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Safrole oxide induces apoptosis by activating caspase-3, -8, and -9 in A549 human lung cancer cells

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Abstract—Previously we found that 3,4-(methylenedioxy)-1-(2′,3′-epoxypropyl)-benzene (safrole oxide) induced a typical apoptosis in A549 human lung cancer cells. In this study, we further investigated which caspases were activated by safrole oxide during the apoptosis. The data showed that the activity of caspase-3, -8, and -9 was significantly enhanced by the compound, which suggested that safrole oxide might be used as a caspase promoter to initiate lung cancer cell apoptosis.

Apoptosis plays a fundamental role in many normal biological processes as well as in several disease states, including cancer.[1] The poor ability of cancer cells to undergo spontaneous apoptosis is partly due to negative or low expression of the crucial components in cell apoptosis pathway. So, up-regulation of the expression of crucial molecules mediating apoptosis by chemotherapeutic agents has been one of the considerable ways for cancer therapy.[2,3]

A 'library' of small molecules has been established in our laboratory. According to chemical genetics,[4–6] we use the 'library' as a powerful tool for screening-specific drugs to induce cancer cell apoptosis. Previously safrole oxide was synthesized in our laboratory and added into our 'library' (Fig. 1).[7] We found that safrole oxide, 112.36–449.44 μM, induced a typical apoptosis in A549 human lung cancer cells (the cell density being 100,000 cm^{-2}) from 24 to 48 h (Fig. 2).[8] Importantly, safrole oxide showed more powerful ability to induce apoptosis than its derivative, 1-ethoxy-3-(3,4-methylenedioxyphenyl)-2-propanol (EOD).[8,9] However, apoptosis-related molecules activated by safrole oxide in A549 cells remain unclear. In this study, we investigated which caspases were activated by safrole oxide, wishing to use safrole oxide as a 'key' to activate caspases and then to initiate lung cancer cell apoptosis.

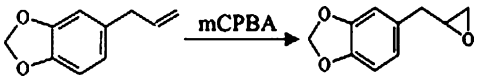

Figure 1. Safrole reacts with 3-chloroperoxybenzoic acid (mCPBA) in chloroform to yield 3,4-(methylenedioxy)-1-(2′,3′-epoxypropyl)-benzene (safrole oxide).[7]

Caspases, intracellular cysteine proteases, play an essential role in apoptosis.[10] Among the caspases, caspase-3 is involved in the execution phase of apoptosis.[11,12] So, we measured the changes in caspase-3 activity by colorimetric assay. Compared with the control group, safrole oxide-treated group showed a higher light absorption at 405 nm (0.375 vs 0.155, $P < 0.001$, $n = 3$) (Fig. 3). Since the solvent control group, ethanol, 0.045% (v/v), had no effect on the activity of caspase-3 ($P > 0.05$, $n = 3$).[9] Obviously, caspase-3 was activated by safrole oxide in A549 cells. Once caspase-3 is activated, the cell has no alternative but death.[10,11]

In addition to executioner caspase-3, initiator caspase-8 and -9 are also important for drug-induced apoptosis.[13,14] Our study showed that safrole oxide enhanced the activity of caspase-8 to 2.05-fold ($P < 0.01$, $n = 2$) (Fig. 4). Interestingly, fluorescence assay showed a dramatic increase in caspase-9 activity to 6.13-fold by the compound ($P < 0.001$, $n = 3$) (Fig. 5). Though

Keywords: Safrole oxide; A549 cells; Caspase promoter; Apoptosis.
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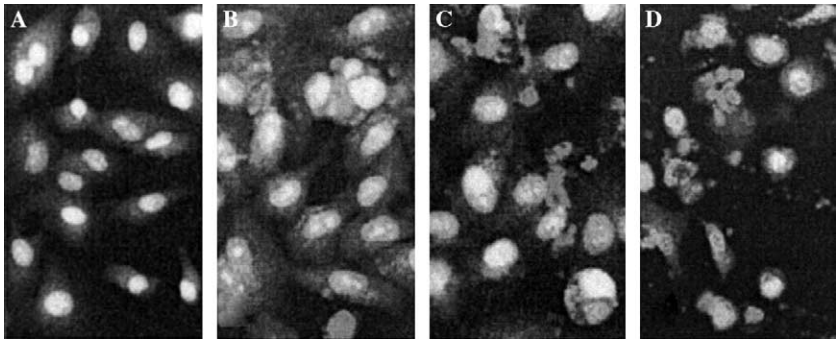

Figure 2. Effect of safrole oxide on nuclear fragmentation of A549 cells at 24 h.[8] (A) Cells in the control group; (B) cells treated by safrole oxide 112.36 μM; (C) cells treated by safrole oxide 224.72 μM; and (D) cells treated by safrole oxide 449.44 μM. (600×).

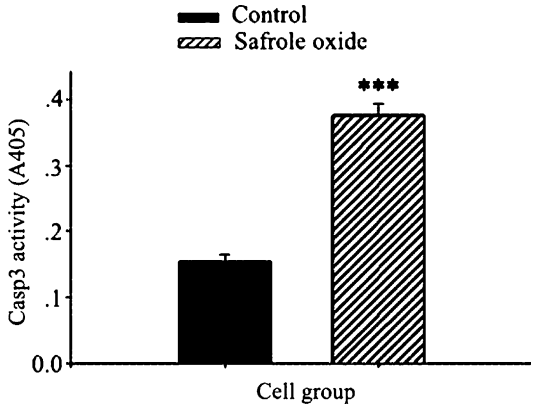

Figure 3. Colorimetric assay for caspase-3 activity at 24 h. Data are means ± SE from three independent experiments ($^{***}P < 0.001$ vs the control group).

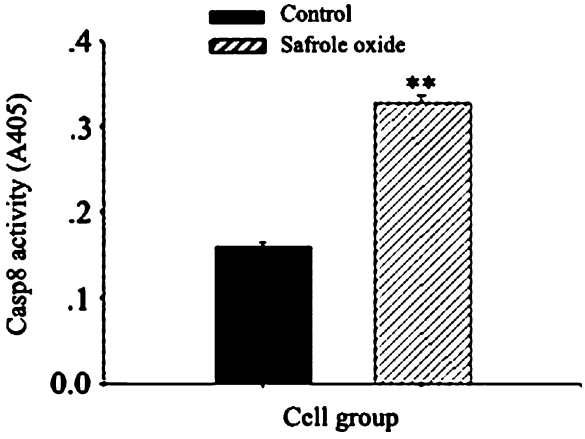

Figure 4. Colorimetric assay for caspase-8 activity at 24 h. Data are means ± SE from two independent experiments ($^{**}P < 0.01$ vs the control group).

caspase-6 is one of the downstream caspases of caspase-9, the activity of caspase-6 was hardly affected by safrole oxide ($P > 0.05$, $n = 3$) (Fig. 6). Furthermore, we found that in the solvent control group, ethanol, 0.04% (v/v), hardly affected the activity of caspase-8,[9] -9 or -6 ($P > 0.05$).

Based on these results, we reported that safrole oxide markedly activated caspase-8, -9, and -3, while caspase-6 might be irrelevant to safrole oxide-induced apoptosis. Once initiator caspases are activated, they can trigger a cascade to activate downstream executioner

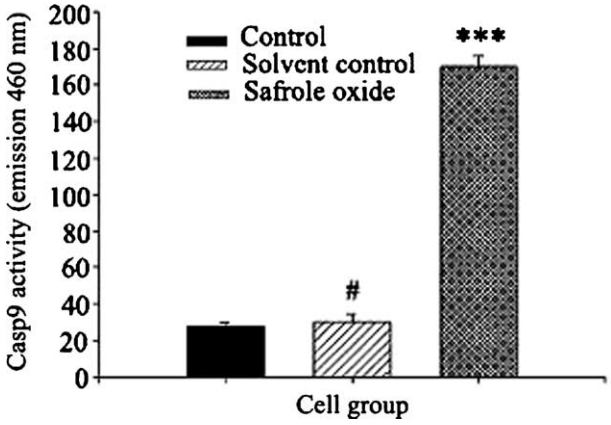

Figure 5. Fluorescence assay for caspase-9 activity at 24 h. Data are means ± SE from three independent experiments ($^{***}P < 0.001$ vs the control group).

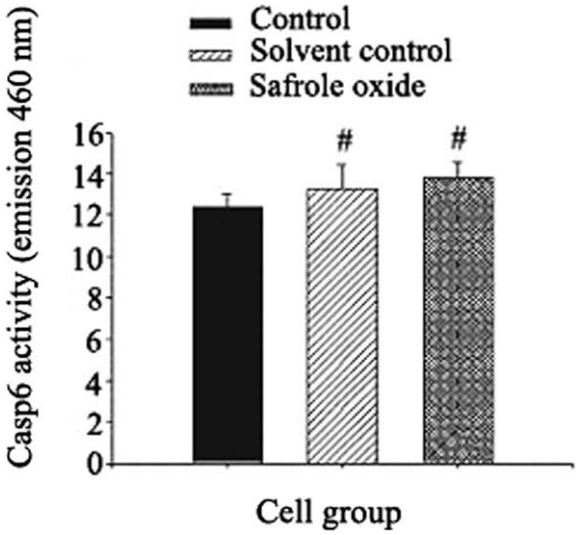

Figure 6. Fluorescence assay for caspase-6 activity at 24 h. Data are means ± SE from three independent experiments ($^{\#}P > 0.05$ vs the control group).

caspases.[15] These executioner caspases subsequently cleave proteins that will ultimately lead to the morphological manifestations of apoptosis, such as DNA condensation and fragmentation, membrane blebbing, and apoptotic body formation.[16]

So far, it has been reported that some reagents, including interferon-γ,[17] proinflammatory cytokine IL-4,[18] ZD1839 (Iressa, quinazoline derivative),[19] and gossypol,[20] initiate apoptosis in A549 human lung cancer cells by activating fas and caspases. Compared with interferon-γ and IL-4, safrole oxide has such advantages as: higher membrane permeability, ease of preparation, and lower cost. Interferon-γ, Iressa, and gosspol can trigger the activation of caspase-8, but not caspase-9. In contrast, in addition to activation of caspase-8, safrole oxide also elevated the activity of caspase-9 dramatically to 6.13-fold. Multiple kinds of caspases activation greatly enhance the effects of safrole oxide on the apoptosis of A549 human lung cancer cells. Activation of caspases is a critical component of execution phase of cell death in most forms of apoptosis. Therefore, factors affecting caspase activation might be important determinants of drug sensitivity.[21] Moreover, Xie et al. reported the feasibility of using tissue-specific expression of inducible caspase-9 as a nonmutagenic alternative modality for prostate cancer suicide gene therapy.[22] Similarly, activation of caspases in lung cancer cells would provide a novel strategy for lung cancer therapy.

In summary, interference of safrole oxide with the apoptosis pathway in A549 cells might take place by activating caspase-3, -8, and -9. The small compound might be used as a significant promoter of caspase-3, -8, and -9 to initiate lung cancer cell apoptosis. These findings would lead us to further investigate the mechanism by which safrole oxide activates caspases. Nevertheless, we hypothesized that the small molecule might be used as a 'key' to the target proteins to activate caspases and then initiate cancer cell apoptosis, which would lay the foundation for us to gain insight into the new strategies for lung cancer therapy.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.09.050.

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