Curcumin as a Possible Lead Compound against Hormone-Independent, Multidrug-Resistant Breast Cancer

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We examine the possible evidence that the phytochemical curcumin may overcome resistance to hormonal and cytotoxic agents in breast cancer. We present our observations on MCF-7R, a multidrug-resistant (MDR) variant of the MCF-7 breast cancer cell line. In contrast to MCF-7, MCF-7R lacks aromatase and estrogen receptor α (ERα) and overexpresses the multidrug transporter ABCB1 and the products of different genes implicated in cell proliferation and survival, like c-IAP-1, NAIP, survivin, and COX-2. Nevertheless, in cytotoxicity and cell death induction assays, we found that the antitumor activity of curcumin is substantial both in MCF-7 and in MCF-7R. We elaborated the diketone system of curcumin into different analogues; the benzyloxime and the isoxazole and pyrazole heterocycles showed remarkable increases in the antitumor potency both in the parental and in the MDR MCF-7 cells. Furthermore, curcumin or, more potently, the isoxazole analogue, produced early reductions in the amounts of relevant gene transcripts that were diverse (i.e., they were relative to Bcl-2 and Bcl-X_L in MCF-7 and the inhibitory of apoptosis proteins and COX-2 in MCF-7R) in the two cell lines. Thus, the two compounds exhibited the remarkable property of being able to modify their molecular activities according to the distinct characteristics of the parental and MDR cells. We discuss also how curcumin may (1) exert antitumor effects in breast cancer through ER-dependent and ER-independent mechanisms; and (2) act as a drug transporter-mediated MDR reversal agent. Overall, the structure of curcumin may represent the basis for the development of new, effective anticancer agents in hormone-independent MDR breast cancer.

Key words: **breast cancer; multidrug resistance; hormone-independence; curcumin; analogues**

Introduction

Breast cancer is the most frequent cause of cancer-related death in women. Though different options, like hormonal, chemotherapeutic, and targeted agents, are available for its systemic treatment, metastatic breast cancer at presentation or relapse remains eventually incurable, because drug resistance develops. This process can be due to several distinct mechanisms, possibly also co-occurring in the same tumor. They include molecular modifications altering the hormone-responsive status, increased expression of multidrug efflux transporters, and hyperactivation of signaling

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pathways or factors, all of which allow the cells to grow and survive in the presence of different drugs.

Clearly, the multiplicity of the causative determinants renders it difficult to satisfy the need of new therapeutic agents capable of contrasting drug resistance in breast cancer as well as in other tumor types. However, reasonably, multitargeted agents might be more capable than single-targeted ones of performing this task. In this respect, here we shortly review the possible evidences from our and other studies that a phytochemical like curcumin can, owing to its pleiotropic activities, overcome resistance to hormonal and cytotoxic agents in breast carcinoma.

Effects of Curcumin and Analogues

This section discusses the effects of curcumin and its analogues on the breast cancer cell line MCF-7 and its multidrug-resistant (MDR), estrogen-independent variant, MCF-7R. Curcumin (diferuloylmethane), extracted from *Curcuma longa* L. and present in curry spice, has a long story of use in Indian medicine for antiinflammatory and other therapeutic purposes; it has exhibited definite tumor-suppressive and -preventive activities in many *in vitro* or *in vivo* models.[1,2] The compound, like other different plant polyphenols, appears to be endowed with the remarkable property of interfering with a vast array of mechanisms profoundly hyperactivated in transformed cells, thereby allowing for only a limited toxicity for the host.[1,2] The ability of curcumin to selectively induce apoptosis in cancer cells undoubtedly contributes to underlining its high antitumor potential, and many research groups have considered this molecule a very good lead compound in the design of a large variety of analogues as potential new antitumor drugs. Despite the promising results, curcumin has not been approved as a clinical therapeutic agent yet; one major issue to resolve is that of improving through adequate derivatives or formulations the low systemic bioavailability of the drug. On the other hand, the results of the structural modifications have been only seldom investigated within the tumor drug-resistance aspect.[3]

Curcumin is endowed with a diketone function, which appears to be important for its antitumor activity.[4] Depending on its concentration, the compound may show either pro- or anti-oxidant effects, which both may, at least in part, be linked to this structural moiety.[4] In particular, at higher concentrations, the α, β-unsaturated ketone, as a Michael acceptor, can form adducts with the –SH groups and generate reactive oxygen species, which in turn may lead to induction of cell death through different possible mechanisms. Inhibition of the signaling of transcription factors like NF-κB, STATs, and AP-1, as well as suppression of the Bcl-2 family anti-apoptotic factors and of the inhibitory of apoptosis proteins (IAPs), are also considered to be important for the cell death-inducing activities of curcumin.[1,2,5]

We have recently synthesized different curcumin analogues: in particular, the diketonic system of the compound was elaborated into different oximes and in the isoxazole or pyrazole heterocycles.[4,6] The cytotoxic and pro-apoptotic effects of curcumin and of these analogues were studied in the breast cancer cell line MCF-7, in its MDR variant MCF-7R, as well as in the hepatocellular carcinoma HA22T/VGH cell line, which is known to innately produce remarkable amounts of drug resistance and anti-apoptotic factors.[4,6]

MCF-7R was established treating the wild-type cells with gradually increasing concentrations of doxorubicin and eventually developed a stable resistance of about 92-fold to this drug. In MCF-7R, multiple differences in the expression of proliferative, prosurvival, and drug resistance genes with respect to MCF-7 were found. First, they were shown to lack aromatase and estrogen receptor alpha (ERα) and were hormone-unresponsive in assays testing the cytotoxicity of tamoxifen; further, they exhibited overexpression of the multidrug efflux pump

ABCB1, of some IAPs (c-IAP-1, NAIP, and survivin) and of COX-2. In contrast, c-IAP-2 and Bcl-2 were significantly more expressed in the parental cells.[6]

Cell growth inhibition (MTT) assays revealed that the cytotoxic activity of curcumin in MCF-7R is at least equivalent or even slightly stronger than in the parental variant (after 72 h of treatment: the IC_{50} of curcumin was of 29.3 ± 1.7 μM in MCF-7 and of 26.2 ± 1.6 μM in MCF-7R). The benzyl oxime (IC_{50} of 7.1 ± 0.2 μM in MCF-7 and of 9.3 ± 1.7 μM in MCF-7R), isoxazole (IC_{50} of 13.1 ± 1.6 μM in MCF-7 and of 12.0 ± 2.0 μM in MCF-7R), and pyrazole (IC_{50} of 7.3 ± 1.1 μM in MCF-7 and of 2.9 ± 1.0 μM in MCF-7R) derivatives exhibited far more potency than curcumin, again with a comparable or stronger activity in the MDR cell line; these results, which were well confirmed by assaying cell death induction by the compounds, implied that the diketone fragment of curcumin is not indispensable for the growth inhibition and pro-apoptotic activities. The increased potencies of the curcumin analogues were observed also by performing MTT and cell death induction assays in the HA22T/VGH cell line.[4,6]

The molecular effects of curcumin and of the isoxazole analogue were investigated after relatively brief (4 and 8 h) periods of exposure of MCF-7 or MCF-7R cells to the compounds, in order to detect possible early changes responsible for the onset of cytotoxicity and cell death. Curcumin and, more potently, its isoxazole analogue produced clear modifications in the amounts of relevant gene transcripts, which, interestingly, were mostly diverse (i.e., concerning decreases in Bcl-2 and Bcl-X_L and increases in the Bcl-X_S/Bcl-X_L ratio in MCF-7 vs. reductions in the IAPs c-IAP-1, XIAP, and NAIP as well as in COX-2 in MCF-7R) in the two cell lines. There were no significant effects of the treatments on the mRNAs of other factors like ABCB1, ERα, c-IAP-2, livin, Bcl-X_s, Bax, COX-1, c-Myc, cyclin D1, and hTERT in either cell line.[6]

Discussion

Overall, our results have indicated that the growth-inhibitory and cell death-inducing effects of curcumin and of its more potent analogues are not reduced or even increased by the drug-resistant and estrogen-independent condition of the breast cancer MCF-7R cells.

MCF-7R overexpresses the ABCB1 multidrug efflux pump, and different authors have found that the antitumor activity of curcumin is not affected in tumor cells endowed with this relevant drug resistance mechanism so frequently expressed in refractory breast cancer.[3,7–10] Of note also, when compared to normal human mammary epithelial cells, another MDR variant of MCF-7, the MCF-7/TH cell line, showed hypersensitivity to the cytotoxic and apoptotic effects of curcumin, thus underlining the potential chemotherapeutic index of the compound.[7]

It must be added that inhibitory effects of curcumin on the function of ABCB1, as well as of other drug transporters, like MRPs (ABCC1 and others) and BRCP (ABCG2) have been reported; they were associated with sensitization of MDR tumor cells to established substrates of these efflux pumps, like vinblastine, etoposide, and mitoxantrone.[3] Although curcumin is not itself extruded by ABCB1, its MDR reversal effects can depend on direct interactions with the pump with inhibition of substrate binding or of ABCB1 ATPase activity. Downregulation of the *ABCB1* gene expression may also occur as a mechanism of curcumin in certain MDR cells.[3] However, compared to other MDR reversal agents, like verapamil, curcumin displays low potency, leading to the search of curcumin mimics with improved MDR reversal activities.[11] Compatible with these last findings, in contrast to what seen with verapamil, we have not observed any reversal activity of ABCB1-mediated resistance to doxorubicin by curcumin in MCF-7R;[6] analysis of the MDR reversal activities of our diketone-modified analogues is in progress.

On the other hand, curcumin has revealed an impressive ability to chemosensitize tumor cells to many different anticancer drugs through several other mechanisms that do not depend on the inhibition of drug transporters function or expression; one of the activities that has been particularly frequently put forth to explain chemopotentiation from curcumin is its interference with the signaling of NF-κB.[12] We ourselves have previously reported on the fine synergy that occurs between curcumin and cisplatin in hepatic carcinoma cells showing constitutive activation of this transcription factor.[5]

MCF-7R was shown to lack aromatase and ERα expression, as well as responsiveness to anti-estrogens, a characteristic already described in MDR variants of MCF-7;[13] speculatively, the finality of such a co-selection might be explained by the recent observation that estrogen signaling is able to downregulate posttranscriptionally ABCB1 in the same cells.[14] Interestingly, some studies have shown that curcumin may exert antitumor effects in both estrogen receptor-positive and -negative breast cancer cells through, respectively, ER-dependent and -independent gene suppression mechanism.[15,16] It also known that curcumin may inhibit a phosphorylation step on ERα necessary for its binding to its cognate DNA targets.[17] Notably, also in prostate cancer the therapeutic potential of curcumin has been reported to encompass both androgen-dependent and androgen-independent tumor cells.[18,19]

We performed immunocytochemistry analyses of ERα expression in MCF-7 cells after incubation with curcumin or its isoxazole analogue at 25 μM for 24 h; these analyses showed that the compounds almost zeroed the number of ERα-positive cells, suggesting indeed their interference with the growth of the estrogen-responsive cell population. For the explanation of the results in the MCF-7R ERα-negative cells, we took in consideration the possible effect of curcumin on transcription factors like NF-κB and STATs, since it is well-known that they can upregulate the expression of the genes specifically affected (the IAPs and COX-2) by the compound in the same cells; on the other hand, as stated before it is well-documented that curcumin can inhibit at different stages the activation of NF-κB or STAT3.[1,2,5,6,12]

Nevertheless, we found that the very low nuclear levels of activated NF-κB or STAT3 present in MCF-7 underwent only slight, though significant, elevations in MCF-7R. Moreover, curcumin or the isoxazole analogue did not cause changes in the activation of NF-κB or STAT3 that could account for their different effects on gene expression in the two cell lines. Thus, alternative mechanisms remain to be examined, considering that reportedly, curcumin may influence gene transcription by other means, including interference with other transcription factors (e.g., AP-1) as well as with the processes of histone acetylation.[1,2,20,21] Such further investigations might also shed more light on the (not yet completely defined) processes that regulate the expression of the IAPs and other drug resistance factors. On the other hand, clearly, the antitumor effects of curcumin may not only depend on influences on gene transcription, but also involve other mechanisms, including direct interactions with specific target molecules; there are also some cases (e.g., like that concerning COX-2) in which the agent can affect both the function and the expression of a given molecule.[1,2,12,22] Also, these further aspects are worth investigating in our breast cancer cell model.

In conclusion, our and other researchers' studies have shown that curcumin exhibits the remarkable property of modifying its molecular activities according to the distinct characteristics of the parental and MDR, estrogen-independent, breast cancer cells. Our data show also that relatively small modifications can give access to compounds presenting antitumor potencies much superior to those of curcumin also in drug refractory cells. This result is also encouraging, considering

the low systemic bioavailability of the parent compound.

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Conflicts of Interest

The authors declare no conflicts of interest.

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