More Than Spice: Capsaicin in Hot Chili Peppers Makes Tumor Cells Commit Suicide

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Hot red chili peppers, which belong to the plant genus *Capsicum,* are among the most heavily and frequently consumed spices throughout the world. Their principal pungent ingredient is the phenolic substance capsaicin (8-methyl-*N*-vanillyl-6-nonenamide). Although capsaicin can cause neurogenic inflammation *per se* under certain physiologic conditions, it also has analgesic and anti-inflammatory activities and is used currently in topical creams and gels (e.g., Axsain and Zostrix) to mitigate neurogenic pain. A receptor for capsaicin and other structurally related substances was identified and cloned *(1–3).* This receptor, vanilloid receptor subtype 1 (VR1), forms a nonselective cation channel in the plasma membrane that mediates some of the pleiotropic effects exerted by capsaicin and its analogues, which are collectively named vanilloids.

The role of capsaicin in carcinogenic processes is quite controversial. Although some investigators suspect that capsaicin is a carcinogen, co-carcinogen, or tumor promoter, others have reported that it has chemopreventive and chemotherapeutic effects [reviewed in *(4–8)* and references therein]. Interestingly, capsaicin has been found to preferentially repress the growth of some transformed human and mouse cells *(9,10).* Although the antiproliferative activity of capsaicin has been ascribed to its ability to induce apoptosis *(9–18),* relatively little is known about the molecular basis for the programmed cell death induced by this edible phytochemical. In an excellent study published in this issue of the Journal, Hail and Lotan *(19)* have conducted a series of elegant experiments that afford important insights into mechanisms underlying the apoptogenic action of capsaicin at the cellular level. The authors report that capsaicin-induced apoptosis in cultured cells derived from human cutaneous squamous cell carcinoma (SCC) occurs through inhibition of mitochondrial respiration.

Although the work by Hail and Lotan addresses the importance of the mitochondrial redox system as a primary target for capsaicin in SCC-derived cells, other investigators have demonstrated that capsaicin-induced apoptosis in some transformed cells *(9,10,15)* and in activated T cells *(17)* is associated with the suppression of plasma membrane NADH-oxidoreductase (PMOR), an enzyme that transfers electrons from cytoplasmic NADH via coenzyme Q (ubiquinone) to external electron acceptors such as oxygen. PMOR is thought to be involved in the control of cell growth and proliferation *(20)* by maintaining the proper NAD^+/NADH ratio required for cell viability. However, PMOR activity in normal tissues and in nontransformed cells is responsive to growth factors and hormones, whereas PMOR activity in tumor tissues and transformed cells is not *(20,21).* Although the capsaicin analogue dihydrocapsaicin and the ultrapotent vanilloid receptor agonist resiniferatoxin also caused apoptotic death, the apoptogenic activity of these vanilloids, as well as that of capsaicin, does not appear to be mediated by the vanilloid receptor, at least in human blood cell lines, because treatment with capsazepin, a prototype vanilloid receptor antagonist, failed to block the apoptosis induced by those compounds *(15,18).*

Coenzyme Q is a lipophilic and mobile electron carrier of the plasma membrane electron transport system that is essential for cell growth and for the cellular response to redox changes. Preincubation of human lymphoblastoid cells with coenzyme Q prevents capsaicin-induced apoptosis *(15),* suggesting that capsaicin, a quinone analogue, induces apoptosis by competing with coenzyme Q in the plasma membrane redox system. Because capsaicin can also inhibit the NADH:coenzyme Q oxidoreductase (i.e., complex I) activity of the mitochondrial electron transport system *(22,23),* the question remained as to whether cap-

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saicin triggered apoptosis by blocking mitochondrial complex I activity, PMOR activity, or both. The biochemical mechanisms that mediate vanilloid-induced apoptosis through PMOR inhibition are unclear, but it has been suggested that an enzyme antagonist, such as capsaicin, may interfere with the coenzyme Q binding site, which may redirect the normal electron flow in the complex and generate excess reactive oxygen species (ROS) and a pro-oxidant environment in the plasma membrane *(15).* The possible involvement of ROS in vanilloid-induced apoptosis has also been reported by Macho et al. *(16–18).* They showed that capsaicin- or resiniferatoxin-treated transformed human T cells (i.e., Jurkat cells) displayed a loss of nuclear DNA and a concomitant increase in the proportion of subdiploid (apoptotic) cells and that this vanilloid-induced apoptosis was preceded by an increase in ROS generation and disruption of the mitochondrial transmembrane potential ($\Delta\psi_m$) *(16),* both invariant features of early programmed cell death. The pro-oxidative environment generated by perturbations in the plasma membrane redox system can result in oxidation of thiol groups in mitochondrial permeability transition pores, leading to dissipation of $\Delta\psi_m$, which is a prerequisite for the induction of apoptosis. Therefore, overproduction of extramitochondrial ROS due to the inhibition of PMOR could contribute to the apoptosis that is induced by capsaicin and related vanilloids. However, one cannot exclude the possibility that some of the ROS generation promoted by vanilloid treatment is attributable to a disruption of the mitochondrial respiratory system.

In this context, Hail and Lotan *(19)* have made a major contribution to the field by identifying an alternative route that mediates vanilloid-induced apoptosis. The authors used two different human cutaneous SCC cell lines (i.e., COLO 16 and SRB-12 cells) and respiration-deficient (ρ^0) clones derived from them to examine their susceptibilities to vanilloid-induced apoptosis. When the COLO 16 cells were treated with either capsaicin or resiniferatoxin, more than half underwent apoptosis, which was associated with progressive dissipation of $\Delta\psi_m$ and enhanced superoxide production, reflecting the disintegration of mitochondria and subsequent malfunction of mitochondrial electron transport *(19).* Exposure to these vanilloids also promoted a rapid induction of hydroperoxide generation in the SCC cells, which occurred much earlier than superoxide production. Hail and Lotan speculate that inhibition of mitochondrial electron transport by capsaicin and resiniferatoxin, presumably at complex I, may promote the production of ROS because of redox cycling of reduced electron carriers upstream of the site of inhibition, which could explain the enhanced hydroperoxide generation induced by these vanilloids in conjunction with induction of mitochondrial permeability transition *(19).* However, capsaicin and resiniferatoxin can interrupt plasma membrane electron transport as well by functioning as coenzyme Q antagonists, as addressed previously by other investigators *(9,10,15).* If the initial pro-oxidant effects of these vanilloids were solely associated with the inhibition of PMOR, the resulting oxidative stress would ultimately lead to disruption of the mitochondrial structure and function which, in turn, could induce mitochondrial permeability transition and, eventually, apoptosis.

All of the above findings suggest that vanilloids can target both mitochondrial and plasma membrane electron transport systems, thereby generating ROS that can mediate apoptosis. To directly assess the relative contributions of mitochondrial and plasma membrane redox systems to initial ROS generation after treatment with capsaicin or resiniferatoxin, the authors measured vanilloid-stimulated hydroperoxide generation in ρ^0 clones derived from the SCC cells. ρ^0 cells exposed to either capsaicin or resiniferatoxin released less hydroperoxide than did DMSO-treated control cells, supporting the notion that the majority of hydroperoxide initially produced in the parental SCC cells after vanilloid treatment was of mitochondrial origin *(19).* Moreover, exposure of the ρ^0 cells to capsaicin or resiniferatoxin failed to disrupt $\Delta\psi_m$ or increase the proportion of cells that were undergoing apoptosis. The authors' explanation—that the association between initial generation of ROS and the induction of apoptosis in the vanilloid-treated SCC cells was a consequence of mitochondrial electron transport perturbation—is reasonable. However, their study did not clarify whether such a mechanism occurs preferentially in transformed cells versus nonmalignant cells. It would be worthwhile to compare the activities of mitochondrial NADH oxidoreductase in SCC cells and in normal skin cells, as well as the relative sensitivities of these cells to vanilloid-induced cytotoxicity.

ROS can differentially affect cell growth or survival, depending on, among other things, the amounts formed, how long a cell is exposed to them, the availability and efficiency of antioxidant capacity, and the cell type. Although excess ROS transiently produced by PMOR inhibitors such as capsaicin and resiniferatoxin can be cytotoxic, milder endogenous redox stress that results from ROS spontaneously generated by an NAD(P)H:quinone oxidoreductase activity has recently been shown to play a functional role in the constitutive activation of the transcription factor, nuclear factor-κB (NF-κB) in several malignant human melanoma cell lines, which may account for the hyperproliferative potential of these cells *(24).* NF-κB is constitutively activated in diverse malignant tumors and in transformed cells *(25–29).* Brar et al. *(24)* found that capsaicin and dicumarol, a known inhibitor of NAD(P)H:quinone oxidoreductase, reduced superoxide production and proliferation of M1619 melanoma cells, suggesting that the redox coupling between NAD(P)H:quinone oxidoreductase and coenzyme Q is a more important source of growth-signaling ROS in transformed cells than it is in normally regulated nonmalignant cells. We have shown that topical application of capsaicin onto the dorsal skin of mice strongly suppresses epidermal NF-κB activation induced by phorbol ester *(30),* which may account for the anti-inflammatory and antitumor promoting effects of this compound. Despite these findings, it is not yet clear that NAD(P)H oxidoreductase is crucial for cell proliferation. The availability of several different genetically tractable animal models, in which the gene encoding NAD(P)H oxidoreductase is mutated or deleted, will help us better understand the functional role of this enzyme and possibly facilitate the discovery of drugs that selectively kill tumor cells by targeting this enzyme.

In summary, the oxidative stress that is stimulated by vanilloid treatment of SCC cells is primarily of mitochondrial origin and contributes to the death of these cells by apoptosis. The exact molecular milieu that characterizes elevated oxidative stress caused by vanilloid treatment is not clear and requires further investigation. It is possible that a high metabolic rate or increased oxygen utilization as a direct or indirect consequence of aberrant electron flow in the mitochondrial respiratory system of malignant cells results in the increased production of oxidants, which may overwhelm cellular antioxidant protections and lead to apoptosis. However, it can be argued that such in-

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appropriate ROS generation may also have a deleterious effect on nonmalignant cells as well, and if this is the case, the vanilloids would be metabolic poisons rather than valuable candidates for use in preventive therapy for skin cancer or other cutaneous disorders.

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