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The anticancer mechanism of capsaicin on various cancer cell lines

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Abstract

It is believed that capsaicin has tumor suppressive effects and this material induces apoptosis in various cancer cell lines such as human KB cancer cells, colorectal cancer cells, human osteosarcoma cancer cells and pancreatic cancer cells. The mechanism of anticancer effects was demonstrated by various techniques such as SRB assay, MTT assay, TUNEL assay, western blot analysis and flow cytometric analysis. These studies investigated whether treatment of capsaicin in a dose dependent manner would induce extrinsic and intrinsic pathway of programmed cell death. Capsaicin stimulates arrest of cell cycle at G2/M section and caused apoptosis in human KB cancer cells. Capsaicin has strong tumor suppressive effect. This material induces apoptosis in various cancer cell lines. The mechanism of anticancer effects was detected in both extrinsic and intrinsic pathway of programmed cell death by many cellular and molecular techniques.

Introduction

It is clear that natural phytochemicals extensively present in daily fruits and vegetables have restrictive effects on numerous kinds of cancers at molecular and cellular levels. Capsaicin one in all these naturally occurring phytochemicals, is the main pungent constituent of hot chili peppers of the genus capsicum that are extensively used as a food additive. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is alkaloid derived from genus capsicum pepper plant, better called chili pepper fruit (1). Capsaicin is a member of the vanilloid family of compounds. Like other vanilloids, capsaicin contains a benzene ring and an extended hydrophobic carbon tail with a polar amide cluster with chemical formula C18H27NO3, the melting point is 62–65°C, and also the molar mass is 305.4 g/mol. While capsaicin is not water-soluble, alcohols and different organic solvents are accustomed solubilize capsaicin in topical preparations (2).

Materials and Methods

This review article discusses the pathophysiological mechanisms of anticancer impact of capsaicin on various cancer cell lines. For this review, we used a variety of sources by searching through Web of Science, EBSCO, PubMed, EMBASE, Scopus, Google Scholar and directory of open access journals (DOAJ). The search was performed using combinations of the following key words and or their equivalents such as; capsaicin, apoptosis, cancer cell line, antioxidant, cancer, colorectal cancer and reactive oxygen species.

Core tip
Capsaicin has strong tumor suppressive effect. This material induces apoptosis in various cancer cell lines. The mechanism of anticancer effects was detected in both extrinsic and intrinsic pathway of programmed cell death by many cellular and molecular techniques.

Antioxidant enzyme system

It has been shown that capsaicin is concerned in several physiological specialty effects for example many reports showed that the utilization of capsaicin will relieve inflammation and pain related to some diseases and cancers (2). Additionally accumulate studies have detected that capsaicin has anti-proliferative effects on various human neoplastic cell lines together with those derived from human KB cancer cells (3), human colorectal cancer cells (4,5), human osteosarcoma cancer cells (6) and pancreatic tumor cells (1,2). These capability of the capsaicin to suppress the expansion of these cancer cells is primarily mediate by induction of programmed cell death which consist the arrest of cell-cycle progression and regulation of transcription issue (3). The failure to manage neoplastic cell death related to the in-

duction of programmed cell death has been thought of to be a critical reason behind resistance against cancer therapy. Apoptosis is a kind of programmed death. As a result of programmed cell death unwanted cells are eliminated during a well-organized sequential method. Programmed cell death is characterized by numerous morphological and biochemical changes like disease, mitochondrial membrane permeability, plasma membrane blebbing, and the activation of proteolytic enzyme cascades. It has been shown that the activation of apoptosis is specially mediated through the outside death receptor pathway and also intrinsic mitochondrial pathway which involve a range of proteolytic enzyme members of the family (7). The outside pathway is started by stimulation of the death receptors that are members of the neoplasm death factor receptor family activated death receptors consist establishment of the death inducing signal complex (DISC) that afterwards promotes activation of caspase-8 (7). The inner pathway initiated by numerous intracellular signals, like DNA injury involves the mitochondrial response. Disruption of mitochondrial membrane through the regulation of the bcl-2 family members dissipates the mitochondrial transmembrane potential leading to discharge of proapoptotic proteins, including cytochrome c and apoptosis-inducing factor from the inter-membrane region into the cytoplasm. Consequently the apoptosome, a complex that stems from the dealings between cytochrome c, apoptosis protease and ATP/dATP activate caspase 9 (7). Both external and internal pathways persuade the activation of caspase 3, 6 and 7 that afterward cleave their substrates together with poly (ADP-ribose) polymerase (PARP) (7).

Anticancer effects of capsaicin on human KB cancer cell line

The effects of capsaicin on human KB cancer cells had been examined. It was found that treatment of KB human cancer cells with capsaicin result in cell cycle arrest and induction of programmed cell death and although mitochondria and caspase members were concerned within the programmed cell death (3). Capsaicin stimulates arrest of cell cycle at G2/M section and caused programmed cell death of KB cancer cells. The capsaicin induced apoptosis was related to mitochondrial membrane permeabilization and protease activation (3). To determine the result of capsaicin on the proliferation of KB human cancer cells many technique were used by researchers such as SRB assay, MTT assay and trypan blue to measure the toxicity of KB cancer cells in a dose dependent manner (1, 50, 100, 150 and 200 μM) 24, 48 and 72 hours at 37°C of this treatment (3). As shown in these techniques analysis resulted strongly dose dependent reduction of variable cells indicating that capsaicin exert a cytotoxic results in KB cancer cells (3). Recent studies established that capsaicin stimulates dissipation of the mitochondrial membrane potential and mitochondrial initiated events are chargeable for the intrinsic pathway of programmed cell death, numerous stress signals are capable of triggering mitochondrial permeabilization that afterwards results in the discharge of cytochrome c to the cytosol. These studies indicated that exposure of human KB cancer cells to capsaicin reduces cell viability, induces cell cycle arrest at G2/M section, and activates programmed cell death that involve mitochondria and caspase members. The programmed cell death of KB cells treated with capsaicin is related to the induction of caspase 3 and 9, similarly as disruption of the mitochondrial membrane potential. In summary, these findings recommend that capsaicin possesses an anti-cancer activity and should be a potential candidate as an anti-cancer agent (3).

Anticancer effects of capsaicin on human colorectal cancer cell lines

It was found that at low concentration of treatment with capsaicin (0-40 μM) human colorectal cancer cells had very little effect on the expansion inhibition, but at high concentration (80-160 μM) 48-72 hours at 37°C treatment with capsaicin considerably repressed cell proliferation (4). Anchorage-independent growth is one in all hallmarks of cell transformation and is taken into account for most correct and demanding in vitro assay for detective work malignant transformation of cells. Therefore investigation focused on the results of capsaicin on the anchorage-independent growth (4). Result of studies showed that capsaicin inhibits powerfully the anchorage independent growth at 40 μM and therefore the number of colonies shaped in soft agar was amazingly decreased. At high concentrations, nearly no colony was discovered. All these results showed that capsaicin had a profound antineoplastic effectiveness in human colon cancer cells in vitro. Further analysis of the cell cycle division of live cells had demonstrated that an oversized proportion of live cells were at G0/G1 phase (70%-80%) after 300 μM capsaicin treatment showed the significant G0/G1 arrest evoked by capsaicin (4). In addition, adopted annexin V–FITC/PI double staining was used to verify that capsaicin considerably induced cell programmed cell death during a dose-dependent manner (4). Application of 300 μM chemical irritant resulted in 20%-30%cancer cells to endure programmed cell death. In recent studies in order to confirm the apoptotic effect of capsaicin, expression of Bax, p21 and cleaved-caspase3 was identified too. Following capsaicin treatment according to the consequence of flow cytometry investigations, the expression of p21, that may be a key regulator of cell cycle progression at G1, was considerably raised, suggesting that capsaicin-induced G0/G1 arrest was intimately correlated with p21 elevation. Bax could be a pro-apoptotic protein and concerned in induction of cell programmed cell death (4). Moreover cleavage of PARP is taken into account to be an important marker for detection of programmed cell death. Western blotting result revealed that the expression of Bax and cleaved PARP were remarkably magnified during a dose-dependent manner after capsaicin treatment. All these results confirmed that capsaicin treatment potently evoked cell cycle G0/G1 arrest and apoptosis in human colon cancer cells (5). Capsaicin treatment resulted in a rise of p53 expression in a dose-depen-

dent and time-dependent mode. As a short-life protein, p53 was ruined frequently via ubiquitination under traditional condition. Therefore, improvement of its stability was necessary for p53 to perform its performance (5). The aim of other studies was to adopt cycloheximide to block protein synthesis in HCT116 cancer cells and detected the expression of p53 with capsaicin treatment. The results showed that p53 was rapidly degraded and the half-life was about 30 minutes, but in capsaicin treatment cluster, the half-life of p53 was dramatically elongated and expanded to 90 minutes (5). Verifying the steadiness of p53 was obviously increased. In order to examine the transcriptional activity of p53 capsaicin treatment, pGL3-p53 firefly luciferase reporter plasmid was transfected into HCT116 cancer cells. Due to the expression of luciferase was below the management of p53 transcriptional activity therefore it was a tendency to check p53 activity via measuring luciferase activity (5). After 40 μM adding capsaicin, the activity of luciferase was raised nearly 4-fold as compared with the management group, which suggested transcriptional activity of MDM2-p53 communication, MDM2-mediated p53 ubiquitination was remarkably reduced with capsaicin treatment that contributed to the stabilization of p53 and also the extension of p53 half-life with capsaicin treatment. Additional investigation established the extension of p53 half-life was related to the separation of p53 from MDM2 (5). Earlier studies had shown stress signals were usually generated after capsaicin treatment in different cell varieties and these signals would cause a series of post-translational modifications of p53 (8-10).

Anticancer effects of capsaicin on human osteosarcoma (MG63) cell line

Recent studies revealed that capsaicin induced apoptosis in MG63 human osteosarcoma cells and its underlying molecular mechanisms. TUNEL assay, flow cytometric analyze and western blot analysis, confirmed that the anticancer effect of capsaicin resulted in morphological changes, decreased cell capability and apoptosis in the MG63 cells (6). These results showed that capsaicin was able to restrain cell viability and growth and persuade apoptosis. The molecular factors that were involved in the programmed cell death of capsaicin treated MG63 cells. The MAPKs are expressed in all mammalian cell varieties and have independently different roles in the regulation of specific cell reactions. MAPKs have been confirmed to be composed of three parallel kinase components, including ERK, JNK and p 38 MAPK (11,12). As shown in several studies, the MAPK signaling pathway is important in the regulation of cellular growth, differentiation, endurance, angiogenesis and programmed cell death (13-15). Accordingly, it was suggested that the MAPK signaling pathway was occupied in the cellular response of capsaicin induced apoptosis. Applying groups pretreated with MAPK inhibitors, it was revealed that MAPKs exerted no exact effect in capsaicin induced apoptosis in the MG63 cells. It has been revealed that caspase, belongs to the group of enzymes identified as cysteine proteases, the cell death gene, CED 3 (16). Caspases have multi faceted functions in almost every feature of physiology, such as in growth and development, senescence and apoptosis (17,18). Moreover, the parts of the caspase cascade exist in various cells in the form of inactive zymogens, which are then activated to transmit the apoptotic signal (19). Additionally, it has been recommended that the caspase cascade may persuade the apoptotic reaction (20). The results showed that the caspase cascade regulated capsaicin induced programmed cell death, examined through cell viability, western blot analysis and flow cytometry. In recent studies it was demonstrated that the antioxidant enzyme system was also involved in the capsaicin induced apoptosis. The antioxidant enzyme system has been pointed to be important in the control of apoptosis (21,22). Moreover, antioxidant enzymes protect cells from oxidative hurt, such as reactive oxygen species (ROS) production (23-25). ROS cooperate with a wide range of cell and factors cause damage to cell structures, including the membrane, and are controlled with apoptosis (26-29). According to the results of current studies, it was verified that the antioxidant enzyme system was particularly useful in capsaicin induced apoptosis in the MG63 cells, as confirmed using a variety of methods. Therefore, it was revealed that ROS were part of the capsaicin induced programmed cell death pathway in the MG63 cells. The present investigations elucidate the molecular mechanisms that were implicated in the induction of apoptosis. In combination, the results revealed that capsaicin induced programmed cell death in the MG63 cells and the caspase cascade and antioxidant enzyme mechanism were the underlying regulatory signaling pathways occupy in the capsaicin induced apoptosis. The present results indicated that capsaicin showed an anticancer effect in osteosarcoma cells (6).

Anticancer effects of capsaicin on pancreatic cancer cell lines

Anticancer result of capsaicin was established by flow cytometry. Adding BxPC-3 and AsPC-1 cells with 150 mM capsaicin for 24 hours resulted in about 2.5-5 folds raise in apoptosis. Interestingly, capsaicin failed to tempt apoptosis in normal HPDE-6 cells. The apoptosis inducing effect of capsaicin was additionally confirmed by western blotting. As capsaicin treatment caused notably activation of caspase-9, caspase-3 and PARP as evident by their individual cleavages in a dose dependent manner. Conversely, capsaicin treatment did not cause any cleavages of caspases or PARP in normal HPDE-6 cells. In a time dependent analyze, cleavage of caspase3, 9 and PARP were obvious by 16 and 24 hours of capsaicin treatment. Mitochondrial ETC complexes are the main producer of ROS in cells and tissues. Since ROS generation was monitored by capsaicin, to observe if mitochondria are involved in this process. Therefore the enzymatic activities were determined and expression of mitochondrial complex-1, complex-2, complex-3 and complex-4 in capsaicin treated BxPC-3, AsPC-1, HPDE-6 and BxPC-3 ρ0 (which lack mitochondrial DNA) cells. Capsaicin treatment reduced complex-activ-

ity by about 5%–20% in BxPC-3 and 2.5%–9% in AsPC-1 cells respectively as measured up to controls. As expected, capsaicin failed to slow down complex-1 action in BxPC-3 cells ρ0 and normal HPDE-6 cells. The results revealed that pretreatment of cells with catalase or considerably stopped the reductions in complex-1 action by treatment of capsaicin. Further capsaicin treatment significantly reduced the protein stages of complex-1 protein complex after 4 hours of treatment in a time dependent analyze and catalase or EUK-134 stopped this change. Likewise, complex-3 action by capsaicin was inhibited by 8%–75% in both BxPC-3 and AsPC-1 cells. Nevertheless, capsaicin failed to reduce complex-3 action in BxPC-3 ρ0 cells. An unassuming decrease in complex-3 action was however monitored in HPDE-6 cells by capsaicin treatment. The decrease in complex-3 activity in BxPC-3 cells by capsaicin was reduced by catalase and EUK-134. In consonant with activity data, expression of complex-3 protein complex was radically reduced in BxPC-3 cells following capsaicin treatment. The effect of capsaicin on the protein stage of complex-3 was abrogated by catalase and EUK-134. The results show that mitochondrial complex-3 is more involved in capsaicin mediated ROS generation in contrast with complex-1. Capsaicin had no effect on complex-2 and 4. Taken together, these results imply that inhibition of mitochondrial complex-1 and complex-3 by capsaicin cause ROS production.

To investigate TRPV1-independent apoptosis induced by capsaicin in human carcinoid cell line (BON), it was focused on adjustment of ROS. Previous investigation found that exposure of cells to capsaicin increases intracellular ROS production (30,31). Therefore it was suggested that extreme ROS production is relevant for cell death induction by capsaicin. ROS is known to present proapoptotic activities of capsaicin. Therefore, inhibition of ROS production by antioxidants was showed to protect against capsaicin induced programmed cell death (30,32). Unexpectedly current results revealed that capsaicin reduces intracellular ROS substance. Despite the enormous majority of reports presentation a connection between increased ROS generation and cell death induction by capsaicin, contradictory results with regard to the role of ROS by capsaicin-induced cell death were reported (33,34). As described above, ROS over production may enhance proapoptotic signaling. However, it should be noticed that at physiological stages ROS controls many basic cellular procedures, including viability and production (35,36). Consequently, it was earlier proposed that both, overproduction as well as suppression of ROS generation may act a role in the initiation of cell death in response to treatment with capsaicin (33). The relation between capsaicin-reduced ROS production and apoptotic cell death initiation was expansively studied by Lee et al (33). The authors of this work informed that in human glioblastoma A172 cells, capsaicin administration reduced ROS generation, while apoptotic cell death enhanced. Moreover, application of an antioxidant N-acetylcysteine (NAC) led to apoptosis. In contrast, supplementation with exogenous H2O2 avoided glioblastoma cells from capsaicin-induced apoptosis (33). This information suggested that ROS inhibition may be involved in initiation of programmed cell death, as a minimum in A127 glioblastoma cells. Furthermore, despite the fact that NAC improved capsaicin-reduced ROS production there was no consequence of NAC on capsaicin-inhibition of cell proliferation or capsaicin-stimulation of apoptosis (33).

Conclusion

Cancer therapy resistance is a major problem that causes cancer therapy failure. According to on top of finding capsaicin is an antioxidant material that can resolve this problem in various types of cancers.

Author's contribution

SMA was the single author of paper.

Conflicts of interest

The author declared no competing interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the author.

Finding /Support

None

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