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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**Keywords:** Apoptosis, Jurkat cells, PCR array

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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.

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**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 Pasteure 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 x 10^{-5} and after 48 hr were the optimum concentration and time of culture...
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, BCL2, BCL2L1, BCL2L10, BCL2L11, BCL2L12, BCLAF1, BFAQ, BID, BIK, NAIP, BIRC2, BIRC3, XIAP, BIRC6, BIRC8, BNIP1, BNIP2, BNIP3, BNIP3L, BRF, 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, TNFRSF1A, TNFRSF1B, TNFRSF1F1A, TNFRSF21, TNFRSF25, CD27, TNFRSF9, TNFRSF10, CD70, TNFRSF8, 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 RT² 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 (Pc 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](image1.png)

**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 (Pc 0.001)

![Figure 2](image2.png)

**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

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**Table 1.** The table shows 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.

| Gene  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-------|---|---|---|---|---|---|---|---|---|----|----|----|
| A     | ABL1| ACT1| APAF1| BAD| BAG1| BAG3| BAG4| BAK1| BAX| BCL10| BCL2| BCL2A1|
| B     | BCL2L1| BCL2L10| BCL2L11| BCL2L2| BCLAF1| BFAQ| BID| BIK| NAIP| BIRC2| BIRC3| XIAP|
| C     | BIRC6| BIRC8| BNIP1| BNIP2| BNIP3| BNIP3L| BFAQ| BID| BIK| NAIP| BIRC2| BIRC3|
| 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| TNFRSF1B| TNFRSF1F1A| TNFRSF21| TNFRSF25| CD27|
| G     | TNFRSF9| TNFRSF10| CD70| TNFRSF8| TP53| TP53BP2| TP73| TRADD| TRAF2| TRAF3| TRAF4|

**Table 2.** The table shows the expressions 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.

| Gene  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-------|---|---|---|---|---|---|---|---|---|----|----|----|
| A     | ABL1| ACT1| APAF1| BAD| BAG1| BAG3| BAG4| BAK1| BAX| BCL10| BCL2| BCL2A1|
| B     | BCL2L1| BCL2L10| BCL2L11| BCL2L2| BCLAF1| BFAQ| BID| BIK| NAIP| BIRC2| BIRC3| XIAP|
| C     | BIRC6| BIRC8| BNIP1| BNIP2| BNIP3| BNIP3L| BFAQ| BID| BIK| NAIP| BIRC2| BIRC3|
| 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| TNFRSF1B| TNFRSF1F1A| TNFRSF21| TNFRSF25| CD27|
| G     | TNFRSF9| TNFRSF10| CD70| TNFRSF8| TP53| TP53BP2| TP73| TRADD| TRAF2| TRAF3| TRAF4|

**Table 3.** The table shows the expressions 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 death agonist | -1.4775 | B |
| BAG3        | BCL2-associated death agonist 3 | -1.2643 | B |
| BAG4        | BCL2-associated death agonist 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 |
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 pre-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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