



A review of the relatively complex mechanism of JS-K induced apoptosis in cancer cells

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Abstract: Nitric oxide (NO) works as a signaling molecule, toxicant, and antioxidant in the body's physiological and pathological processes. JS-K is designed to be activated by glutathione-S-transferase (GST) and release NO in a sustained and controlled manner within the tumor cells. JS-K also promotes apoptosis in cancer cells through mitogen-activated protein kinase (MAPK) pathway, ubiquitin-proteasome pathway, cell factor β -catenin/T (TCF) signaling pathway, and other mechanisms. In future studies, we should further develop new NO precursors, so that new drugs in the treatment of cancer can become more efficient, more accurate, and have less adverse reactions.

Keywords: JS-K; cancer; apoptosis

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Introduction

Nitric oxide (NO) works as a signaling molecule, toxicant, and antioxidant during physiological and pathological processes in the human body in addition to other environments. Numerous investigations have revealed that NO is both pro- and anti-tumor depending on its concentration, cell environment, release rate, and source (1,2). Research on apoptosis caused by NO has sharply increased in recent years, and the main focus has been on the development and utilization of NO pro-drug as an anti-tumor drug.

NO initiates apoptosis of tumor cells in prostate cancer, rectal cancer, acute myeloid leukemia (AMEL), and multiple myeloma (3-6). The drug concentration of NO in inducing apoptosis in cancer cells usually restricts its application in clinical practice. Upon comparison with previous NO precursor drugs, JS-K (O₂-(2, 4-dinitrophenyl) 1-[(4-ethoxycarbonyl) piperazin-1-yl] diazen-1-ium-1, 2-diolate) elicits higher cytotoxicity in leukemia cells

and multiple myeloma cells sensitive to conventional therapy as well as in multidrug-resistant multiple myeloma and patient-derived multiple myeloma cells. It is also noteworthy that JS-K can selectively target tumor cells and perform their cytotoxic function. Moreover, in a previous study, the concentration of JS-K did not show any obvious cytotoxicity in peripheral blood mononuclear cells or bone marrow stromal cells but inhibited the proliferation of multiple myeloma cells (7). JS-K, a NO precursor, shows a promising potential application in the treatment of cancer, and thus, in this paper, we have reviewed the likely mechanism of the pro-apoptotic activity of JS-K.

Chemical and biochemical properties of JS-K

JS-K is designed to be activated by glutathione-S-transferase (GST) and release NO in a sustained, and controlled manner within the tumor cells, wherein GST is often overexpressed (8). JS-K is dissoluble in DMSO (dimethyl sulfoxide) and stable in aqueous solvents without

GSH, its hydrolysis rate is $1 \times 10^{-6}/s$, and it has a half-life period of over 1 week (9). Moreover, JS-K is almost dissoluble in environments where GSH is present. It has a secondary reaction rate of $1.0 \text{ mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$ (9), and its IC_{50} range is about 0.2~1.2 μM . During the reaction of JS-K releasing NO activated by GST, the catalytic activity of μ type of isomers (GSTM) is almost 100 times larger than that of π type isomers (GSTP) (9,10).

JS-K regulates the initial stages and progression of cancer

JS-K regulates cancer cells mainly by releasing NO. JS-K regulates the initial stages and progression of cancer cells depending on the release rate and the concentration of NO (11). One study (12) showed the relation between apoptosis and ubiquitin-proteasome pathway in cancer cells treated by JS-K and JS-43-126 (analog of JS-K, which do not release NO); they found that JS-43-126 could not induce apoptosis just like the 0 μM JS-K. Also, JS-K showed the ability to inhibit the invasion of basement membrane in breast cancer cells, but the same phenomenon was not demonstrated in JS-43-126 (13). Another study (14) found that JS-K also induces apoptosis in breast cancer cells but that JS-43-126 does not. Additionally, JS-K works as an arylating agent and might induce apoptosis in cancer cells through arylation of the glutathione (GSH) and sulfhydryl groups (15). In conclusion, JS-K performs a pro-apoptotic function through NO release and/or the ability of arylation.

The complex mechanisms through which JS-K promotes apoptosis in cancer cells

JS-K induces apoptosis in cancer cells through activating the mitogen-activated protein kinase (MAPK) pathway and caspase cascade reaction. A large quantity of research has shown that JS-K can inhibit proliferation and induce apoptosis in various cancer cells in a concentration-dependent manner as was observed in leukemia cells, prostate cancer cells, liver cancer cells, multiple myeloma cells, and non-small cell lung cancer cells, both *in vitro* and *in vivo* (7,9,11,16-18).

JS-K was also found to induce apoptosis with the concomitant activation of the MAPK members, ERK, JNK, and p38, along with their downstream effectors, c-Jun and AP-1; meanwhile, JS-K was antagonized by using NO inhibitor. These results indicate that the possible mechanisms for the apoptosis-induced effects of JS-K are

NO-induction of MAPK pathway or possible arylation reactions (18). As a member of the MAPK pathway, the SAPK/JNK pathway is associated with apoptosis induced by JS-K in NSCLC cells. Oxidative/nitrosative stress and DNA damage have been observed in NSCLC cells treated by JS-K, while oxidative stress and DNA damage have been widely observed to activate a SAPK/JNK pathway and its downstream effectors, transcription factors ATF2 and c-jun, with rapid phosphorylation (19). Another investigation (20) showed that JS-K causes phosphorylation of p38, SAPK/JNK and their downstream targets ATF2 and c-jun, while also promoting the upregulation of their protein level in human leukemia U937 cells; in order to determine whether the induction of stress signaling was associated with cell death, markers of apoptosis such as Caspase-7,8, and PARP were also observed. In addition, JS-K can induce apoptosis in glioma U87 cells and activate caspase-3; however, it was demonstrated that pan-caspase inhibitors Z-VAD-FMK and Q-VD-OPH could diminish the action of JS-K in inducing apoptosis (21), while U0126, an MEK/ERK inhibitor, could also diminish apoptosis induced by JS-K, the results of which indicate the mechanism of pro-apoptosis caused by JS-K is involved with the MAPK pathway (22). Furthermore, it was (15) indicated that JS-K induces the MEL cells to undergo caspase-associated apoptosis, with the potential mechanisms being the inhibition of both PI3K and MAPK pathways and the subsequent activation of FoxO3a (transcription factor that activates genes that induce apoptosis); inhibition of either of these kinases permits the translocation of FoxO3a from the cytoplasm to the nucleus where it can activate genes associated with inducing apoptosis.

JS-K promotes apoptosis in cancer cells through the ubiquitin-proteasome pathway

The ubiquitin-proteasome pathway works in multiple steps: firstly, ubiquitin is activated from its precursor through the addition of ubiquitin-activating enzyme (E1); secondly, activated ubiquitin is transferred to the ubiquitin-conjugating enzyme (E2); thirdly, E2 interacts with the ubiquitin-protein ligase (E3) and transfers ubiquitin to the target protein finally, and selective labeling and degradation of specific intracellular protein are enabled according to the type of ubiquitin modification on the protein substrate. Interestingly, one study (23) discovered that abnormal ubiquitination is found during the formation and progression of cancer.

Another prior study showed that E1 is a target for NO release by JS-K. JS-K also led to a decrease in total cellular ubiquitylation and an increase in cellular p53 levels (23). According to the fact that NO can modulate active cysteine residues which existed in E1 (24), we suspect that JS-K could inactivate E1 through targeting cysteine residues. Another investigation (12) found that E1 was S-nitrosylated by JS-K but not by JS-43-126 in preserved human retinal pigment epithelium cells. Western blotting revealed that JS-K reduces the formation of thioester bonds between ubiquitin and E1. However, this effect is diminished by dithiothreitol (DTT), which blocks cysteine residues of E1. All these results point to the possibility that JS-K-released NO inactivates E1 through targeting cysteine residues. Other researchers (25) found that NO precursor PABA/NO reduces the formation of thioester bonds between ubiquitin and E1 in varying degrees in ovarian cancer cells.

Programmed cell death 4 (Pcd4) is a tumor suppressor protein that inhibits the mutation of cells *in vitro* and the formation of tumor *in vivo*. One recent study (26) showed that TGF- β 1 also inhibits the activation of S6 kinase 1 which phosphorylates the serine 67 residues of PDCD4 and leads to phosphorylation of serine 71 and serine 76 in the β -TRCP binding sequence. This phosphorylation sequence causes protein degradation in the ubiquitin-proteasome system. Other research (12) also found that JS-K increases PDCD4 protein levels. The main function of tumor suppressor gene p53 is to regulate different target genes as a transcription factor, which can lead to growth arrest, senescence, and the apoptosis of the cancer cells. p53 selectively kills the mutant cells by inducing apoptosis. Generally, p53 is kept at very low levels by ubiquitin-proteasome degradation. Hdm2 works as a ubiquitin E3 ligase and can mediate ubiquitin-proteasome degradation of p53 and itself (23). JS-K can inhibit the ubiquitin-proteasome degradation of p53 mediated by Hdm2, which leads to the accumulation of p53 and thus, apoptosis (12). About this function of the ubiquitin of E3 ligase, in 2017, we published a paper in BMC cancer reporting that JS-K might have been able to inhibit proliferation and induce apoptosis via regulation of the ubiquitin-proteasome degradation pathway. As p53 operated as a key regulator in the apoptotic process, JS-K inhibited Mdm2's ubiquitin ligase ability, which mediated ubiquitin-proteasome degradation of p53 (27).

JS-K promotes apoptosis of tumor cells by acting on the cell factor β -catenin/T (TCF) signaling pathway

β -catenin plays a central role in the Wnt signaling pathway

that regulates cell-cell adhesion and proliferation of leukemia cells (28,29). Increasing the levels of the β -catenin target gene (such as cyclin D1) can induce genetic instability and lead to tumor formation (30). β -catenin is expressed in almost all T cells, tumor cells with the hematopoietic origin, and primary leukemia cells but not in the normal peripheral blood T cells. β -catenin has low expression in myeloid leukemia HL-60 cells, but the high expression in Jurkat T cells (31). JS-K can induce differentiation and apoptosis in myeloid leukemia HL-60 cells (17). One study (32) showed that JS-K could induce apoptosis according to the NO release rate. Using NO scavenger PTIO as a control to assess the effect of NO released by JS-K on β -catenin, it was found that JS-K could significantly inhibit the activity of β -catenin/TCF transcription factor, which is involved with the nitrosation of β -catenin, by releasing NO. JS-K also inhibits the expression of cyclin D1 which is regulated by β -catenin and interrupts the cell cycle, inducing apoptosis of the cells.

JS-K promotes apoptosis of tumor cells by causing DNA damage

Cell toxicity leads to DNA damage by causing a double-strand break. One study showed that NO could cause double-strand breaks of DNA in mammalian cells (33). Since JS-K can release NO, it has been used to explore the correlation between DNA damage and apoptosis in tumor cells. Recent studies have indicated that JS-K increased the expression of DNA-damage-associated proteins in the phosphorylation of H2AX, the phosphorylation of checkpoint kinase (Chk1, Chk2), ataxia-telangiectasia mutated (ATM) kinase, the phosphorylation of ataxia-telangiectasia mutated rad3-related (p-ATR) kinase. Meanwhile, apoptotic-associated proteins cleaved caspase-3, caspase-7, and poly ADP-ribose polymerase (cleaved PARP) in human multiple myeloma cells or HBV-positive hepatocellular carcinoma HepG2.2.15 cells (7,34,35). All these findings suggest JS-K could be a potential anti-cancer drug associated with DNA damage.

JS-K induces apoptosis in tumor cells through other mechanisms

The mechanism of apoptosis induced by JS-K is complex. It was found that JS-K can affect the mitochondrial membrane potential and induce the release of cytochrome C from mitochondria to the cytoplasm, which activates the caspase

cascade reaction and further promotes the activation of endogenous apoptosis (36-38). Another investigation (4) showed that reductase thioredoxin 1 plays a complicated role in the growth of cells by inhibiting apoptosis and providing favorable conditions for the cells being treated by oxidants, which can cause genetic mutations. After the silencing of reductase thioredoxin 1, the toxic function of JS-K upon cells was significantly enhanced, and the expression of PARP and active Caspase3 was increased. These results are consistent with the hypothesis that weakening of the thioredoxin system can promote apoptosis in cells. As is widely known, reactive oxygen species (ROS) are chemical species and responsible for inducing carcinogenesis. JS-K presented an anti-cancer function of gastric cancer through inducing a significant accumulation of ROS and inhibiting the expression of antioxidant enzymes, including copper-zinc-containing superoxide dismutase (SOD1) and catalase, which contributed to the decrease of antioxidant enzyme activity and the subsequent inhibition of ROS clearance (38). Furthermore, our investigations (39-41) showed similar results of JS-K-induced apoptosis in prostate cancer cells, bladder cancer cells, and in renal cancer cells through regulating the ROS level and nitrites resulting from oxidation of NO having a continuous apoptosis-inducing effect. Lately, studies on JS-K-activated p53- and TNF- α mediated apoptotic pathways, caspase activation, and anti-angiogenesis in Hep3B cells or renal carcinoma cells were carried out (40,42). Subsequently, another investigation (37) showed that JS-K significantly induced apoptosis through activating PP2A-C (a member of the phosphoprotein phosphatase family) and dephosphorylated PP2A substrates including β -catenin, c-Myc, and p-Bcl-2 (Ser70) in HCC cells, the effect of which was abolished by PP2A inhibitor okadaic acid. In the meantime, another research team reported that JS-K induced apoptosis in glioblastoma multiforme cells by causing mitochondrial dysfunction and induced COX expression and storage of COX-2 in the mitochondria; they also found that acetylsalicylic acid could significantly enhance these anti-tumor effects synergistically (43).

The application and innovation of JS-K

In the process of developing new anti-cancer drugs, it is crucial to understand how to block tumor cell survival and the drug resistance mechanism. Recent investigations have shown that NO can achieve both by inhibiting proliferation, promoting apoptosis, and reversing the drug resistance of cancer cells, which significantly improves the

sensitivity of traditional treatments of tumor cells (44). It is also important to know that NO is a “double-edged sword”: high concentrations of NO can promote apoptosis and inhibit the proliferation of tumor cells, while low concentrations of NO act conversely. However, a high concentration of NO has not yet been used in large-scale animal experiments and clinical trials. A concentration of NO that causes two kinds of different biological effects needs to vary in 2 orders of magnitude, which means that concentration at which NO can induce apoptosis is 10~100 times higher than that at which it promotes cell growth (45). Therefore, it is possible to kill tumor cells by producing a high concentration of NO locally. JS-K, activated by GST could release a large amount of NO and significantly inhibit the proliferation of tumor cells. One investigation (46) has recently designed a nano-vehicle containing JS-K, which can effectively release NO and improve the prognosis of head and neck cancer patients. Results of previous studies show that this nano-JS-K carrier can release NO in prostate cancer cells and produce strong cytotoxicity. They could also be targeted to the tumor cells and effectively inhibit proliferation through binding to specific tumor antigens and antibodies. These nano-carriers also slowed the growth of transplanted tumors and survival time was thus prolonged; NO produced by nano-JS-K carriers localized in the tumor tissues and did not show any obvious toxicity in the normal cells or lead to weight decline of the animals. On the other hand, after intravenous injection of JS-K, inflammation, and necrosis was observed at the injection site. This preliminary investigation shows that nano-JS-K-carriers are safer and more effective for the treatment of cancer than traditional methods.

Application and prospect of JS-K in clinical treatment

Compared with the previous NO precursors, JS-K, activated by GST, can release high concentrations of NO in a controlled and sustained manner and has no obvious toxicity toward normal tissues. JS-K can also inhibit the growth of tumor cells through the conversion of GST—a key factor in the drug resistance mechanism of tumor cells—into a favorable form. In-depth research about JS-K has confirmed that it can induce apoptosis and reverse chemotherapeutic drug resistance in various cancer cells, while investigations into the development of nano-carriers have also seen promising results indicating this novel method’s potential clinical application. We should further

study the relationship between JS-K and apoptosis in cancer cells so that we can furnish more new ideas and methods in clinical practice.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.07.20>). The authors have no conflicts of interest to declare.

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