Differential effects of curcumin on modulation of Reactive Oxygen Species in Chronic Lymphocytic Leukemia cell models

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BACKGROUND

Reactive Oxygen Species (ROS) have an important role in signaling and induction of apoptosis, producing DNA damage and activating different molecules that can finally trigger the mitochondrial pathway. Curcumin is a natural polyphenol largely studied for its anticancer potential, with high selectivity for cancer cells. Although curcumin has antioxidant activity, it has also been reported to induce apoptosis in cancer cells through intracellular induction of oxidation. We have studied ROS production by curcumin in three different Chronic Lymphocytic Leukemia (CLL) cell models. CLL is a chronic disease caused by an abnormal expansion of B cells, with reduced sensitivity to drug-induced apoptosis rates. The aim of this study was to determine curcumin effects on ROS production as a potential way to induce apoptosis in CLL.

MATERIALS AND METHODS

I83, EHEB and Mec1 CLL cell lines at a concentration of $5x10^5$cells/mL were incubated at 37ºC and 5% CO_2 for 0.5, 24, 48 and 72 hours in presence of different concentrations of curcumin (0, 1, 5 and 10µM). For negative and positive controls, DMSO and tert-butyl hydroperoxide (TBHP) 200µM were respectively used. TBHP and curcumin were also simultaneously tested.

After incubation, samples were stained with CellROX™ Deep Red Flow Cytometry Assay Kit (Thermo Fisher) for 30 minutes in a cell incubator. Prior to analysis, cells were stained with Propidium Iodide (PI) to detect cellular membrane disruption.

Flow cytometry acquisition was performed on the Attune™ NxT flow cytometer (Thermo Fisher), using 637 nm excitation and 670/14 nm emission for CellROX Deep Red Reagent, and 561 nm excitation and 620/15 nm emission for PI. These stains were chosen to avoid crosstalk with broad band curcumin fluorescence, which has an absorption spectrum from 250 to 550 nm.

RESULTS AND DISCUSSION

Curcumin induced production of ROS at 24 hours in I83, Mec1 and EHEB in a dose dependent manner (Figure 1). When compared with DMSO treatment, curcumin 1, 5 and 10µM respectively, increased CellROX mean fluorescence intensity 6.94, 28.9 and 129.77% in I83; 10.22, 29.04 and 33.58% in Mec1; and -15.38, 2.29 and 7.48% in EHEB.

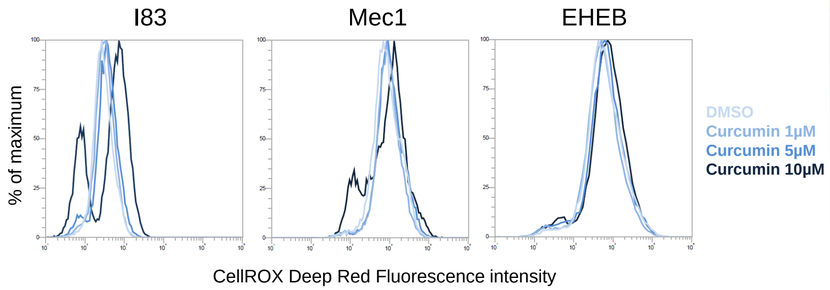

Figure 1. Curcumin induction of ROS after 24h incubation. Curcumin increased ROS in all three tested cell models in a dose-dependent manner.

TBHP, a potent pro-oxidant, strongly induced oxidation at 30 minutes (data not shown). Long-term TBHP treatment caused massive cell death in all cell models. When combined, curcumin (0, 1, 5 or 10µM) reduced toxicity of TBHP. In I83, curcumin at 1 and 5 µM had this protective effect, allowing survival of a small fraction of cells (Figure 2). This effect was higher with the lowest tested dose, 1µM, and disappeared with the highest dose, 10µM. In Mec1, the effect was dependent of dose, with higher protective effects at higher doses (Figure 2). Curcumin at 10µM, which produced mortality in I83, produced the highest protection in Mec1. EHEB was the most resistant cell line to TBHP toxicity effects. Curcumin at 1µM slightly reduced this toxicity. Higher doses, however, had no protective effect and slightly increased TBHP toxicity (Figure 2).

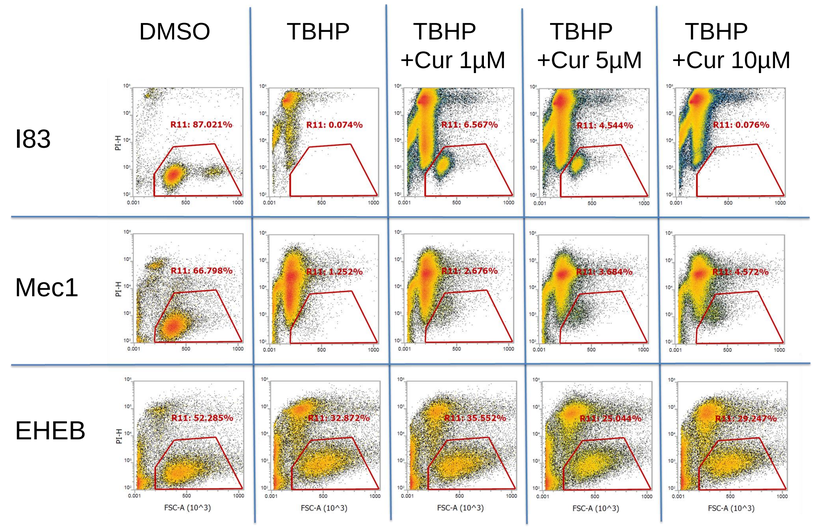

Figure 2. Curcumin has protective effects against TBHP induced toxicity, Forward Scatter vs. PI plots. TBHP potently caused cell death on al three tested cell lines, reducing the number of cells with intact cell membranes (R1). Curcumin relatively protected cells, with differences depending on curcumin concentration and the cell model used.

Despite this protective effect, curcumin has shown a synergistic effect on toxicity induced by different drugs in previous studies. As an example, curcumin's synergistic effects on camptothecin and colchicine induced cell membrane disruption are shown (Figure 3).

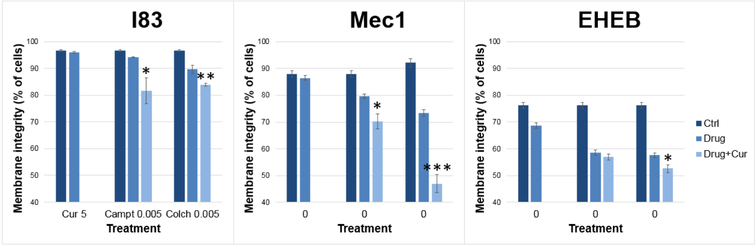

Figure 3. Curcumin 5µM significantly enhances toxicity of camptothecin (0.005µM) and colchicine (0.005µM) at 72 hours of incubation. P<0.05*, P<0.005**, P<0.0005***.

CONCLUSIONS

Curcumin induced ROS in all CLL cell models tested, supporting the hypothesis that it may contribute to increased apoptosis sensitivity. Surprisingly, it also produced a considerable protective effect against TBHP. This effect is coherent, since curcumin is an antioxidant and has demonstrated cell protective effects against oxidative stress induced by ischemia. This dual effect may explain how curcumin selectively damages cancer cells, by reducing overexpressed pro-survival pathways and restoring apoptosis in vitro, but has low effect in healthy cells. Moreover, ROS induction by curcumin was dose-dependent, whilst protective effect was higher in I83 and EHEB at lower doses, which could indicate an hormetic effect. More studies will be needed to determine the potential mechanisms underlying these effects. Although we previously demonstrated that curcumin enhanced apoptosis-inducing potential of proapoptotic drugs on these cell models, an unexpected antagonistic effect when combined with pro-oxidant drugs like TBHP cannot be ruled out.

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