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Comparative analysis of apoptosis-inducing activity of codeine and codeinone.

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The authors previously found that codeinone, an oxidation metabolite of codeine, among 10 opioids, showed the highest cytotoxic activity (DNA fragmentation-inducing activity) against human promyelocytic leukemic cell lines (HL-60). These findings prompted us to perform a more detailed study of apoptosis induction after codeinone treatment. Apoptosis was induced by treating HL-60 cells for 1-6 h with codeine or codeinone. DNA fragmentation was assessed by both agarose gel electrophoresis and fluorometric determination of the fragmented DNA after staining with diamidinophenylindole (DAPI). The release of cytochrome c and cytochrome oxidase from mitochondria and activation of caspase 3 were monitored by Western blot analysis. Intracellular caspase 3-like activity was confirmed by FACS, using cell permeable substrate. Mitochondrial manganese-containing superoxide dismutase activity and mRNA expression were assayed by activity staining after separation on the polyacrylamide gel electrophoresis, and reverse transcriptase-polymerase chain reaction (RT-PCR), respectively. Codeinone induced internucleosomal DNA fragmentation and production of Annexin-positive apoptotic cells more potently than codeine in HL-60 cells. Codeinone stimulated the release of both cytochrome c and cytochrome oxidase, and cleavage of procaspase 3 without significant changes in both the activity and expression of MnSOD. Codeinone was found to possess both apoptosis and necrosis-inducing activity, in addition to the reported antinociceptive activity, further substantiating its antitumor potential.