP4	CASP5	CASP6	CASP7	CASP8	CASP9	CD40	CD40LG	CFLAR
E	CIDEA	CIDEB	CRADD	DAPK1	DFFA	FADD	FAS	FASLG	GADD45A	HRK	IGF1R	LTA
F	LTBR	MCL1	NOL3	PYCARD	RIPK2	TNF	TNFRSF10A	TNFRSF10B	TNFRSF11B	TNFRSF1A	TNFRSF21	TNFRSF25
G	CD27	TNFRSF9	TNFSF10	CD70	TNFSF8	TP53	TP53BP2	TP73	TRADD	TRAF2	TRAF3	TRAF4

Figure 2. The Figure showed relative expression levels of evaluated molecules which participate in apoptosis in opium treated Jurkat cells in comparison to no treated cells. Red and green colors demonstrated increase and decrease, respectively, expression of the molecules

Table 1. Relative expression levels of apoptosis related genes in opium treated Jurkat cells in comparison to untreated cells

Gene symbol	Gene description	Fold Regulation	Comments
ABL1	Abelson murine leukemia viral oncogene homolog 1	2.779	B
AKT1	V-akt murine thymoma viral oncogene homolog	1.1942	B
APAF1	Apoptotic protease activating factor 1	-1.0559	C
BAD	Bcl-2-associated death promoter	-1.0559	C
BAG1	BCL2-associated athanogene 1	-1.4775	B
BAG3	BCL2-associated athanogene 3	-1.2643	B
BAG4	BCL2-associated athanogene 4	-1.0559	C
BAK1	Bcl-2 homologous antagonist/killer	-1.0559	C
BAX	Bcl-2-associated X protein	1.6994	B
BCL10	B-cell CLL/lymphoma 10	-1.0559	C
BCL2	B-cell CLL/lymphoma 2	3.7425	B
BCL2A1	BCL2-related protein A1	-1.0559	C
BCL2L1	BCL2-like 1	1.2633	B
BCL2L10	BCL2-like 10	-1.0559	C
BCL2L11	BCL2-like 11	1.1283	B
BCL2L2	BCL2-like 2	-1.0965	B
BCLAF1	Bcl-2-associated transcription factor 1	-2.8543	B
BFAR	Bifunctional apoptosis regulator	1.1417	B
BID	BH3 interacting-domain death agonist	1.7735	B
BIK	Bcl-2-interacting killer	-1.0559	C
NAIP	NLR family, apoptosis inhibitory protein	1.1244	B
BIRC2	Baculoviral IAP repeat containing 2	-1.0559	C
BIRC3	Baculoviral IAP repeat containing 3	-1.0559	C
XIAP	X-linked inhibitor of apoptosis	1.9629	B
BIRC6	Baculoviral IAP repeat containing 6	1.9136	B
BIRC8	Baculoviral IAP repeat containing 8	-1.0559	C
BNIP1	BCL2/adenovirus E1B 19kDa interacting protein 1	-1.0559	C
BNIP2	BCL2/adenovirus E1B 19kDa interacting protein 2	-1.1135	B
BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	2.7924	B
BNIP3L		1.2793	B
BRAF	V-raf murine sarcoma viral oncogene homolog B1	-1.1081	B
NOD1	Nucleotide-binding oligomerization domain-containing protein 1	2.0889	B
CARD6	Caspase-associated recruitment domain 6	-1.0559	C
CARD8	Caspase-associated recruitment domain 8	-1.0559	C
CASP1	Caspase 1, apoptosis-related cysteine peptidase	1.2434	B
CASP10	Caspase 10, apoptosis-related cysteine peptidase	-1.0559	C
CASP14	Caspase 14, apoptosis-related cysteine peptidase	-1.0559	C
CASP2	Caspase 2, apoptosis-related cysteine peptidase	-1.0559	C
CASP3	Caspase 3, apoptosis-related cysteine peptidase	2.447	B
CASP4	Caspase 4, apoptosis-related cysteine peptidase	2.3947	B
CASP5	Caspase 5, apoptosis-related cysteine peptidase	1.3686	B
CASP6	Caspase 6, apoptosis-related cysteine peptidase	2.914	B
CASP7	Caspase 7, apoptosis-related cysteine peptidase	1.5241	B
CASP8	Caspase 8, apoptosis-related cysteine peptidase	-1.0559	C
CASP9	Caspase 9, apoptosis-related cysteine peptidase	-2.3438	B
CD40	CD40 molecule	-1.0559	C
CD40LG	CD40 ligand	-1.0559	C
CFLAR	Caspase 8 and FADD-like apoptosis regulator	1.362	B
CIDEA	Cell death activator CIDE-A	-1.0559	C
CIDEB	Cell death activator CIDE-B	-1.0559	C
CRADD	Death domain-containing protein CRADD	1.4186	B

DAPK1	Death-associated protein kinase 1	-1.0559	C
DFFA	DNA fragmentation factor subunit alpha	3.696	B
FADD	Fas-Associated protein with Death Domain	-1.0559	C
FAS	Fas (TNF receptor superfamily, member 6)	-1.0559	C
FASLG	Fas ligand	-1.0559	C
GADD45A	Growth arrest and DNA-damage-inducible protein GADD45 alpha	-1.0559	C
HRK	Activator of apoptosis harakiri	-1.0559	C
IGF1R	Insulin-like growth factor 1 receptor	1.1879	B
LTA	Lymphotoxin alpha	-1.0559	C
LTBR	Lymphotoxin beta receptor	3.0613	B
MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1.9438	A
NOL3	Nucleolar protein 3	2.7685	B
PYCARD	Apoptosis-associated speck-like protein containing a CARD or ASC	-1.0559	C
RIPK2	Receptor-interacting serine/threonine-protein kinase 2	-2.0398	B
TNF	Tumor necrosis factor	1.675	B
TNFRSF10A	Tumor necrosis factor receptor superfamily, member 10a	-1.0559	C
TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b	-1.0559	C
TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b	-1.0559	C
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1a	2.3709	B
TNFRSF21	Tumor necrosis factor receptor superfamily, member 21	1.1407	B
TNFRSF25	Tumor necrosis factor receptor superfamily, member 25	-1.0559	C
CD27	CD27 molecule	-3.8684	B
TNFRSF9	Tumor necrosis factor receptor superfamily, member 9	-1.0559	C
TNFSF10	Tumor necrosis factor superfamily, 10	-1.0559	C
CD70	CD70 molecule	1.0352	B
TNFSF8	Tumor necrosis factor superfamily, 8	-1.0559	C
TP53	Tumor protein p53	-1.2672	B
TP53BP2	Tumor suppressor p53-binding protein 2	3.059	B
TP73	Tumor protein p73	-1.0559	C
TRADD	Tumor necrosis factor receptor type 1-associated DEATH domain	-1.1042	B
TRAF2	TNF receptor-associated factor 2	-1.6347	B
TRAF3	TNF receptor-associated factor 3	-1.1836	B
TRAF4	TNF receptor-associated factor 4	1.1343	B

A: Although, expression levels of the gene is increased in opium treated Jurkat cells in comparison to untreated cells, but the difference is not significant

B: The differences between groups are significant (Negative: decreased, Positive: Increased)

C: Although, expression levels of the gene is decreased in opium treated Jurkat cells in comparison to untreated cells, but the difference is not significant

Discussion

Our previous studies demonstrated that opium induced apoptosis in *in vivo* and *in vitro* models (8, 11). The results of current study also demonstrated that apoptosis were increased in opium treated Jurkat cells. The results revealed that expression levels of pro-apoptotic molecules including ABL1 have been increased in the opium treated Jurkat cells. Previous studies demonstrated that ABL1 is a cytoplasmic and nuclear protein tyrosine kinase which participates in several cell functions including differentiation, division, adhesion and stress response (14). Based on the fact that differentiation is decreased in cancer cells, hence, according to our results it appears that opium components may increase differentiation and reduce immortal properties of cells. The results also demonstrated that mRNA levels of BNIP3, NOD1, LTA, TNFRSF1A and TP53BP2, as pro-apoptotic molecules, were increased more than two folds in opium treated Jurkat cells. BNIP3 is a mitochondrial protein and results in apoptosis, even in the presence of BCL2. Our results showed that mRNA levels of BCL2, as anti-apoptotic molecule, were also increased in the opium treated cells. According to the results it appears that increased expression of BNIP3 overcome BCL2 and induced apoptosis in the opium treated cells. NOD1 is an inflammasome which participates in induction of apoptosis via activation

of NF-κB (15). Based on the fact that NODs have a LRR (Leucine-rich repeated) domain it appears that some opium derivatives may directly or via unknown intermediate molecules were recognized by NOD1 to induce apoptosis in Jurkat cells. LTBR is an receptor for pro-apoptotic molecule, lymphotoxin (LTA), which produced by lymphocytes and LTBR/LTA interaction induces apoptosis (16). LTBR induces apoptosis via interaction with second mitochondria-derived activator of caspases (SMAC) and consequently activation of caspases (17). Additionally, TNFRSF1A is a subunit of TNF receptor for TNF-α which in association with TNFRSF1B plays key roles in stimulation of apoptosis through TNFR1 (18). Moreover, it has been reported that LTA can use TNFR1 to induce apoptosis (16). Therefore, it appears that the LTA/LTBR, LTA/TNFR1 and TNF/TNFR1 pathways play crucial roles in induction of opium induced apoptosis in the Jurkat cell line. Interestingly, mRNA levels of TP53, a tumor suppressor molecule, were not changed after opium treatment but TP53BP2 were increased (Table1). This molecule is a binding protein which interacts with TP53 and multiplied function of this molecule and, hence, induces apoptosis (19). TP53BP2 promotes transcription of pro-apoptotic molecules via enhances the DNA binding and trans activation function of TP53 on the promoter region of the related genes (20). Therefore, it appears that opium increased apoptotic features of TP53 via promotion of its function rather than its expression. Additionally, expression levels of CASP3, CASP4 and CASP6 were also increased after opium treatment (Table1). These molecules are the intermediated pro-apoptotic molecules which mediate apoptosis via intrinsic and extrinsic pathways in a common manner (21). Based on the fact that mRNA levels of CASP9 were decreased more than two folds in the opium treated cells, hence, it seems that opium mainly induces apoptosis via extrinsic pathway, which is confirmed by increased expression of LTA and TNFRSF1A molecules. In contrast with apoptotic features of opium treated Jurkat cells, mRNA levels of some of the anti-apoptotic molecules, including BCL2, DFFA and NOL3 were increased in the cells. As mentioned in the previous sentences, BNIP3 can induce apoptosis even in the presence of anti-apoptotic molecules such as BCL2. Based on the fact that extrinsic pathway may be the main route for induction of apoptosis in the opium treated cells, it seems that increased expression of NOL3 is a normal response of the cells to escape from death through suppress function of CASP8, because NOL3 inhibits apoptosis via interaction with CASP8 (22).

The results revealed that three pro-apoptotic molecules including BCLAF1 (a transcriptional repressor factor from Bcl-2 family proteins) CD27 (a member of the TNF-receptor super family) and RIPK2 (containing of a C-terminal caspase recruitment domain (CARD)) were also down-regulated in the opium treated group. Thus, it may be concluded that opium induced apoptosis in BCLAF1, CD27 and RIPK2 independent manner. Although, there are several studies regarding the roles of alkaloids on the apoptosis and also the roles of opium on the induction of apoptosis but its molecular mechanisms are yet to be identified. Our study elucidates some of important pathways which participate in the induction of apoptosis by opium.

Conclusion

Collectively, it seems that opium leads to induction of apoptosis in Jurkat cells via both intrinsic and extrinsic pathways. Additionally, although opium induces apoptosis in the cells but elevated expression of some anti-apoptotic molecules may be a normal resistance of the cell for death.

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