Redox modulatory activities of dimethoxycurcumin, a synthetic analogue of curcumin

Amit Kunwar and K. Indira Priyadarsini
Radiation & Photochemistry Division

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Abstract

To understand the anti-tumor activity of dimethoxycurcumin (Dimc), an analogue of curcumin, the redox modulatory activities have been examined and compared with those of curcumin. Reactions with reactive oxygen species (ROS), indicated Dimc to be as efficient as curcumin in scavenging superoxide radicals, while its reaction with peroxyl radicals being much slower. These results were also supported by the observations on the scavenging of basal ROS levels in lymphocytes and in vitro antioxidant studies. Like curcumin, Dimc was a pro-oxidant and generated ROS in tumor cells. Accordingly, the compounds were non-toxic to lymphocytes, while exhibiting comparable cytotoxicity to tumor cells. Additionally, both curcumin and Dimc showed selective higher uptake in tumor cells than in normal lymphocytes.

Introduction

Curcumin, the major pigment from turmeric has been extensively researched for a wide variety of medicinal properties including antioxidant, anticancer, and anti-inflammatory activities [1]. New synthetic and natural analogues of curcumin are being explored for better metabolic stability and increased biological activity [2]. Among several analogues, dimethoxycurcumin (Dimc), a methoxylated derivative of curcumin, (Structure given in Scheme 1) showed superior anti-tumor activity and more metabolic stability over curcumin [3].

Although not fully established, it is proposed that the stability and pro-oxidant nature of Dimc could play a major role in its anti-tumor activity [3,4]. In order to understand the role of redox modulatory properties in anti-tumor activity, we compared the results on antioxidant and pro-oxidant effects of Dimc with those of curcumin under similar treatment conditions.

Results

Differential antioxidant activity

Using pulse radiolysis technique, the rate constants for the reaction of curcumin with peroxyl radicals such as lipid peroxyl and trichloromethyl peroxyl radicals, was estimated to be $7.3 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ and $1.5 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ respectively [5]. Reactions of above radicals with curcumin produced phenoxyl radicals, which could be regenerated back to curcumin by water-soluble antioxidants like

\begin{align*}
\text{Scheme 1} \\
\text{Curcumin; } & R_1=R_3=\text{OCH}_3, R_2=R_4=\text{OH} \\
\text{Dimethoxy curcumin (Dimc); } & R_1=R_2=R_3=R_4=\text{OCH}_3
\end{align*}
ascorbic acid. Similar studies on reactions of peroxy radicals with Dimc have also been attempted. However, due to the absence of the phenolic OH group, this reaction kinetics with Dimc was much slower and no intermediate phenoxy radicals could be identified during such reactions. This observation confirmed, that the phenolic-OH is essential, and the central CH$_2$ group of the β-diketone moiety is not involved in the peroxy radical scavenging reactions [5]. Cyclic voltammetric studies of curcumin and Dimc in methanol indicated that, under identical experimental conditions, the peak potential for the oxidation of curcumin is −150 mV less than that of Dimc. This suggested that the phenolic OH group is not only responsible for the reducing power but also the peroxy radical scavenging of curcumin [5]. Although Dimc is less efficient than curcumin in reacting with peroxy radicals, it is as efficient a scavenger of O$_2$· radicals as curcumin [6], indicating the involvement of the diketo group of curcumin or Dimc. The bimolecular rate constants for the reaction of O$_2$· radical with curcumin and Dimc were estimated to be 1.0 x 10$^5$ M$^{-1}$s$^{-1}$ and 0.8 x 10$^5$ M$^{-1}$s$^{-1}$ respectively [6]. Further, when curcumin and Dimc were checked for their ability to prevent the ROS induced lipid peroxidation in liposomal model system, curcumin was three to four times more effective than Dimc [5]. In line with these results, both curcumin and Dimc significantly reduced the basal levels of intracellular ROS in lymphocytes; however, curcumin was more effective than Dimc. All these studies portray that curcumin is a better antioxidant than Dimc under in vitro models.

**Differential pro-oxidant activity**

Extensive research in the last decade has indicated that, Curcumin exhibits pro-oxidant activity depending on the cell type through ROS production and alteration of the cellular redox homeostasis (e.g., depletion of GSH). Our group investigated the pro-oxidant activity of Dimc in human breast carcinoma, MCF7 cells as measured in terms of changes in cellular basal levels of ROS and GSH. The results on dihydroethidium (DHE) stained MCF7 cells, treated with Dimc for 2 h revealed that, it significantly elevated the basal ROS levels. Further, treatment with Dimc also showed a dose (5-50 μM) dependent decrease of GSH/GSSH at 2 h, followed by an increase of this ratio at 6 h as estimated by change in the fluorescence of o-phthalaldehyde, which is a selective binder of free thiols. Comparing these results with another study by Shang et al [7] on curcumin modulating intracellular levels of ROS and GSH in MCF7 cells under similar treatment conditions suggested that, pro-oxidant effects of curcumin and Dimc are comparable.

**Differential cytotoxicity in MCF7 cells vs. spleen lymphocytes**

The pro-oxidative behaviour of curcumin and Dimc could be correlated with cytotoxicity. Therefore, we determined the relative cytotoxicity of Dimc and curcumin in two different cell types, MCF7 cells and normal splenic lymphocytes. The cell viability was monitored by both 3-4,5-dimethyithiazol-2-yl-2,5-diphenyl-tetrazolium bromide (MTT) assay and also by propidium iodide (PI)-staining assay. The results indicated that there was an increase in the cytotoxicity in both cell types with increasing concentrations of curcumin or Dimc treatment and both of them displayed similar cytotoxicity in MCF7 cells, however, Dimc was less toxic than curcumin in splenic lymphocytes.

**Differential uptake in MCF7 cells vs. spleen lymphocytes**

The cytotoxicity of any agent would be primarily governed by intracellular availability. Earlier we had reported, that the MCF7 cells selectively take up more curcumin than normal cells like lymphocytes [8,9]. Similar attempt was made to quantitatively estimate the uptake of Dimc in splenic lymphocytes and MCF7 cells. The results showed that the uptake levels of Dimc and curcumin were comparable in both the cells. MCF7 cells showed nearly two times more uptake than lymphocytes, for both curcumin and Dimc [10].

**Conclusions**

New improved analogues of curcumin are necessary to overcome the limited bioavailability and bio-stability problem of curcumin. Dimc, is more stable towards
hydrolysis and chemical oxidation than curcumin. This attracted many researchers to test its biological activity in different model systems. The results so far indicated that the pro-oxidant activity of Dimc is comparable to that of curcumin, while its antioxidant activity is much lower than that of curcumin. Since an ideal anti-cancer agent acts as an inhibitor of oxidative stress (antioxidant) in normal cells while acting as its inducer (pro-oxidant) in tumor cells, Dimc has the potential to be developed as an effective anti-tumor agent.

References


