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Review

Relationship between the *in vitro* efficacy, pharmacokinetics and *in vivo* efficacy of curcumin

Gordon T. Bolger [a], Kresimir Pucaj [b], Yvonne O. Minta [c], Peter Sordillo [d]

[a] GO-TO-PK SOLUTIONS, Ajax, ON, Canada
[b] CroToxPath Consulting, Toronto, ON, Canada
[c] Nucro-Technics, Department of Toxicology, Toronto, ON, Canada
[d] SignPath Pharma, Sandy, UT, United States

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ABSTRACT

Considerable interest continues to be focused on the development of curcumin either as an effective stand-alone therapeutic or as an adjunct therapy to established therapies. Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5- dione; also called diferuloylmethane) is a polyphenolic phytochemical extracted from the root of curcuma longa, commonly called turmeric. Despite evidence from in vitro (cell culture) and preclinical studies in animals, clinical studies have not provided strong evidence for a therapeutic effect of curcumin. The relevance of curcumin as a drug has been questioned based on its classification as a compound with pan assay interference and invalid metabolic panaceas properties bringing into question the relevance of the therapeutic targets identified for curcumin. To some extent this is due to the lack of a complete understanding of the link between the in vitro (cell culture activity), pharmacokinetics and *in vivo* activity of curcumin. In this review and using NF-κB as a cellular target for curcumin, we have investigated the relationship between the potency of curcumin as an inhibitor of NF-κB in cell culture, the pharmacokinetics of curcumin and curcumin's anticancer and anti-inflammatory effects in preclinical models of cancer and inflammation. Plausible explanations and rationale are provided to link these activities together and suggest that both curcumin and its more soluble Phase II metabolite curcumin glucuronide may play a key role in the treatment effects of curcumin *in vivo* mediated at NF-κB.

1. Introduction

Considerable interest continues to be focused on the development of curcumin either as an effective stand-alone therapeutic or as an adjunct therapeutic to established therapies. According to PubMed, between 2020 and 2022, there were 5,006 citations for curcumin either as well written reviews or as research articles. Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione; also called diferuloylmethane, Fig. 1) is a polyphenolic phytochemical.

extracted from the root of curcuma longa, commonly called turmeric. Curcumin, the active principle of turmeric, is a bis-R,-unsaturated diketone which exhibits keto–enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium. Commercial curcumin contains approximately 77 % diferuloylmethane, 17 % demethoxycurcumin, and 6 % bisdemethoxycurcumin, all active curcuminoids [1,2]. Potential therapeutic applications for curcumin have been suggested on the basis of its pleotropic activity in *in vitro* and *in vivo assays*. The therapeutic potential for curcumin has been detailed in a number of comprehensive reviews [1–4 and references cited therein]. In brief, curcumin has shown promise as an antioxidant, anti-inflammatory, anti-infective, and anticancer agent. Curcumin has been shown to protect against liver, kidney and heart damage, suppress clot formation and possess neuroprotective activity in neurodegenerative and neurological disorders such as

Abbreviations: BSA, Bovine serum albumin; C_{av}, Average plasma concentration; CHO, Chinese Hamster Ovary; Cur, Curcumin; CGluc, Curcumin glucuronide; CXCL, C-X-C motif chemokine 5; CXCR, Chemokine receptor; Diff, Diffusion; DNA, Deoxyribonucleic acid; ED50, effective dose generating a 50% response; β-Glrndase, β-Glucuronidase; HSA, Human serum albumin; IL, Interleukin; IP, Intraperitoneal; IV, Intravenous; LogP, Logarithm of the partition coefficient between an aqueous and lipophilic phase; μM, Micromolar; MCP-1, Monocyte chemoattractant protein-1; MTX, methotrexate; NTHi, Non-typeable Haemophilus Influenzae; NF-κ-B, nuclear factor-κ-B; OM, otitis media; OAT, Organic anion transporter; OATP, Organic cation transporter polypeptide; OCT, Organic cation transporter; PAINS, Pan assay interference; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PP, Plasma proteins; ROS, reactive oxygen species; THC, tetrahydrocurcumin; TNF-α, tumour necrosis factor-α; TSS, toxic shock syndrome; TSST-1, toxic shock syndrome toxin-1

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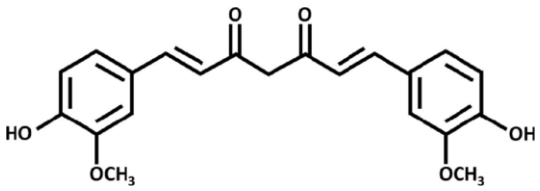

Fig. 1. Structure of Curcumin.

Alzheimer's disease, Parkinson's disease, and Huntington's disease. Furthermore, curcumin has been shown to limit cytokine release and is of potential benefit in the treatment microbial infections mediated by bacteria and viruses.

Despite evidence from *in vitro* (cell culture) and preclinical studies in animals, clinical studies have not provided strong evidence for a therapeutic effect of curcumin [3]. This is likely due to its poor pharmacokinetic properties linked to unattractive physical chemical properties such as poor aqueous solubility at physiological pH, chemical instability, metabolic instability, poor oral absorption and rapid elimination all of which contribute to low systemic concentrations and limited blood–brain barrier permeability [2,3 and references cited therein]. As a consequence, several attempts have been made to improve systemic levels of curcumin following dosing using formulation approaches that range from complexation with adjuvants and inclusion in nanoparticles, liposomes, and phospholipid micelles to the design of novel analogs with better oral absorption [4,5]. More recently, a number of investigators have claimed that the lack of efficacy by curcumin seen in numerous clinical trials is due to the fact that it is a Pan-Assay Interference (PAINS) compound, which frequently gives false-positive results during testing [3,6]. A characteristic of this type of compound is that it is not directed toward a single molecular target, but against many, disparate, targets or diseases. We have noted that NF-κB (Nuclear factor kappa B) is suppressed by curcumin in inflammation, cancer and many other diseases [7–9]. Others have made similar arguments, noting curcumin can affect many different genes [10,11]. Recent clinical studies have shown that the failure of earlier clinical trials using curcumin compounds was due to the inability in these studies to achieve adequate blood levels of curcumin. Indeed, curcumin's poor systemic exposure following oral administration suggests that there is a need for higher and more sustained plasma levels of curcumin to optimize its treatment effects. Thus, it has been suggested that high exposure of the gastrointestinal tract to curcumin following oral administration may provide therapy with respect to gastrointestinal cancers and intravenous administration of formulated curcumin may provide high enough plasma levels of curcumin for systemic treatment [3]. Greil et al, in a cancer study using escalating doses of an intravenously administered liposomal preparation of curcumin, were able to achieve blood levels 1000-times that of standard oral curcumin [12]. A clear dose–response was seen, with reductions in cancer markers (carcinoembryonic antigen, prostate-specific antigen) seen only at the highest doses of liposomal curcumin administered. Furthermore, as a consequence of its poor pharmacokinetics, there has been considerable speculation as to whether curcumin or its metabolites are responsible for the *in vivo* activity of curcumin [2,13]. It is therefore important to gain a better understanding of the relationship between targets identified for curcumin *in vitro* (cell culture assays), curcumin's pharmacokinetics and the activity of curcumin at its targets *in vivo*.

In this review, we have investigated the nature of the relationship between the *in vitro* (cell culture) efficacy, pharmacokinetics and *in vivo* efficacy of curcumin in preclinical models of cancer and inflammatory disease. As part of this investigation and for simplicity, we have made the assumption that curcumin's most potent treatment effects are associated with the parent molecule and are less likely associated with its metabolites, although clearly, several of curcumin's metabolites (i.e. tetrahydrocurcumin (THC)) do possess some bioactivity [3]. NF-κB was chosen as the target both in cell culture and *in vivo* given the fact that curcumin has been well researched as an inhibitor of NF-κB [7–9,14 and references cited therein] and there exists a substantial amount of information in the literature pertaining to curcumin's inhibition NF-κB mediated responses in cell culture and its anticancer and anti-inflammatory effects *in vivo*. Plausible explanations and rationale are provided to link the cell culture activity of curcumin at NF-κB with its pharmacokinetics and *in vivo* treatment efficacy in preclinical models of cancer and inflammation.

2. Evidence for an interaction of curcumin with NF-κB: Studies in cell culture

The nuclear transcription factor NF-κB is a transcription factor found in the cell cytosol of a wide variety of cells and regulates the expression of distinct sets of genes that encode proteins involved in mediating a number of cellular functions including, cellular proliferation, inflammatory responses, and cell adhesion [15]. It is therefore a target of interest for the development of anticancer and anti-inflammatory drugs. The functionally active NF-κB exists mainly as a hetero-dimer consisting of p50 and the proto-oncogene rEL-associated proteins, which are normally sequestered in the cytosol as an inactive complex by binding to the inhibitory protein IκBα. Phosphorylation and subsequent ubiquitination of IκBα upon exposure of cells to various extracellular stimuli leads to a rapid degradation of this inhibitory subunit by proteosomes. The resulting free NF-κB translocates to the nucleus, where it binds to the specific κB binding sites that are located in the promoter region of the target genes, thereby controlling their expression [16–18].

A number of studies in cultured cells have either provided evidence either for curcumin's direct inhibition of NF-κB or curcumin's inhibition of the expression of gene products regulated by NF-κB (i.e. cytokines/chemokines). Generally, the potency of curcumin to inhibit these processes ranges from the low to high micromolar concentrations that are not associated with a cytotoxic effect of curcumin. Data concerning curcumin's direct inhibition of NF-κB and inhibition of cytokine/chemokine release is presented in Table 1. Other studies not included in Table 1 have also characterized the inhibition of NF-κB by curcumin. Curcumin at concentrations ranging from 1 to 10 μM was found to inhibit 12-Otetradecanoylphorbol-13-acetate-induced activation of NF-κB by preventing the degradation of the inhibitory protein IκBα and the subsequent translocation of the rEL-associated subunit to the nucleus in cultured human promyelocytic leukemia cells and by a direct interruption of the binding of NF-κB to its consensus DNA sequences [15]. Using an in vitro model of human tenocytes, curcumin at concentrations of 5–20 μM inhibited IL-1β-induced inflammation and apoptosis in cultures of human tenocytes by blocking NF-κB mediated down-regulation of gene products that mediate matrix degradation (matrix metalloproteinase-1, -9, and -13), prostanoid production (cyclooxygenase-2) and apoptosis (B-Cell lymphoma-2 associated X Protein and activated caspase-3) [19]. In the same study, curcumin at a concentration of 20 μM suppressed IL-1β-induced NF-κB activation via inhibition of phosphorylation and degradation of inhibitor IκBα, inhibition of IκB-kinase activity, and inhibition of the nuclear translocation of NF-κB. Using, quantitative PCR and flow cytometry, curcumin (20 μM) was shown to increase the chemotherapeutic effects of prednisone, 6-mercaptopurine, dexamethasone, cyclophosphamide, l-asparaginase, vincristine, daunorubicin, doxorubicin, methotrexate and cytarabine in an acute lymphoblastic leukemia cell line via enhancement of caspase-3 and downregulation of nuclear NF-κB activation.

Taken together, studies in cultured cells indicate that curcumin produces direct inhibition of NF-κB resulting in an inhibition of NF-κB - dependent cytokine/chemokine gene expression.

Of particular importance with respect to the inhibition of NF-κB by curcumin is the dose-dependence of its inhibition. Unfortunately, many

Table 1
Decreased NF-κB Activation and Cytokine and Chemokine Release Mediated by Curcumin in Cell Culture.

Cultured Cells and Treatment	Endpoint	Concentration of Curcumin and Inhibition of NF-κB	Reference
NF-κB			
Breast carcinoma	Activation of NF-κB	14 μM; 18 and 37 %↓ NF-κB activation in two breast cancer cell lines	[29]
Mouse macrophages		10, 30, 50 and 100 μM; 24, 30, 38 and 62 %↓ NF-κB activation, respectively	[14]
Human monocytes		10, 30 and 100 μM pre-exposure; 12, 16 and 46 %↓ NF-κB activation upon exposure to stimulus, respectively	[23]
Intestinal epithelial cells		100 μM; 96 %↓ NF-κB activation	[24]
Prostate cancer cells		15 μM; 20 %↓ NF-κB activation	[25]
Intestinal epithelial cells		6.8 μM; 100 %↓NF-κB activation by either cytosine arabinoside or MTX	[26]
Human Neutrophils		50 μM; 69 %↓ NF-κB activation	[27]
Cytokine and Chemokine Release			
Mouse macrophages	TNF-α, IL-1β and IL-6 release in response to lipopolysaccharide challenge	5, 10 and 50 μM; 0, 4 and 95 %↓ TNF-α, 2, 47 and 93 %↓ IL-1β, 8, 62 and 98 %↓ IL-6, respectively.	[14]
Mouse macrophages	IL-2 and TNF-α release in response to concanavlin A and lipopolysaccharide	30 μM; 65 %↓ IL-2, 85 %↓ TNF-α	[20]
Middle ear epithelial cells infected with non-typeable haemophilus influenzae	Upregulation of chemokine ligand 5 (CXCL5) following infection with non-typeable haemophilus influenzae	10, 20 and 50 μM; 26, 53, and 89 %↓ CXCL5, respectively.	[22]
Mouse splenic macrophages treated with Lipopolysaccharide	IL-12 production	1.4, 2.7, 6.7 and 13.6 μM; 18, 39, 65 and 74.6 %↓ IL-12, respectively.	[22]
Human monocytes	IL-1, IL-6, TNF-α production	30 and 100 μM; 24 and 43 %↓ IL-1; 21 and 64 %↓ IL-6; 10, 30 and 100 μM; 46, 68 and 88 %↓ TNF-α	
Intestinal epithelial cells	IL-1 mediated IL-8 secretion	75 and 100 μM; 59 and 86 %↓ IL-8, respectively.	[24]
Prostate cancer cells	Chemokine receptor-2 (CXCR-2) elevation	15 μM; 59 %↓ CXCR-2	[25]
Intestinal epithelial cells	MCP-1, TNF-α production	6.8 μM; 99 %↓ MCP-1 and 42.3 %↓ TNF-α	
Human Neutrophils	IL-8 production	50 μM; 66 %↓ IL-8	[27]
Pancreatic carcinoma cell lines	IL-8 release	At IC_{50} concentrations of curcumin for inhibition of cell growth in five cell lines of 5, 2.5, 2, 37.8, 6.75 and 4.8 μM; 27, 61, 36, 73, 77 and 27 %↓ IL-8, respectively	[28]

↓-Inhibition.

of the studies mentioned above used either a single or two concentrations of curcumin. However, for the studies conducted by investigators employing mouse macrophages [14], *T*-lymphocytes and macrophages [20], splenic macrophages [21], middle ear epithelial cells [22] and human monocytes [23], curcumin decreased NF-κB activity in a concentration-dependent manner. To further explore the concentration-dependence of the effects of curcumin in cell culture, the information presented in Table 1 was illustrated by constructing scatterplots of the concentration of curcumin in cell culture versus the curcumin mediated percent decrease of either direct activation of NF-κB or NF-κB cytokine/chemokine release (Fig. 2). Both for direct inhibition of NF-κB and inhibition of cytokine/chemokine release, lower concentrations of curcumin produced a wide range of inhibition (lower to higher) which tended clearly towards a higher inhibition as the cell culture concentrations of curcumin increased. This suggests that a concentration-dependence for the direct inhibition of inhibition NF-B and of cytokine/chemokine release by curcumin does exists, the latter possibly reaching an apparent maximum with increasing concentrations of curcumin. The similar concentration range of curcumin for direct inhibition of NF-κB

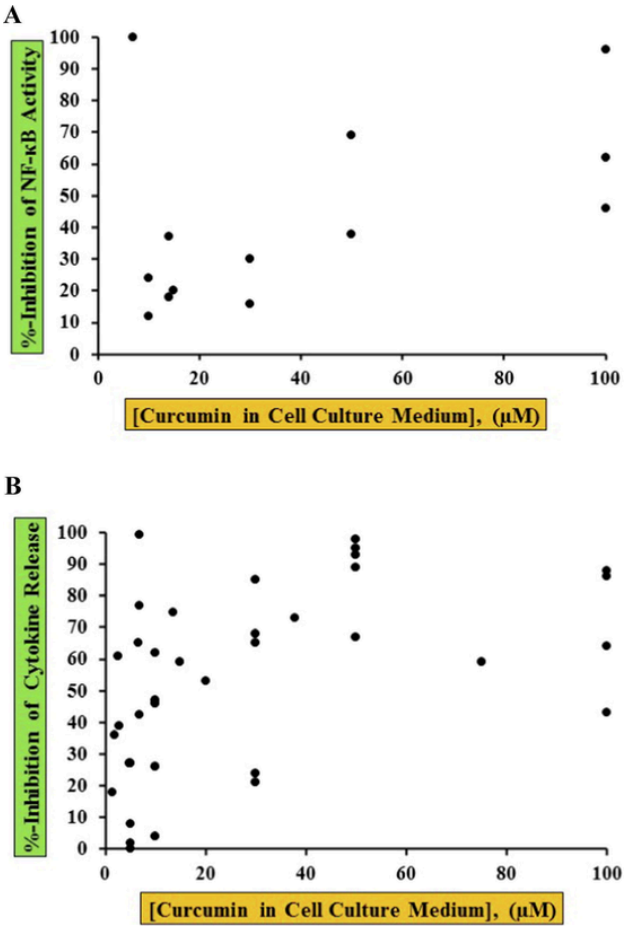

Fig. 2. Relationship Between the Concentrations of Curcumin in Cell Culture and Either the Effect of Curcumin on NF-κB or Cytokine Release. The concentrations of curcumin in cell culture shown in Table 1 were plotted against the curcumin mediated decreases of activation of NF-κB (panel A) or cytokine release (panel B).

and cytokine/chemokine release is consistent with the role of NF-κB in controlling the expression of cytokine genes in the cell. The analysis presented here is also supported by several studies indicating a concentration-dependent effect for curcumin at NF-κB.

In addition to demonstrating that curcumin could inhibit NF-κB in cell culture in a concentration-dependent manner, results from several studies suggest that the inhibition of NF-κB by curcumin may be long-lived and possibly irreversible. This property of curcumin was clearly demonstrated by the work of Kang et al. [21] using *T*-lymphocytes and macrophages. In addition, an apparent long-lived/irreversible activity was also noted for curcumin with respect to the growth arrest of chronic myelogenous leukemia lymphoblastic K562 cells as a consequence of curcumin's activity on the ROS (reactive oxygen species) metabolic pathway. In this study, curcumin and imatinib both were shown to suppress the proliferation of K562 cells. However, after removal of compound, cells treated with imatinib started to proliferate again, whereas curcumin-treated cells remained growth-inhibited and gradually lost their viability [30]. The potential for a long-lived/irreversible interaction of curcumin at the cell level may figure critically in the relationship between the pharmacokinetics of curcumin and its efficacy at NF-κB *in vivo*. No clear mechanism for the production of a long-lasting inhibition of NF-κB was presented, however, it has been speculated that curcumin may chemically react and irreversibly modify the proteins it associates with (i.e. via alkylation of critical amino acid residues) [2,4,31]. Curcumin's potential to produced a long lived/irreversible interaction at its target may contribute to the apparent potent effect of curcumin observed in some cell culture studies. In summary, in cell culture curcumin directly inhibits NF-κB and NF-κB mediated expression of cytokines and chemokines concentration-dependent manner and at concentrations ranging from 1 to 100 μM. Curcumin also has the potential to produce a long-lasting inhibition of NF-κB.

3. Cellular uptake of Curcumin: Impact on the inhibition of NF-κB

Of relevance to a discussion of the relationship between the cell culture concentration-dependent activity of curcumin as a NF-κB inhibitor and curcumin's activity *in vivo* is a discussion of what is currently known about the cellular uptake of curcumin. From the preceding analyses, it was observed that curcumin inhibited NF-κB and NF-κB dependent cytokine release over a concentration range of 1–100 μM. Given this concentration range, it is of importance to consider the aqueous solubility of curcumin, as it is the soluble form of curcumin which would be taken up by cells in culture. Published data exist on the water solubility of curcumin where values ranging from 11 ng/mL [32] to 1340 ng/mL [33] (0.030 μM–3.64 μM) have been reported; the large range of solubilities likely related to the crystal structure of the starting material (i.e. either amorphous or mixture of amorphous and crystalline curcumin). In the Pubchem data base curcumin, is listed as having a LogP of 3.29 and is referred to as a "fat soluble" dye, ideal for targeting the plasma membrane of the cell. It is readily soluble in solvents such as DMSO, ethanol and vegetable oil and in basic solutions, but is chemically unstable in basic solution and more stable in acidic solutions [34]. Additionally, there is evidence that in pure water curcumin both aggregates [35] and can self associate to form dimeric structures [36] possibly limiting its ability to permeate cells. Given its limited solubility in water, the presence of bovine albumin (BSA) in cell culture medium (at concentrations from 55 to 80 μM) likely aids in increasing the solubility of curcumin in cell culture medium (at concentrations up to 100 μM) due to its reported 1:1 stoichiometric binding with serum albumin at both high and low affinity binding sites [37]; binding to BSA altering, but not completely preventing the ability of drugs to form aggregates [38]. Thus, curcumin's limiting aqueous solubility, potential to aggregate and form multimeric structures and binding to BSA are just some of the barriers against its ready uptake into cells.

One way for curcumin to enter cells is simply by diffusion. However, this route is hindered for larger molecular weight compounds that have a high degree of plasma protein binding. Curcumin has a molecular weight of 368.38 Daltons, large enough to hinder ready passage through the plasma membrane and it binds to plasma proteins further limiting its diffusion into cells. Studies utilizing fluorescence spectroscopy and both normal and cancerous cells provided additional information into the diffusion of curcumin into cells following treatment with either an aqueous solution of curcumin, curcumin bound to human serum albumin (HSA) or curcumin complexed in phosphatidyl choline containing liposomes [39,40]. Four hours of incubation with 5 to 10 nmoles of curcumin per million cells resulted in differing cellular locations for curcumin. For non-cancerous cells curcumin was clearly located in the cell membrane and the nucleus, while for the cancerous cells with much larger nuclei and little cytoplasm, curcumin was located throughout the cell. In general, a liposomal preparation of curcumin delivered more curcumin into the cells then when either complexed with HSA or added as free curcumin. Cancerous cells had higher levels of curcumin consistent with their larger nuclei; higher levels of curcumin were also observed in peripheral blood mononuclear cells (PBMC) isolated from patients with chronic lymphocytic leukemia compared to PBMC isolated from patients with chronic lymphocytic leukemia [41]. Thus, for diffusion of curcumin into cells, it would appear that the initial step in gaining entry into the cell would be the association of curcumin with the cell membrane followed by diffusion out of the membrane to the cytoplasm and subsequently localization to the nucleus with the overall uptake and distribution of curcumin within the cell dependent on the cell type.

More recent studies have indicated that not only curcumin, but its major metabolites, THC (tetrahydrocurcumin) and curcumin glucuronide may also access cells by another route, active uptake, using the organic anion transporter polypeptide (OATP) as well as the organic anion transporter (OAT). These routes of cell uptake can handle a wide variety of compounds of diverse molecular weight and varying degrees of plasma protein binding. Both the OATP and OAT transporters have been well studied and exist ubiquitously in a variety of cells [42]. Several organs, such as the liver, kidney and lung have particularly high levels of these transporters [43–45]. In a very eloquent study, it was demonstrated in human embryonic kidney cells transfected to express human OAT and OATP transporters that the rate of cellular uptake of both curcumin and curcumin glucuronide were increased compared to non-transporter expressing cells [46]. In addition, in the same study, both curcumin and curcumin glucuronide inhibited rosuvastatin uptake by OATP-1B1 and OATP-1B3 and increased the plasma concentration of rosuvastatin upon coadministration in rats and dogs. Evidence was also presented for the uptake of curcumin sulfate into transfected cells. In a more recent study however, transfected Chinese Hamster Ovary (CHO) cells expressing OATP-1B1 and OATP-1B3 transported curcumin, THC and curcumin sulfate, but the transport of curcumin glucuronide was not detected [47]. The method of detection in the latter study was HPLC UV detection compared to HPLC tandem mass spectrometry in the earlier study. The use of a lower sensitivity HPLC method may have resulted in an inability to detect curcumin glucuronide in CHO cells expressing OATP transporters as the rate of transport of curcumin glucuronide by OATP expressing cells was much lower than for curcumin [46]. Nonetheless both of these studies provide evidence that curcumin and its metabolites are substrates for cellular uptake transporters.

As a consequence of these more recent studies, the entry of curcumin (as well as its metabolites) into cells is likely by two major routes, diffusion and active transport. Clearly active transport will be the most effective way for curcumin to gain entry into the cell particularly for lower extracellular concentrations of curcumin. Irrespective of how curcumin gains entry into the cell, its presence in the cytosol and localizing to the nuclei clearly favour the two major sites of interaction for curcumin, inhibition of the phosphorylation of the NF-κB/IκBα com-

plex in the cytosol and inhibition of the binding of NF-κB to DNA. As was outlined previously, from cell culture studies, the concentrations of curcumin that inhibited the activity of NF-κB did so at concentrations ranging from 1 μM to 100 μM, with incubation times ranging from 1 to 96 h. This rather large range of concentrations might be related to how curcumin gains entry into cells, either by diffusion or active uptake, the latter being dependent on how well uptake transporters are expressed in the cell lines used to conduct the study. As a general rule, uptake transporters are either not expressed or expressed at a low level in cultured cell lines; their expression highly dependent on the conditions used to culture the cells. This fact played largely into the reason to use primary cultures of hepatocytes as opposed to cell lines to design a model to predict the human liver partitioning of drugs [48]. Implementation of the use of the human hepatocyte transformed cell line, HepG2, in the design of the model was ruled out due to, amongst other differences from human hepatocytes, HepG2 cells failing to display OATP-1B1, OATP-1B3, the organic cation transporter (OCT-1), and the sodium taurocholate co-transporting polypeptide, in quantifiable levels [49]. Thus, transformed cell lines either may not or only partially express the uptake transporters now implicated in the cellular uptake of curcumin and therefore the cellular uptake of curcumin into transformed cells may be largely diffusional in nature. The impact of this mode of cell entry by curcumin on its activity in cell culture activity can be predicted as follows. As discussed previously, curcumin a relatively large molecule, is solubility limited in an aqueous environment and is protein bound. Its preferential binding to proteins would restrict its diffusion across the cell membrane. To reach effective enough concentrations of curcumin at NF-κB either in the cytosol or in the nucleus within the incubation time of the experiment, the applied concentration of curcumin would have to be high enough (in this case in the upper μM range) to drive curcumin into the cells. Thus, the building up of sufficient quantities of curcumin into the cell membrane for diffusion into the cell (a time and concentration-dependent process) appears to be a critical limiting step for curcumin's cell culture activity. The potential for curcumin to utilize the plasma membrane as a "reservoir" for maintaining intracellular concentrations of curcumin may contribute to the long-lasting effects of curcumin in cell culture. Furthermore, the differing composition and surface area of the plasma membrane in different cells, may contribute how much curcumin can be incorporated into the plasma membrane and how much curcumin will enter into cells resulting in varying efficacies for inhibiting NF-κB dependent cellular processes. These premises are supported by several studies determining the effects of curcumin on NF-κB dependent cellular proliferation. Curcumin produced a concentration-dependent inhibition of cellular proliferation in six pancreatic carcinoma cell lines designated as, AsPC-1, BxPC-3, Capan-1, Capan-2, HS766-T and MκaPaCa2 with ED_{50} values of 5.0, 2.5, 2.0, 37.8, 6.75 and 4.8 μM, respectively [28]. In general, curcumin inhibited cellular proliferation more potently with longer incubations times (72–96 hrs: ED_{50} values ranging from 2.0 to 37.8 μM [20, 28] compared to shorter incubations times (1–48 hrs, where either no effect was observed or ED_{50} values of ~25 μM and greater were observed. Therefore, cell type and exposure time play a critical role in curcumin's inhibition of NF-κB dependent cellular processes, a phenomenon more consistent with the diffusion of curcumin into the cell as opposed to active transport.

While the activity of uptake transporters in cultured cells cannot be completely ruled out, the implications would be that active uptake of curcumin into cells would reduce the extracellular concentration of curcumin required to inhibit NF-κB. In fact, in the previous cell culture activity data presented for curcumin, there was a high variability of cell activity (little to marked activity) for curcumin at lower concentrations (i.e. 1–10 μM). This may be linked to the expression of uptake transporters on cells leading to a marked activity of curcumin or a lack of uptake transporters and diffusion dominated cell uptake leading to lower cell activity for curcumin. As the cell culture concentrations of curcumin increased, higher cell activity for curcumin predominated due to adequate permeation of curcumin into the cell by diffusion in addition to active uptake. The potential for multiple pathways for curcumin to gain access into cells provides a plausible explanation for the observation of variable responses of NF-κB to curcumin at lower concentrations in cell culture.

4. *In vivo* interaction of curcumin with NF-κB: Animal studies

Evidence also exists from animal studies indicating that oral and parenteral dosing with curcumin leads to the inhibition of NF-κB dependent-cellular processes and in some cases thorough studies have been performed to show inhibition of NF-κB dependent processes in cell culture (see studies cited above) that correlate with the proliferation of cancer in animals. However, as will be discussed, these significant effects at NF-κB *in vivo* occur at very low measured concentrations of curcumin in plasma.

Otitis media (OM) is a common childhood bacterial infection and leading cause of conductive hearing loss. Non-typeable Haemophilus influenzae (NTHi) is a major bacterial pathogen for OM. Consistent with the effects of curcumin on middle ear epithelial cells infected with NTHi, either pre-treatment of C57/BL6 mice for 1 h by intraperitoneal (IP) injection of 50 mg/kg curcumin followed by *trans*-tympanic inoculation with NTHi or treatment with curcumin (50 mg/kg) (IP) 1 h after NTHi, inhibited NTHi-induced CXCL5 expression *in vivo* [23]. Curcumin suppressed CXCL5 expression by direct inhibition of the phosphorylation of the NF-κB IκB subunit and inhibition of p38 via induction of the negative regulator mitogen activated protein kinase-1. In a rabbit model of toxic shock syndrome (TSS), instillation of 1.62 μmoles of curcumin into the vagina of rabbits protected against the lethal effects of TSS mediated by intravaginal administration of toxic shock syndrome toxin-1(TSST-1) toxin [50]. Many of the vaginal epithelial genes that are upregulated in response to TSST-1 are known NF-κB -regulated genes [50]. *Ex-vivo* analysis of vaginal tissue from rabbits treated with curcumin showed low levels of TNF-α compared to control rabbits and ~13 nmoles of curcumin remaining 70 h following treatment. These findings indicate that low topical exposure of vaginal tissue to curcumin resulted in significant protective effects against the lethal effects of TSS toxin and resulted in reductions of cytokine production mediated by NF-κB. Employing female rats and a model of chemotherapy induced intestinal mucosal barrier induced by methotrexate (MTX) and mediated by NF-κB generated mediators of inflammation, the induction of NF-κB -related cytokines and chemokines was detected upon treatment with MTX [26]. Curcumin administered in the diet at a dose of 2.5 mg/kg/day for four days inhibited NF-κB driven cytokine production and ameliorated mucosal injury following MTX administration. In BALB/c mice infected with Klebsiella pneumoniae B5055 by intranasal instillation, fifteen days of oral treatment with curcumin at 150 mg/kg/day resulted in a significant decrease in neutrophil influx into the lungs and a significant decrease in the production of malondialdehyde and nitrous oxide. As well curcumin treatment reduced the activity of myeloperoxidase and decreased TNF-α levels in the absence of a decrease in bacterial load [51]. Using an orthotopic murine model of ovarian cancer [52] employing athymic mice the effects of oral curcumin (suspended in Sesame seed oil) at doses ranging from 100 to 2000 mg/kg were assessed on the modulation of NF-κB, angiogenic cytokines, cellular proliferation, angiogenesis and apoptosis. *In vitro* experiments using human ovarian cancer cell lines and monitoring NF-κB activity were also conducted. Curcumin (10 μM) was found to inhibited inducible NF-κB activation in response to TNF-α and suppressed tumor cell proliferation. *In vivo* dose-finding experiments revealed that 500 mg/kg curcumin administered orally for two days was the optimal dose needed to suppress NF-κB and signal transducers and activators of transcription 3-activation and decrease angiogenic cytokine expression. Furthermore, oral dosing for 19 days with 500 mg/kg of curcumin twice per day re-

sulted in a 44%–53 % reduction of mean ovarian tumor growth compared to controls was observed. As a consequence of NF-κB's role in in regulating cell growth and proliferation, the ability of curcumin to be antiproliferative and pro-apoptotic in pancreatic cells lines and to reduce the growth of pancreatic tumors in mice was investigated [28]. *In vivo,* in female athymic nu/nu mice (3–5 weeks old), intravenous (IV) administration of 40 mg/kg of liposomal curcumin three times per week for two weeks resulted in a suppression the growth of pancreatic cell tumours and a down regulation of NF-κB dependent markers of tumor angiogenesis.

Taken together, these studies indicate that curcumin when administered to animals can inhibit the activation of NF-κB-dependent processes *in vivo* and comparatively in cell culture. As mentioned above, the effects of curcumin at NF-κB following systemic administration are observed at low plasma concentrations of curcumin. Thus, it is important to gain a further understanding of the relationship between the plasma levels of curcumin, its *in vivo* treatment effects and how these relate to curcumin's concentration-dependent effects as an inhibitor of NF-κB in cell culture.

5. Pharmacokinetics and tissue and cell distribution of curcumin

The pharmacokinetics of curcumin following both oral and parenteral routes (IP and IV) of administration have been extensively investigated both in preclinical animal models and humans and have been well described in several key reviews [2,3]. In brief, following oral administration, the plasma levels of curcumin attained in the plasma are very low as a consequence of poor absorption. Curcumin's poor absorption is due its low solubility and the metabolic instability of curcumin during absorption process at the brush border membrane where extensive Phase II glucuronidation of curcumin occurs mediated primarily by intestinal UGT1A8 and UGT1A10, [13,53,54]. Once absorbed into the circulation and into the liver and likely other tissues, plasma curcumin undergoes extensive phase I metabolism (i.e., action of reductase enzymes on alkene bonds resulting in the conversion of curcumin into THC, as well the reduction of ketone groups to form various alcohol metabolites all of which possess varying degrees of biological activity). Plasma curcumin is also subjected to Phase II metabolism, primarily glucuronidation by UGT1A1 in the liver [54] and a much smaller component of sulfation [47–49] followed by active transport into the blood and excretion in the urine. While biologically inactive, the glucuronides of curcumin can be deconjugated to yield active curcumin via the action of tissue and plasma β-glucuronidase [13,57–59]. Curcumin glucuronide is the major component of total plasma curcuminoids (curcumin plus metabolites).

Compared to oral dosing, parenteral routes of administration resulted in higher plasma levels of curcumin being observed [12,13, 61–68]. Intravenous infusion studies of liposomal curcumin in dogs and humans clearly demonstrate the ability to maintain sustained levels of curcumin during infusion, but upon termination of infusion the plasma levels of curcumin fall very rapidly to almost undetectable with mean residence times of 36 min in dogs and 6–15 min in humans. Similarly, intravenous bolus doses of curcumin provide for short lived high plasma concentrations of curcumin. More recent studies have demonstrated that curcumin is metabolized by red blood cells in a species-dependent manner with THC being identified as a metabolite [60]. Given the abundance of red bloods, the red blood cell metabolism in addition to hepatic metabolism of curcumin likely contributes to the rapid decrease of plasma curcumin levels following intravenous dosing. In contrast, since curcumin has the potential to accumulate in the cell membrane, red blood cells may also serve as vehicles to distribute curcumin into tissues in much the same manner as binding to plasma proteins which increase the systemic distribution of drugs.

IP dosing with curcumin can result in higher plasma levels of curcumin over a somewhat longer period as a consequence of the lack of first pass metabolism and sustained absorption from the peritoneum. However, both enteral and parenteral routes of administration result in changes in the profile of curcumin metabolites, particularly the Phase II metabolites, where, for example, higher levels of curcumin glucuronide are observed following oral administration, particularly in rodents compared to humans.

In contrast to curcumin's plasma pharmacokinetics, the tissue distribution of curcumin has been less well investigated in preclinical animal models. In rats, bolus intravenous administration of 25 mg/kg of either curcumin or curcumin as a nanoparticle preparation resulted in detectable average tissues levels of curcumin over 60 min in liver (755 ng/g), heart (253 ng/g), spleen (477 ng/g), lung (748 ng/g), kidney (1000 ng/g) and brain (337 ng/g), with the nanoparticle preparation of curcumin delivering more curcumin into tissues, particularly in the lung and the spleen (22-fold and 212-fold more, respectively) compared to curcumin on its own [69]. However, the tissue half lives and mean residence times were very short ranging from 12.6 to 48.8 min and 20.4–75.7 min, respectively. Similarly, in mice, administration of 20 mg/kg of curcumin by bolus tail vein injection resulted in detectable average curcumin levels over 720 min in plasma (149 μg/mL, 413 μM), liver (24 ng/g tissue), kidney (30 ng/g tissue) and brain (24 ng/g tissue) with plasma and tissue half-lives of 32.3, 15.6, 16.8 and 6.1 min, respectively [70]. In rats, three different formulations of curcumin were evaluated for their ability to distribute curcumin into the brain, liposomal curcumin, polymeric nanocurcumin curcumin and polyacticglycolic acid co-polymer (PLGA) curcumin following intravenous administration [71,72]. Of the three formulations, the nanocurcumin formulation (5 mg/kg) produced plasma levels of 3913 and 1980 ng/mL at 1 hr and 2 hrs following administration. Brain stem levels of curcumin at 1 hr post injection were 120 ng/g tissue and increased to 235 ng/g tissue at 2 hrs post injection. In contrast, the administration of liposomal curcumin resulted in plasma levels of 166 and 15.4 ng/mL at 1 hr and 2 hrs following administration, respectively. Brain stem levels were 43.0 and 62.0 ng/g of wet tissue, increasing slightly at 2 hrs post injection despite a huge drop in plasma levels. Several interesting points can be made from these observations. Firstly, despite a there being 24-and 128-fold higher plasma curcumin levels produced by the nanocurcumin formulation compared to the liposomal formulation of curcumin at 1 hr and 2 hrs post injection, brain stem levels of curcumin were only 2.8 and 3.8-higher following administration of the nanocurcumin formulation suggesting that elevating plasma levels of curcumin may not contribute that much to greater tissue levels of curcumin, quite possibly due to limitations for curcumin penetrating the blood brain barrier. Given the observation that the tissue levels of curcumin rose modestly to moderately at 2 hrs compared to 1 hr post injection, for both formulations, it is likely that the length of tissue exposure to circulating curcumin in the blood has an important influence on the distribution of curcumin into tissues. Quite clearly, despite the rapid elimination of curcumin from the plasma, it can still distribute into tissues, the longer the exposure to curcumin the greater the tissue distribution of curcumin.

More eloquent studies evaluating and relating the pharmacokinetics of curcumin to its tissue distribution were carried out employing intravenously infused Lipocurc™ (a liposomal nanocurcumin preparation [66,72]) and have shed additional light on the relationship between tissue levels of curcumin and the time of exposure to circulating levels of curcumin in the blood. Lipocurc™ was intravenously infused into Beagle dogs at doses of 5 mg/kg/hr for 2 h (total dose 10 mg/kg) and 1.25 mg per kg for 8 h (total dose 10 mg/kg). In either case, 15 min following infusion, tissues were harvested and analyzed for their curcumin content in a media containing phosphoric acid to stabilize tissue curcumin. Maximum plasma concentrations and plasma exposure to curcumin were 319 ng/mL and 394 ng-hr/mL for the 2 h of infusion and 66 ng/mL and 187 ng-hr/mL for the 8 h of infusion. For either infusion, the lung, the tissue with the highest fraction of total systemic blood

flow and a high expression of uptake transporters [45], contained the highest levels of curcumin (22.9 ng/g) followed by the liver (1.82 ng/g) for the 2 h of infusion. What was particularly interesting was that despite the lower apparent exposure to curcumin during the 8 hrs of infusion, tissue levels of curcumin were higher in general amongst most tissues, but most notably by 11-fold (250.75 ng/g) and 16-fold (28.28 ng/g) in the lung and liver, respectively. These differences were even greater when one considers the tissue partition coefficients for the lung and liver which for the 2 h of infusion were 0.14 and 0.01 and for the 8 h of infusion were 24.2 and 2.7, respectively. Tissues levels of THC, although lower than for curcumin, displayed a similar pattern to curcumin with respect to the short and longer infusions times for curcumin and confirmed the entry of curcumin into the cells making up the tissue. It can thus be concluded from these data that, consistent with the studies of Chiu et al. [71], longer tissue exposure to circulating levels of curcumin may result in higher tissue levels of curcumin in a manner disproportionate to the plasma levels of curcumin.

The distribution of curcumin in the form of Lipocurc™ has also been investigated in a variety of freshly isolated or cryopreserved but viable blood cell types including PBMC and red blood cells from dog and human, PBMC from healthy and chronic lymphocytic leukemia patients, B-lymphocytes and multiple myeloma cells lines following incubation with curcumin at concentrations between 2 and 3 μM in the form of Lipocurc™ for up to 30 min in the presence of 50 % plasma supplemented medium [73]. The results of these studies based on the intracellular conversion of curcumin to THC demonstrated a notably higher distribution of curcumin into cancerous cells compared to normal cells consistent with a 2–3-fold higher uptake of curcumin in a breast cancer cell line compared to wild-type cells [40]. A role for uptake transporters in the distribution of curcumin into normal PBMC has been proposed in the literature [74]; uptake transporters being assessed as targets for the development of anticancer agents [75]. Thus, it would appear that plasma curcumin is available for diffusion mediated and active uptake into blood cells as well as tissues.

Given the metabolic instability of curcumin, its rapid removal from the plasma and the low sustained plamsa concentrations of curcumin, a number of research groups have questioned whether the therapeutic benefits of curcumin are derived from curcumin's metabolites [2,3,76], more specifically with the conversion of curcumin glucuronide to curcumin as curcumin glucuronide is present in considerably higher concentrations compared to curcumin in the plasma. A recent study demonstrated that curcumin glucuronide, when administered by the IV route to rats resulted in significant plasma concentrations of curcumin [13], likely via interaction with tissue and plasma β-glucuronidases [57,77]. In the same study, administration of curcumin glucuronide intravenously to mice with colon carcinoma xenographs resulted in a significant treatment effect. Thus, *in vivo*, curcumin glucuronide likely serves as an important reservoir of curcumin for tissue uptake as has been proposed by Wang et al. [71]. As curcumin glucuronide should be more soluble than curcumin and less protein bound (similar to the distinction between estradiol and estradiol-β-glucuronide [78]; data for curcumin-β-glucuronide are not available in the literature), it should also be better distributed between the plasma and interstitial fluid. Thus, curcumin glucuronide would likely be more available for direct uptake into cells by active transport than highly plasma protein bound curcumin. Curcumin glucuronide would also serve a source of interstitial curcumin following conversion to curcumin by interstitial fluid β-glucuronidase activity for active transport uptake into cells. In support of this premise, a recent study demonstrated that the distribution of doxorubicin into bronchial carcinoma tissue was found to be considerably greater when administered as the glucuronide due to conversion of doxorubicin glucuronide to doxorubicin by the action of tissue glucuronidases [59]; cancerous tissues containing an increased activity of β-glucuronidase compared to normal tissues [79].

In summary, taking all the aforementioned observations into consideration, one could predict that compared to cell culture studies, the ability of curcumin to gain access to the cells *in vivo* would be superior based on 1) the greater patency of uptake transporters in whole tissues and 2) the presence of the better distributed curcumin glucuronide in interstitial fluid. Curcumin glucuronide could either be directly transported into the cells and converted into curcumin by intracellular β-glucuronidase or converted to curcumin extracellularly by β-glucuronidase for effective uptake transport into cells. Thus, relating the treatment effects of curcumin to its plasma levels may be misleading when comparing plasma levels of curcumin to the cell culture concentrations of curcumin required to achieve activity at NF-κB. Nonetheless, it is important to assess such a relationship to confirm the relevance of NF-κB as a target for curcumin *in vivo*.

6. Relationship between plasma levels of curcumin and treatment response in preclinical animal studies

Data from cell culture and *in vivo* studies have revealed a number of properties for curcumin that may have a direct bearing on the relationship between its pharmacokinetics and *in vivo* efficacy as an inhibitor of NF-κB. These include the potential impacts of tissue perfusion, the length of exposure of tissues and cells to curcumin, a role for curcumin glucuronide and the impact of both passive and active uptake transport for gaining access into the cell. Nonetheless, it remains to be established whether a relationship exists between plasma levels of curcumin and its treatment effects (mediated principally or in part due to inhibition of NF-κB) in preclinical models of disease with the caveat that plasma levels of curcumin reflect the total plasma level of curcumin and its metabolites. For this exercise, cancer and inflammatory disease preclinical models in rodents have been chosen to monitor the treatment effects of curcumin as inhibition of NF-κB has been implicated as an important mediator in cancer and inflammatory disease. Quite clearly, there are a number of other proposed intracellular targets for curcumin that may play a role in cancer and inflammatory disease, however, the initial event for all these targets is the uptake of curcumin by tissues and curcumin's entry into the cell.

A more recent review by Tomeh et al. [80] summarizes the treatment effects of curcumin in a number of preclinical animal models of cancer that involve the implantation of tumour cells (i.e. mouse xenograph models) and where cell culture studies have indicated inhibition of NF-κB as the major mechanism mediating treatment. These include prostate, colorectal, breast and brain cancer. By virtue of its inhibition of NF-κB and reduction of cytokine formation, curcumin has also been shown to be effective in a number of preclinical models of inflammation of which include rheumatoid arthritis, asthma, colitis, pancreatitis, vascular inflammation and renal perfusion injury. While there are many examples of curcumin's treatment effects in preclinical models of cancer and inflammation, relatively few of these studies have simultaneously monitored plasma concentrations of curcumin. As a consequence, evaluating the relationship between the plasma concentrations of curcumin and its treatment effects in all preclinical disease models assessed was not possible. Studies characterizing the plasma pharmacokinetics of curcumin prepared as powder in aqueous or non-aqueous medium and administered by the routes used in the evaluation of curcumin's treatment potential in preclinical models, namely, enteral (inclusion in diet and oral dosing) and parenteral routes (intraperitoneal and intravenous) do exist [13,61–64,68,81]. Average plasma concentrations of curcumin (C_{av}) were obtained from these studies. Oral dosing of a 1000 mg/kg dose of curcumin in mice resulted in a C_{av} for curcumin of 32 ng/mL over a 24 h period [68] a value similar to the C_{av} of 26 ng/mL over a 24 h period observed in one other study [81]. Oral consumption of curcumin in the diet at a concentration of 0.5 % w/w resulted in a C_{av} for curcumin of 10 ng/mL [62]. IP dosing a 100 mg/kg dose of curcumin in mice resulted in a C_{av} for curcumin of 65 ng/mL

over a 24 h period [81]. Such information was useful to provide an estimate the plasma levels of curcumin attained in preclinical disease models in relation to its treatment effects.

In light of the difficulty of using plasma concentrations of curcumin to relate to its treatment effects in preclinical disease models, the dose of curcumin administered was related to its treatment effect. The treatment endpoints used for comparison were based on established endpoints for cancer (i.e. tumour volume, angiogenesis, number of metastases) and inflammatory disease (degree of inflammation, tissue/plasma chemo/cytokine levels). The comparisons are shown below for preclinical cancer models in Table 2 for enteral (dietary supplementation with curcumin and fixed oral doses of curcumin) and Table 3 for parenteral IP, IV and subcutaneous) routes of administration of curcumin. For inflammatory disease preclinical models, the comparison for both enteral and parenteral routes of administration are presented in Table 4.

Clearly, from a review of the data presented in Tables 2 and 3, curcumin administered by various routes either resulted or did not result in a treatment effect depending on the dose of curcumin administered. This interesting observation was illustrated by plotting the data presented in Tables 2 and 3 as the dose of curcumin versus the treatment effects (percent decrease of endpoint for monitoring progression of cancer). The results are presented for enteral administration in Fig. 3 and parenteral administration in Fig. 4. IV dosing data [13,28] and subcutaneously implanted curcumin [84] were not included in this comparison. For inflammatory disease models, no graphical analysis of the data was made and the treatment effects of curcumin at the estimated plasma concentrations were reviewed independent of whether the route of administration was oral or IP.

For preclinical cancer models and both the oral and intraperitoneal routes of administration, there was a reasonable relationship between increasing dose and a greater treatment effect. Based on pharmacokinetic data reported previously, a range of C_{av} concentrations was estimated for the various routes of administration based on the dose of curcumin administered compared to the dose at which the C_{av} was determined for curcumin. Thus, for curcumin, in the diet at concentrations ranging from 0.1 to 2 % w/w, dosed orally at doses of 5–3000 mg/kg and dosed IP at doses of 5–120 mg/kg, the estimated C_{av} ranges would

Table 2
Comparison of Dose with Treatment Effect for Curcumin Following Enteral Administration in Preclinical Animal Models of Cancer.

Curcumin Dose Over 24 Hours	Tumour Model, Length of Treatment and Species	Treatment Effect	Reference
Curcumin Administered in the Diet			
1 % w/w	Prostate carcinoma in mice, 5 weeks	19 %↓ Tumour volume; 50 %↓ Tumour metastases	[25]
0.5 % w/w	Leukemic tumours, daily 3 weeks prior to tumour cell injection, daily 4 weeks following tumour cell injection, mice	No effect on leukemia tumour volume	[61]
2 % w/w	Prostate carcinoma, daily, two weeks and four weeks, mice	73 and 80 %↓ Tumour volume, respectively	[82]
0.6 % w/w	Breast carcinoma, 5 weeks, mice	44 %↓ Tumour volume	[87]
1 % w/w	Breast cancer metastases model, 35 Days, mice	Little to no lung metastases in 68 % of treated mice	[84]
1 % w/w	Breast carcinoma, 1 year, rat	67 %↓ Incidence of mammary tumours.	[90]
0.1 % w/w	Estrogen-Induced Mammary Carcinogenesis in rats, 12 weeks	No effect on breast cancer tumour volume	[91]
Curcumin Administered Orally			
1000 mg/kg	Ovarian carcinoma, daily 19 days, mice	50 %↓, 53 %↓, 44 %↓ Tumour volumes for 3 ovarian tumour cell lines	[52]
1000 mg/kg	Colorectal carcinoma, daily 30 days, mice	11 %↓ Tumour volume	[83]
100 mg/kg	Breast carcinoma, daily 32 Days, mice	No effect on tumour volume.	[85]
500, 1000, 1500 mg/kg	Cervical carcinoma, daily 30 days, mice	4, 26 and 48 %↓ Tumor volume, respectively	[86]
300 and 3000 mg/kg	Liver carcinoma, daily 21 days, mice	15 and 69.2 %↓ Angiogenesis, respectively	[88]
500 mg/kg	Prostate carcinoma, three times per week, 4 weeks, mice	27 %↓ Tumour volume	[89]
40 mg/kg	Breast Carcinoma, daily-three weeks, mice	40 %↓ Tumour volume	[92]
5 mg/kg	Prostate carcinoma in mice, three times per week, 4 weeks	43 %↓ Tumour volume	[93]
360 mg/kg	Prostate carcinoma, daily, 5 days per week, 4 weeks, mice	56 %↓ Tumour volume	[94]

↓-Decrease.

Table 3
Comparison of Dose with Treatment Effect for Curcumin Following Parenteral Administration in Preclinical Animal Models of Cancer.

Curcumin Dose Over 24 Hours	Tumour Model, Length of Treatment and Species	Treatment Effect	Reference
Curcumin Administered IP			
20 mg/kg	Pancreatic carcinoma, three times a week for 31 days, mice	44 %↓ Tumour volume	[28]
5 mg/kg	Leukemic tumours, every-two days, 4 weeks, mice	No effect on tumour volume	[61]
30, 60, 120 mg/kg	Intracranial human glioma carcinoma, daily 30 days, mice	10, 43 and 53 %↓ Tumour volume, respectively	[95]
60 mg/kg	Subcutaneous human glioma carcinoma, daily 30 days, mice	67 %↓ Tumour volume	[95]
15 and 30 mg/kg	Leukemic tumours, daily 15 days, mice	17and 42 %↓ Tumour volume, respectively	[96]
50 mg/kg	Breast carcinoma, daily, every 5 days, 22 days, mice	44 %↓ Tumour angiogenesis	[97]
20 mg/kg	Pancreatic carcinoma, three times a week for 31 days, mice	42 %↓ Tumour volume	[98]
45 mg/kg	Lung adenocarcinoma, every-five days for two weeks, mice	28 %↓ Tumour volume	[99]
25 and 50 mg/kg	Ehrlich ascites solid tumours in mice, daily for 15 days, mice	74 and 55 %↓ Tumour volume, respectively	[100]
7.5 mg/kg	Prostate carcinoma, three times a week for 4 weeks, mice	25 %↓ Tumour volume	[101]
50 mg/kg	Colorectal carcinoma, every 5 days for 4 weeks, mice	24 %↓ Tumour nodules	[102]
Curcumin/Curcumin Glucuronide Administered by Other Parenteral Routes (IV and SC)			
Curcumin Glucuronide 45 mg/kg, intravenous	Colon carcinoma, three times per week, 21 Days, mice	67 %↓ Tumour volume	[13]
40 mg/kg, intravenous	Pancreatic carcinoma, three times per week, 17 – 20 days, mice	66–82 %↓ Tumour volume	[28]
Subcutaneous curcumin implants, 200 mg	Estrogen-Induced Mammary Carcinogenesis in rats, 12 weeks	34 %↓ Tumour volume	[84]

↓-Decrease.

Table 4
Comparison of Dose with Treatment Effect for Curcumin in Preclinical Animal Models of Inflammation.

Curcumin Treatment Regimen	Inflammation Model, Length of Treatment and Species	Treatment Effect	Reference
200 mg/kg, intraperitoneal	Pancreatitis, single dose over a 48-hr period, rat	IL-6↓, TNF-α↓, c-reactive protein↓ and pancreatic inflammation↓ by 57, 61, 56 and 100 %, respectively	[65]
200 mg/kg, oral	Arthritis, daily-three weeks, rats	30 %↓ Arthritic score; 48 %↓ Serum IL-1β; 42 %↓ Serum TNF-α	[103]
3.3 mg/kg, oral	Arthritis, every other day for two weeks, mice	58 % and 65 %↓ Arthritic index and incidence, respectively; 57 % and 65 %↓ IL-1β and TNF-α levels, respectively	[104]
15 mg/kg, oral	Pulmonary inflammation, daily 8 Days, mice	38 %↓ Bronchiolar lavage inflammatory cell counts; 26 %↓ TNF-α messenger ribonucleic acid expression; 73 %↓ Eosinophil infiltration	[105]
50–200 mg/kg, oral	Pulmonary inflammation, daily 7 days, mice	20–40 %↓, Airway resistance	[106]
100 mg/kg, oral	Vascular inflammation, daily 14 Days, mice	71 and 61 %↓ for IL-1β and IL-6, respectively; 62 %↓ MCP-1; 100 %↓ Matrix metallopeptidase-9	[107]
12.5 mg/kg, intraperitoneal	Renal inflammation, daily 2 Days, rats	↓86 % Creatinine clearance; ↓75 % serum levels of TNF-α	[108]

↓-Decrease.

be <1–40 ng/mL, <1–96 ng/mL and 3–80 ng/mL, respectively. The range of estimated C_{av} for curcumin for eliciting a therapeutic response upon either oral or IP administration clearly overlapped for each dosing route. Consistent with this observation was a similar range of treatment effects observed for curcumin for each dose route. Both for the oral and intraperitoneal dosing routes, the variability of the treatment response to curcumin decreased with increasing dose, similar to observations made in cell culture where the variability of the response decreased at higher cell culture concentrations of curcumin. Several factors may have contributed to the variability of the repsonses to curcumin at lower doses of curcumin including the frequency of dosing, the length of treatment and the involvement of tissue uptake transporters for curcumin. Nonetheless for disease models of cancer, there appears to be a relationship between the dose of curcumin and its treatment effects which supports a specific interaction between NF-κB and curcumin in targeting cancer. Another observation made from this analysis was that the highest C_{av} estimated for curcumin to elicit a similar treatment effect of curcumin following oral and IP dosing in cancer preclinical models was 96 ng/mL (0.26 μM) and 80 ng/mL (0.22 μM), respectively. These estimated concentrations of curcumin are lower than the 1–100 μM range of concentrations of curcumin that were observed to inhibit the activation of NF-κB and release of cytokines in cell culture, strongly suggesting, as previously discussed, that there are a number of factors that may contribute to the low measured plasma concentration of curcumin associated with its treatment effects *in vivo*.

In contrast to what was observed in animal models of cancer, for inflammatory disease models, there was no relationship between dose and the treatment response to curcumin, the range of estimated C_{av} values for curcumin in preclinical models of inflammation following oral administration and intraperitoneal administration ranging from 0.1 to 65 ng/mL. Possible reasons for this finding are discussed below.

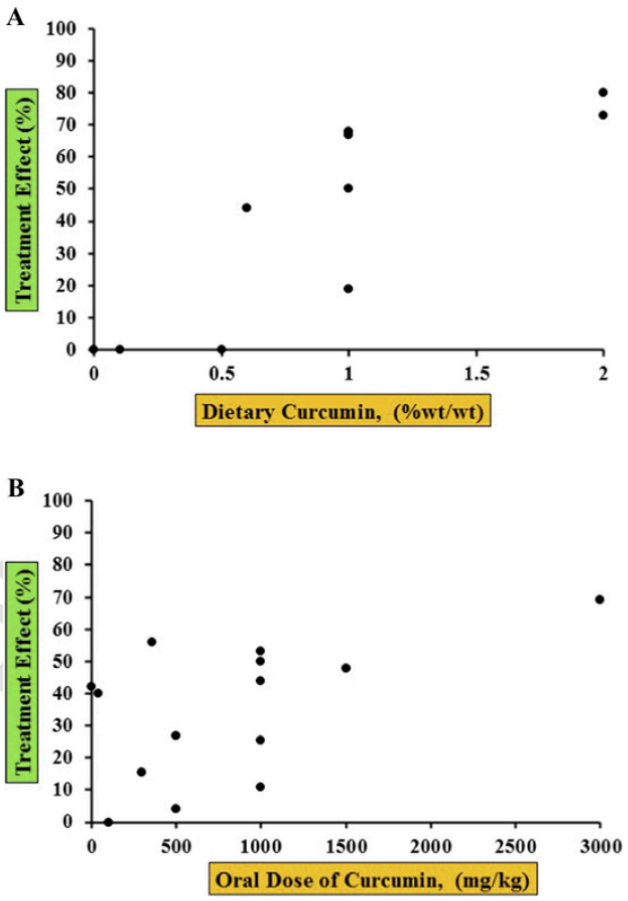

Fig. 3. Relationship Between the Dose of Curcumin Administered Orally and the Treatment Effects of Curcumin in Preclinical Animal Models of Cancer. The oral dose of curcumin administered either in the diet (Panel A) or as a fixed dose (Panel B) shown in Table 2 were plotted against the treatment effects of curcumin.

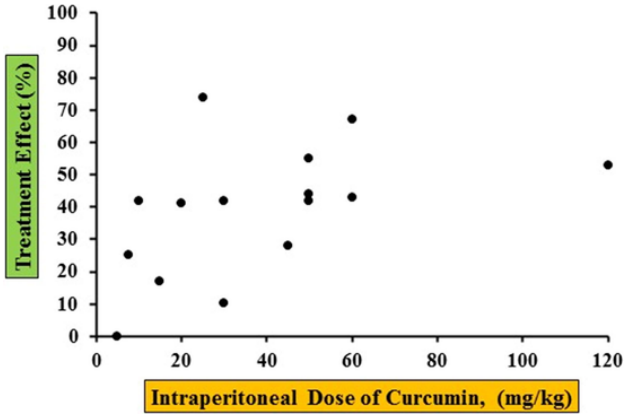

Fig. 4. Relationship Between the Dose of Curcumin Administered Intraperitoneally and the Treatment Effects of Curcumin in Preclinical Animal Models of Cancer. The doses of curcumin administered intraperitoneally and shown in Table 3 were plotted aginst the treatment effects of curcumin.

7. Data interpretation, summary and conclusions

The purpose of this review was to investigated if there was a plausible relationship between the activity of curcumin in cell culture, curcumin's treatment effects in animal models of disease and the pharmacokinetics of curcumin. Direct targeting of NF-κB and NF-κB mediate responses by curcumin was chosen to compare curcumin's cell culture ac-

tivity with its activity in preclinical rodent models of cancer and inflammatory disease, as the interaction between NF-κB and curcumin has been well investigated. Based data existing in the literature and more recent developments in the understanding of how cells handle curcumin, plausible explanations as to the reasons why higher concentrations of curcumin are required to interact with NF-κB in cell culture compared to the estimated plasma concentrations of curcumin that elicited effective treatment in rodent models of disease were provided and are summarized in Fig. 5. Furthermore, pharmacokinetic reasons were provided to help explain the activity of curcumin in rodent models of disease when administered either orally or intraperitoneally.

From an analysis of the cell culture data for curcumin several key features of curcumin were noted. Firstly, the effects of curcumin were concentration-dependent, with a similar range of concentrations producing a direct inhibition NF-κB and NF-κB mediated cytokine/chemokine release; lower concentrations of curcumin displaying a greater variability of inhibition. Factors that may have likely played a role in curcumin's cell culture activity were time of exposure to curcumin and evidence for long lived and possible irreversible inhibition of its cellular targets. Given the evidence that curcumin uses cellular uptake transporters, their presence or lack thereof on the cell membrane would contribute to the variability of curcumin's activity, especially at lower concentrations of curcumin where there would be less of a driving force for simple diffusion of curcumin across the cell membrane. For simple diffusion of curcumin across the cell membrane, increasing the incubation time and concentration with cells would favour greater amounts of curcumin entering the cell and hence a greater but less variable activity which was observed. Nonetheless, the rather high concentrations of curcumin required to elicit cell culture responses are likely related to its poor solubility, high protein binding and a mostly diffusion dominated route of cell entry.

Evidence was presented indicating that curcumin can directly inhibit NF-κB in rodent models of disease and despite its poor pharmacokinetic profile distribute to tissues in an exposure time-dependent manner. Thus, by virtue of its inhibition of NF- κB curcumin would be expected to be active in disease models such as cancer and inflammation. This was indeed the case and compared to the high concentrations of curcumin required for activity at NF-κB in cell culture, curcumin was clearly active at considerably lower estimated plasma concentrations in rodent models of disease whether administered orally or IP. One possible explanation for this difference is the involvement of uptake transporters in the cell uptake of curcumin in animals. In animals, uptake transporters would likely be optimally expressed in comparison to cell lines and primary cell cultures in which transporters may be poorly expressed or absent from the plasma membrane of the cell. However, while the involvement of uptake transporters in tissues may provide an explanation for lower effective estimated average plasma concentrations compared to cell culture, other explanations for the difference between the cell culture activity of curcumin and its activity in preclinical animals likely exist such as the potential of curcumin to irreversibly interact with its targets and the impact of longer plasma exposure to curcumin on the time-dependent increased accumulation of curcumin in tissues. Another plausible explanation may lie in the large number of metabolites produced by the metabolism of curcumin. Important roles for the phase II metabolite of curcumin, curcumin glucuronide, both as a source for plasma and tissue curcumin through metabolism by β-glucuronidase and as a treatment for neuroinflammation have been proposed [13,109]. It has been demonstrated in mice that oral administration of curcumin results in a higher ratio of curcumin glucuronide to curcumin compared to IP administration by 56-fold vs 13-fold, respectively [61] principally due to the high degree of glucuronidation occurring at the intestinal brush border membrane in rodents. In addition,

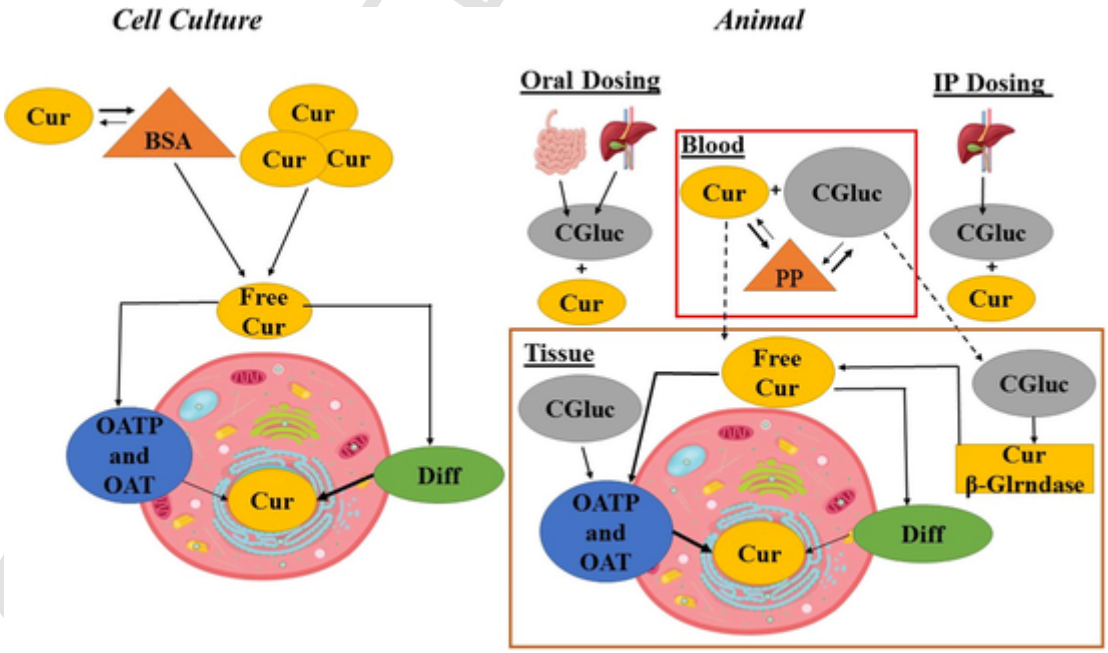

Fig. 5. Schematic diagram showing how the entry of curcumin into cells in cell culture and in tissue differs. In cell culture, BSA present in the culture medium binds curcumin and helps to solubilize it, nonetheless some aggregation of curcumin may occur. Free curcumin accumulates in the cell membrane and diffuses into the cells to interact with NF-κB and within the cell nucleus. The expression of uptake transporters (i.e. OATP and OAT) is likely limited or non-existent and diffusion is likely the dominant pathway for curcumin to gain access into cells in culture. In preclinical animal studies curcumin dosed orally is converted to curcumin glucuronide at the intestinal brush border membrane and within the liver, while when dosed intraperitoneally conversion to curcumin glucuronide occurs in the liver. While curcumin is tightly bound to plasma proteins, curcumin glucuronide is less bound and better distributed to tissues. Curcumin β-glucuronidase breaks down curcumin glucuronide to free curcumin and together with free curcumin not bound to plasma proteins can be taken into cells by the predominant pathway, transporter mediated uptake via well expressed uptake transporters (OATP and OAT) and to a lessor extent diffusion. Curcumin glucuronide may also be taken up into cells and converted to curcumin by cellular curcumin β-glucuronidase. Tissue cells are therefore exposed to effective treatment levels of curcumin compared to the relatively low levels of curcumin measured in the plasma.

the plasma terminal half-life of curcumin glucuronide was found to be 2.8-fold longer than curcumin following oral administration of nano-emulsion curcumin to mice [68]. Given that curcumin glucuronide is more soluble than curcumin and likely is less plasma protein bound, it would more readily gain access to the interstitial space and tissues and subsequently be converted to curcumin by tissue β-glucuronidase; β-glucuronidase activity varying amongst tissues. Curcumin would then be available for diffusion and active-uptake into tumour cells in considerably greater amounts than plasma bound curcumin as was observed for tumour levels of doxorubicin following the administration of doxorubicin β-glucuronide [59]. In addition, OATP uptake transporters have been found to be overexpressed in a number of solid human tumours [110] and β-glucuronidase activity in tumour tissue is elevated [111]. Therefore, the treatment effect of curcumin in preclinical animal models at lower estimated plasma concentrations following oral and intraperitoneal administration compared to its cell culture activity may be related to the presence of higher amounts of curcumin β-glucuronide in tumours which would be converted to curcumin by β-glucuronidase and rapidly taken up by OATP uptake transporters. The variability in the expression of tissue OATP transporters may contribute to the scatter in the data relating the dose of curcumin to its treatment effects. Curcumin glucuronide may also have played a critical role for the better distribution of curcumin into dog tissues with longer infusion times. The conversion of curcumin glucuronide to curcumin by β-glucuronidase in tissues may also contribute to the treatment effects in rodent models of inflammatory diseases observed at low estimated C_{av} concentrations of curcumin. β-Glucuronidase activity is increased at the site of inflammation as a consequence of neutrophil infiltration [1,12]. In concert with curcumin glucuronide and uptake transporters present on inflammatory cells, the metabolism of curcumin glucuroide by β-glucuronidase to curcumin and the resulting active uptake into cells may have resulted in mediation of its treatment effects against intracellular oxidative metabolites at lower than expected plasma concentrations of curcumin [113]. A high degree of variability in the degree of the inflammatory response to curcumin in animal models of inflammation may have contributed to the inability to observate a relationship between curcumin dose and treatment effects.

Given the evidence, it would appear that the conversion of curcumin glucuronide to curcumin within tissues may help to explain the relationship between the lower than expected plasma concentrations of curcumin that mediate its treatment effects following oral and IP dosing compared to its activity in cell culture. If such is the case, given the finding that not much difference was observed between the estimated C_{av} and treatment effects for curcumin following oral and IP administration, it is likely that both the liver and intestine provide more than adequate levels of tissue curcumin glucuronide to mediate its treatment effects.

With respect to the treatment of cancer using curcumin, its well-known antiangiogenic effects have the undesirable effect of reducing the exposure of the tumour to curcuminoids, an apparent critical factor limiting curcumin's treatment effects. Nonetheless, formulating curcumin so that its poor pharmacokinetic properties (poor bioavailability, short plasma half-life are improved) as is the case for nano-formulated curcumin [114] has resulted in better treatment effects. Whether the better treatment effects are either due to a longer exposure to curcumin or a consequence of a role for the more soluble metabolites of curcumin (curcumin glucuronide, curcumin sulfate) should be evaluated. Based on the analysis of the relationship between curcumin's cell culture activity, pharmacokinetics and activity in animal models of disease presented in this review, cell based experiments are clearly very important for identifying potential curcumin targets for treating disease in preclinical animal models despite the high concentrations of curcumin employed. Several reasons were provided to explain the lower potency of curcumin in cell culture compared to estimated C_{av} in animal models of disease at one target for curcumin, NF-κB. Thus, expeditiously run cell culture experiments will remain an important model for identification of therapeutic targets for curcumin in the future. Important considerations for elucidating therapeutic targets for curcumin in cell culture should take into account the potential activity of curcumin glucuronide (and in general more soluble less protein bound metabolites of curcumin) *in vivo* and the activity of cellular uptake transporters. In addition, more focus should be put on elucidating the role of the soluble metabolites of curcumin in the mediation of curcumin's treatment effects *in vivo*. While curcumin and its metabolites alone may not produce a strong therapeutic effect, they may induce a cellular environment that enhances the acivity of targeted drugs in the treatment of disease. In this regard, curcumin combined with cytotoxic anticancer drugs has already shown improved therapeutic outcomes both as a result of reducing the toxicity of standard anticancer therapies and due to its intrinsic anticancer activity [115]. With respect to the PAINS activity of curcumin, compounds possessing PAINS and the potential to aggregate are more likely to possess "off target" activity which is associated with an increased propensity to be toxic at pharmacologically relevant doses [38]. This does not appear to be the case for orally administered curcumin, curcumin is relatively non-toxic even in the intestine where tissue exposures are high [116]. Many successful attempts have been made to increase the concentration of curcumin in the plasma following oral dosing resulting in greater treatment effects, however, there is always a risk that unwanted curcumin toxicities and drug-drug interactions may arise [117]. The use of curcumin glucuronide as a "pro-drug" [13] to supply tissues with active curcumin is a reasonable alternative approach to be considered in the design of curcumin analogs with an even lower toxic liability. The optimization of better distributed curcumin analogs that can be readily acted upon by cellular uptake transporters should also be considered as a way to boost cell levels and treatment effects alone or in combination with known therapeutic drugs.

Uncited references

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CRediT authorship contribution statement

Gordon T. Bolger : Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Kresimir Pucaj :** Conceptualization. **Yvonne O. Minta :** Conceptualization, Writing – review & editing. **Peter SordilloMinta :** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] S.K. Sandur, M.K. Pandey, B. Sung, K.S. Ahn, A. Murakami, G. Sethi, P. Limtrakul, V. Badmaev, B.B. Aggarwal, Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and tumerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism, Carcinogenesis 28 (2007) 1765–1773, https://doi.org/10.1093/carcin/bgm123.

[2] M. Herger, R.F. van Bolen, M. Broekgaarden, M.C. Michel, The molecular basis for the pharmacokinetics and pharmacodynamics of curcumin and its metabolites in relation to cancer, Pharmacol. Rev. 66 (2014) 222–307, https://doi.org/10.1124/pr.110.004044.

[3] K.M. Nelson, J.L. Dahlinb, J. Bisson, J. Graham, G.F. Pauli, M.A. Walters, The essential chemistry of curcumin, J. Med. Chem. 60 (2017) 1620–1637, https://doi.org/10.1021/acs.jmedchem.6b00975.

[4] K.I. Priyadarsini, The chemistry of curcumin: from extraction to therapeutic agent, Molecules 19 (2014) 20091–20112, https://doi.org/10.3390/molecules191220091.

[5] M. Gera, N. Sharma, M. Ghosh, D.L. Huynh, S.J. Lee, T. Min, T. Kwon, D.K. Jeong, Nanoformulations of curcumin: an emerging paradigm for improved remedial application, Oncotarget 8 (2017) 66680–66698, doi: 10.18632/oncotarget.19164.

[6] S.N. Bolz, M.F. Adasme, M. Schroeder, Toward an understanding of pan-assay interference compounds and promiscuity: a structural perspective on binding modes, J. Chem. Inf. Model. 61 (2021) 2248–2262, doi: 10.1021/acs.jcim.0c01227.

[7] H. Yu, L. Lin, Z. Zhang, H. Zhang, H. Hu, Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study, Signal Transduct. Target. Ther. 5 (2020) 209, https://doi.org/10.1038/s41392-020-00312-6.

[8] H. Zhang, S.-C. Sun, NF-κB in inflammation and renal diseases, Cell Biosci. 5 (2015) 63, https://doi.org/10.1186/s13578-015-0056-4.

[9] R.-H. Shih, C.-Y.Wang, C.-M. Yang, NF-kappaB signaling pathways in neurological inflammation: a mini review, Front. Mol. Neurosci. 8 (2015) 77, doi: 10.3389/fnmol.2015.00077.

[10] H. Li, C. Zhong, Q. Wang, W. Chen, Y. Yuan, Curcumin is an APE1 redox inhibitor and exhibits an antiviral activity against KSHV replication and pathogenesis, Antiviral Res. 167 (2019) 98–103, https://doi.org/10.1016/j.antiviral.2019.04.011.

[11] N. Lamanna-Rama, D. Romero-Miguel, M. Desco, M.L. Soto-Montenegro, An update on the exploratory use of curcumin in neuropsychiatric disorders, Antioxidants 11 (2022) 353, https://doi.org/10.3390/antiox11020353.

[12] R. Greil, S. Greil-Ressler, L. Weiss, C. Schönlieb, T. Magnes, B. Radl, G.T. Bolger, B. Vcelar, P.P. Sordillo, A phase 1 dose-escalation study on the safety, tolerability and activity of liposomal curcumin (LipocurcTM) in patients with locally advanced or metastatic cancer, Cancer Chemother. Pharmacol. 82 (2018) 695–706, https://doi.org/10.1007/s00280-018-3654-0.

[13] H. Ozawa, A. Imaizumi, Y. Sumi, T. Hashimoto, M. Kanai, Y. Makino, T. Tsuda, N. Takahashi, H. Kakeya, Curcumin β-D-glucuronide plays an important role to keep high levels of free-form curcumin in the blood, Biol. Pharm. Bull. 40 (2017) 1515–1524, https://doi.org/10.1248/bpb.b17-00339.

[14] A. Olivera, T.W. Mooreb, F. Hua, A.P. Brown, A. Sunb, D.C. Liottab, J.P. Snyderb, Y. Yoond, H. Shimd, A.I. Marcuse, A.H. Miller, T.W.W. Pacea, Inhibition of the NF-κB signaling pathway by the curcumin analog, 3,5-bis(2-pyridinylmethylidene)-4-piperidone (EF31): anti-inflammatory and anti-cancer properties, Int. Immunopharmacol. 12 (2012) 368–377, https://doi.org/10.1016/j.intimp.2011.12.009.

[15] S.-S. Han, Y.-S. Keum, H.-J. Seo, Y.-J. Surh, Curcumin suppresses activation of NF-κB and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells, J. Biochem. Mol. Biol. 35 (2002) 337–342, https://doi.org/10.5483/bmbrep.2002.35.3.337.

[16] C.K. Sen, L. Packer, Antioxidant and redox regulation of gene transcription, FASEB J. 10 (1996) 709–720, doi: 10.1096/fasebj.10.7.8635688.

[17] P. Barnes, M. Karin, Nuclear factor-κB a pivotal transcription factor in chronic inflammatory diseases, N. Eng. J. Med. 336 (1997) 1066–1071, https://doi.org/10.1056/NEJM199704103361506.

[18] M. Biancalana, E. Natan, M.J. Lenardo, A.R. Fersht, NF-κB rel subunit exchange on a physiological timescale, Protein Sci. 30 (2021) 1818–1832, https://doi.org/10.1002/pro.4134.

[19] C. Buhrmann, A. Mobasheri, F. Busch, C. Aldinger, R. Stahlmann, A. Montaseri, M. Shakibaei, Curcumin modulates nuclear factor κB (NF-κB)-mediated inflammation in human tenocytes in vitro; role of the phosphatidylinositol 3-kinase/Akt pathway, J. Biol. Chem. 286 (2011) 28556–28566, https://doi.org/10.1074/jbc.M111.256180.

[20] X. Gao, J. Kuo, H. Jiang, D. Deeb, Y. Liu, G. Divine, R.A. Chapman, S.A. Dulchavsky, S.C. Gautama, Immunomodulatory activity of curcumin: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity and cytokine production in vitro, Biochem. Pharmacol. 68 (2004) 51–61, https://doi.org/10.1016/j.bcp.2004.03.015.

[21] B.Y. Kang, Y.J. Song, K.-M. Kim, Y.K. Choe, S.Y. Hwang, T.S. Kim, Curcumin inhibits Th1 cytokine profile in CD4+ T-cells by suppressing interleukin-12 production in macrophages, Br. J. Pharmacol. 128 (1999) 380–384, doi: 10.1038/sj.bjp.0702803.

[22] A.S. Konduru, B.-C. Lee, J.D. Li. Curcumin suppresses NTHi-induced CXCL5 expression via inhibition of positive IKKβ pathway and up-regulation of negative MKP-1 pathway, Sci. Rep. 6 (2016) 31695, doi: 10.1038/srep31695.

[23] B. Rahardjo, E. Widjajanto, H. Sujuti, K. Keman, Curcumin decreased level of proinflammatory cytokines in monocyte cultures exposed to preeclamptic plasma by affecting the transcription factors NF-κB and PPAR-γ, Biomark. Genomic Med. 6 (2014) 105–115, https://doi.org/10.1016/j.preghy.2014.10.134.

[24] C. Jobin, C.A. Bradham, M.P. Russo, B. Juma, A.S. Narula, D.A. Brenner, R. Balfour Sartor, Curcumin blocks cytokine-mediated NF-κB activation and proinflammatory gene expression by inhibiting inhibitory factor I-κB kinase activity, J. Immunol. 163 (1999) 3474–3483. http://www.jimmunol.org/content/163/6/3474.

[25] P.H. Killian, E. Kronski, K.M. Michalik, O. Barbieri, S. Astigiano, C.P. Sommerhoff, U. Pfeffer, A.G. Nerlich, B.E. Bachmeier, Curcumin inhibits prostate cancer metastasis in vivo by targeting the inflammatory cytokines CXCL1 and -2, Carcinogenesis 33 (2012) 2507–2519, https://doi.org/10.1093/carcin/bgs312.

[26] B. van't Land, N.M.A. Blijlevens, J. Marteijn, S. Timal, J.P. Donnelly, T.J.M. de Witte, L. M'Rabet, Role of curcumin and the inhibition of NF-κB in the onset of chemotherapy-induced mucosal barrier injury, Leukemia 18 (2004) 276–284, doi: 10.1038/sj.leu.2403233.

[27] F. Antoine, J.C. Simard, D. Girard, Curcumin inhibits agent-induced human neutrophil functions in vitro and lipopolysaccharide-induced neutrophilic infiltration in vivo, Int. Immunopharmacol. 17 (2013) 1101–1107, https://doi.org/10.1016/j.intimp.2013.09.024.

[28] L. Li, F.S. Braiteh, R. Kurzrock, Liposome-encapsulated curcumin: in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis, Cancer 104 (2005) 1322–1331, doi: 10.1002/cncr.21300.

[29] Q. Liu, W.T.Y. Loo, S.C.W. Sze, Y. Tong, Curcumin inhibits cell proliferation of MDA-MB-231 and BT-483 breast cancer cells mediated by down-regulation of NF-κB, cyclin D and MMP-1 transcription, Phytomed. 16 (2009) 916–922, https://doi.org/10.1016/j.phymed.2009.04.008.

[30] Y.A. Larasati, N. Yoneda-Kato, I. Nakamae, T. Yokoyama, E. Meiyanto, J.-A. Kato, Curcumin targets multiple enzymes involved in the ROS metabolic pathway to suppress tumor cell growth, Sci. Rep. 8 (2018) 2039, doi: 10.1038/s41598-018-20179-6.

[31] J. Fang, J. Lu, A. Homgren, Thioredoxin reductase is irreversibly modified by curcumin, J. Biol. Chem. 280 (2005) 25284–25290, doi: 10.1074/jbc.M414645200.

[32] H.H. Tønnesen, Solubility chemical and photochemical stability of curcumin in surfactant solutions. Studies of curcumin and curcuminoids, XXVIII, Pharmazie 57 (2002) 820–824.

[33] D. de M. Carvalho, K. Takeuchi, R.M. Geraldine, C.J. de Moura, M.C.L. Torres, Production, solubility and antioxidant activity of curcumin nanosuspension, Food Sci. Technol. Campinas 35 (2015) 115–119, doi: 10.1590/1678-457X.6515.

[34] J.-Y. Wang, M.-H. Pan, A.L. Cheng, L.-I. Lin, Y.S. Ho, C.-Y. Hsieh, J.-K. Lin, Stability of curcumin in buffer solutions and characterization of its degradation products, J. Pharmaceut. Biomed. Anal. 15 (1997) 1867–1876, https://doi.org/10.1016/S0731-7085(96)02024-9.

[35] N.K. Bhatia, S. Kishor, N. Katyal, P. Gogoi, P. Naranga, S. Deep, Effect of pH and temperature on conformational equilibria and aggregation behaviour of curcumin in aqueous binary mixtures of ethanol, RSC Adv. 6 (2016) 103275–103288, doi: 10.1039/C6RA24256A.

[36] T. Masuda, Y. Toi, H. Bando, T. Maekawa, Y. Takeda, H. Yamaguchi, Structural identification of new curcumin dimers and their contribution to the antioxidant mechanisms of curcumin, J. Agric. Food Chem. 50 (2002) 2524–2530, https://doi.org/10.1021/jf011601s.

[37] A.C.P. Reddy, E. Sudharshan, A. Rao, B.R. Lokesh, Interaction of curcumin with human serum albumin – a spectroscopic study, Lipids 34 (1999) 1025–1029, https://doi.org/10.1007/s11745-999-0453-x.

[38] S.R. LaPlante, N. Aubry, G. Bolger, P. Bonneau, R. Carson, R. Coulombe, C. Sturino, P. Beaulieu, Monitoring drug self-aggregation and potential for promiscuity in off-target in vitro pharmacology screens by a practical NMR strategy, J. Med. Chem. 56 (2013) 7073–7083, https://doi.org/10.1021/jm4008714.

[39] A. Kunwar, A. Barik, R. Pandey, K.I. Priyadarsini, Transport of Liposomal and albumin loaded curcumin to living cells: an absorption and fluorescence spectroscopic study, Biochim. Biophys. Acta 1760 (2006) 1513–1520, doi: 10.1016/j.bbagen.2006.06.012.

[40] A. Kunwar, A. Barik, B. Mishra, K. Pruyadarsini, K. Rathinasamy, R. Pandy, Differential up-take and fluorescence of curcumin, a yellow pigment from tumeric in normal vs tumor cells, BARC Newsletter 285 (2007) 202–207.

[41] G.T. Bolger, A. Licollari, A. Tan, R. Greil, L. Pleyer, B. Vcelar, M. Majeed, P. Sordillo, Distribution of curcumin and THC in peripheral blood mononuclear cells isolated from healthy individuals and patients with chronic lymphocytic leukemia, Anticancer Res. 38 (2018) 121–130, https://doi.org/10.21873/anticanres.12199.

[42] M. Roth, A. Obaidat, B. Hagenbuch, OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene |superfamilies, Br. J. Pharmacol. 165 (2012) 1260–1287, https://doi.org/10.1111/j.1476-5381.2011.01724.x.

[43] N.F. Smith, W.D. Figg, A. Sparreboom, Role of the liver-specific transporters OATP1B1 and OATP1B3 in governing drug elimination, Expert Opin. Drug Metab. Toxicol. 1 (2005) 429–445, https://doi.org/10.1517/17425255.1.3.429.

[44] S.K. Nigam, K.T. Bush, G. Martovetsky, S.-Y. Ahn, H.C. Liu, E. Richard, V. Bhatnagar, W. Wu, The organic anion transporter (OAT) family: a systems biology perspective, Physiol. Rev. 95 (2015) 83–123, https://doi.org/10.1152/physrev.00025.2013.

[45] A.A. Zeki, S. Ott, K. Gohil, The organic anion transporter polypeptides (OATP) in the mouse lung and human airway epithelium: important molecular targets in the development of inhaled statins, Am. J. Respir. Crit. Care Med. 197 (2018) A1330.

[46] X. Zhou, F. Zhang, C. Chen, Z. Guo, J. Liu, J. Yu, Y. Xu, D. Zhong, H. Jiang, Impact of curcumin on the pharmacokinetics of rosuvastatin in rats and dogs based on the conjugated metabolites, Xenobiotica 47 (2017) 267–275, https://doi.org/10.1080/00498254.2016.1183060.

[47] N. Jaerapong, Q. Jamil, J. Riha, D. Milovanovic, G. Krupitza, B. Stieger, K. Jarukomjorn, W. Jäger, Organic anion-transporting polypeptides contribute to the uptake of curcumin and its main metabolites by human breast cancer cells: impact on antitumor activity, Oncol. Rep. 41 (2019) 42558–42566, doi: 10.3892/or.2019.701.

[48] J. Duan, G. Bolger, M. Garneau, M. Amad, The liver partition coefficient-corrected inhibitory quotient and the pharmacokinetic-pharmacodynamic relationship of directly acting anti-hepatitis C virus agents in humans, Antimicrob. Agents Chemother. 56 (2012) 5381–5386, https://doi.org/10.1128/AAC.01028-12.

[49] J.R. Wiśniewski, A. Vildhede, A. Norén, P. Artursson, In-depth quantitative analysis and comparison of the human hepatocyte and hepatoma cell line HepG2 proteomes, J. Proteomics 136 (2016) 234–247, doi: 10.1016/j.jprot.2016.01.016. Epub 2016 Jan 26.

[50] M.W. Schaefers, L.M. Breshears, M.J. Anderson, Y.-C. Lin, A.E. Grill, J. Panyam, P.J. Southern, P.M. Schlievert, M.L. Peterson, Epithelial proinflammatory response and curcumin-mediated protection from staphylococcal toxic shock syndrome toxin-1.7 PLoS ONE (2012), doi: 10.1371/journal.pone.0032813.

[51] S. Bansal, S. Chhibber, Curcumin alone and in combination with augmentin protects against pulmonary inflammation and acute lung injury generated during klebsiella pneumoniae B5055-induced lung infection in BALB/c mice, J. Med. Microbiol. 59 (2010) 429–437, https://doi.org/10.1099/jmm.0.016873-0.

[52] Y.G. Lin, A.B. Kunnumakkara, A. Nair, W.M. Merritt, L.Y. Han, G.N. Armaiz-Pena, A.A. Kamat, W.A. Spannuth, D.M. Gershenson, S.K. Lutgendorf, B.B. Aggarwal, A.K. Sood, Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kB pathway, Clin. Cancer Res. 13 (2007) 3423–3430, https://doi.org/10.1158/1078-0432.CCR-06-3072.

[53] S.K. Vareed, M. Kakarala, M.T. Ruffine, J.A. Crowell, D.P. Normolle, Z. Djuric, D.E. Brenner, Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects, Cancer Epidemiol. Biomarkers Prev. 17 (2008) 1411–1417, https://doi.org/10.1158/1055-9965.EPI-07-2693.

[54] S.I. Hoehle, E. Pfeiffer, M. Metzler, Glucuronidation of curcuminoids by human microsomal and recombinant UDP-glucuronosyltransferases, Mol. Nutr. Food Res. 51 (2007) 932–938, https://doi.org/10.1002/mnfr.200600283.

[55] G. Garcea, D.J.L. Jones, R. Singh, A.R. Denneson, P.B. Farmer, R.A. Sharma, W.P. Steward, A.J. Gescher, Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration, Br. J. Cancer 90 (2004) 1101–1015, doi: 10.1038/sj.bjc.6601623.

[56] Y.-M. Jia, T. Zhu, H. Zhou, J.-Z. Ji, T. Tai, H.-G. Xie, Multidrug resistance-associated protein 3 is responsible for the efflux transport of curcumin glucuronide from hepatocytes to the blood, Drug Metab. Dispos. 48 (2020) 966–971, https://doi.org/10.1124/dmd.119.089193.

[57] A.G. Kunihiro, P.B. Luis, J.A. Brickey, J.B. Frye, H.-H.S. Chow, C. Schneider, J.L. Funk, Beta-glucuronidase catalyzes deconjugation and activation of curcumin-glucuronide in bone, J. Nat. Prod. 82 (2019) 500–509, doi: 10.1021/acs.jnatprod.8b00873.

[58] E. Pfeiffer, S.I. Hoehle, S.G. Walch, A. Riess, A.M.S. Lyom, M.D. Metzler, Curcuminoids form reactive glucuronides in vitro, J. Agric. Food Chem. 55 (2007) 538–544, https://doi.org/10.1021/jf0623283.

[59] T.E. Mürdter, B. Sperker, K.T. Kivist, M. McClellan, P. Fritz, G. Friedel, A. Linder, K. Bosslet, H. Toomes, R. Dierkesmann, H.K. Kroemer, Enhanced uptake of doxorubicin into bronchial carcinoma: β-glucuronidase mediates release of doxorubicin from a glucuronide prodrug (HMR 1826) at the tumor site, Cancer Res. 57 (1997) 2440–2445.

[60] G.T. Bolger, A. Licollari, A. Tan, R. Greil, B. Vcelar, M. Majeed, L. Helson, Distribution and metabolism of Lipocurc™ (Liposomal Curcumin) in dog and human blood cells: species selectivity and pharmacokinetic relevance, Anticancer Res. 37 (2017) 483–492, doi: 10.21873/anticanres.11716.

[61] S.J. Zunino, D.H. Storms, J.W. Newman, T.L. Pedersen, C.L. Keen, J.M. Ducore, Oral or parenteral administration of curcumin does not prevent the growth of high-risk T(4;11) acute lymphoblastic leukemia cells engrafted into a NOD/SCID mouse model, Int. J. Oncol. 42 (2012) 741–748, doi: 10.3892/ijo.2012.1734.

[62] S. Perkins, R.D. Verschoyle, K. Hill, I. Parveen, M.D. Threadgill, R.A. Sharma, L. Marion, M.L. Williams, P. William, W.P. Steward, A.J. Gescher, Chemopreventive efficacy and pharmacokinetics of curcumin in the Min/+ mouse, a model of familial adenomatous polyposis, Cancer Epidemiol. Biomarkers Prevent. 11 (2002) 535–540.

[63] S. Bisht, M. Mizuma, G. Feldmann, N.A. Ottenhof, S.M. Hong, D. Pramanik, V. Chenna, C. Karikari, R. Sharma, M.G. Goggins, M.A. Rudek, R. Ravi, A. Maitra, A. Maitra, Systemic administration of polymeric nanoparticle-encapsulated curcumin (NanoCurc) blocks tumor growth and metastases in preclinical models of pancreatic cancer, Mol. Cancer Ther. 9 (2010) 2255–2264, doi: 10.1158/1535-7163.MCT-10-0172.

[64] P. Ramalingam, Y.T. Ko, A validated LC-MS/MS method for Quantitative analysis of curcumin in mouse plasma and brain tissue and its application in pharmacokinetic and brain distribution studies, J. Chromatogr. B 969 (2014) 101–108, https://doi.org/10.1016/j.jchromb.2014.08.009.

[65] Y. Wang, C. Bui, K. Wu, R. Wang, J. Wang, Curcumin protects the pancreas from acute pancreatitis via the mitogen-activated protein kinase signaling pathway, Mol. Med. Rept. 20 (2019) 3027–3034, https://doi.org/10.3892/mmr.2019.10547.

[66] L. Helson, G. Bolger, M. Majeed, B. Vcelar, K. Pucaj, D. Matabudul, Infusion pharmacokinetics of Lipocurc™ (liposomal curcumin) and its metabolite tetrahydrocurcumin in beagle dogs, Anticancer Res. 32 (2012) 4365–4370.

[67] A. Storka, B. Vcelar, U. Klickovic, G. Gouya, S. Weisshaar, S. Aschauer, G. Bolger, L. Helson, M. Woltz, Safety, tolerability and pharmacokinetics of liposomal curcumin (Lipocurc™) in healthy humans, Int. J. Clin. Pharmacol. Ther. 53 (2015) 1–12, https://doi.org/10.5414/CP202076.

[68] L. Zhongfa, M. Chiu, J. Wang, W. Chen, W. Yen, P. Fan-Havard, L.D. Yee, K.K. Chan, Enhancement of curcumin oral absorption and pharmacokinetics of curcuminoids and curcumin metabolites in mice, Cancer Chemother. Pharmacol. 69 (2012) 679–689, doi: 10.1007/s00280-011-1749-y.

[69] Y.-M. Tsai, W.-C. Jan, C.-F. Chien, W.-C. Lee, L.-C. Lin, T.-H. Tsai, Optimised nano-formulation on the bioavailability of hydrophobic polyphenol, curcumin, freely-moving rats, Food Chem. 127 (2011) 918–925, https://doi.org/10.1016/j.foodchem.2011.01.059.

[70] J. Wang, X. Yu, L. Zhang, L. Wang, Z. Peng, Y. Chen, The pharmacokinetics and tissue distribution of curcumin and its metabolites in mice, Biomed. Chromatog. 32 (2018) e4267, https://doi.org/10.1002/bmc.4267.

[71] S.S. Chiu, E. Lui, M. Majeed, J.K. Viswanatha, A. Ranjan, A. Maitra, D. Pramanik, J. Smith, L. Helson, Differential distribution of intravenous curcumin formulations in the rat brain, Anticancer Res. 31 (2011) 907–912.

[72] D. Matabudul, K. Pucaj, G. Bolger, B. Vcelar, M. Majeed, L. Helson, Tissue distribution of (Lipocurc™) liposomal curcumin and tetrahydrocurcumin following two- and eight-hour infusions in beagle dogs, Anticancer Res. 32 (2012) 4359–4364.

[73] G.T. Bolger, A. Licollari, R. Bagshaw, A. Tan, R. Greil, B. Vcelar, M. Majeed, P. Sordillo, Intense uptake of liposomal curcumin by multiple myeloma cell lines: comparison to normal lymphocytes, red blood cells and chronic lymphocytic leukemia cells, Anticancer Res. 39 (2019) 1161–1168, https://doi.org/10.21873/anticanres.13225.

[74] K. Nakagawa, J.M. Zingg, S.H. Kim, M.J. Thomas, G. Dolnikowski, A. Azzi, T. Miyazawa, M. Meydani, Differential cellular uptake and metabolism of curcuminoids in monocytes/macrophages: regulatory effects on lipid accumulation, Br. J. Nutr. 112 (2014), 8–14, doi: 10.1017/S0007114514000567.

[75] K. Brecht, A.M. Schäfer, H.E. Meyer zu Schwabedissen, Uptake transporters of the SLC21, SLC22A, and SLC15A families in anticancer therapy—modulators of cellular entry or pharmacokinetics? Cancers 12 (2020) 2263, doi: 10.3390/cancers12082263.

[76] J. Jankun, M. Wyganowska-Swuatkowska, K. Dettlaff, A. Jelinska, A. Surdacka, D. Watrobska-Swietlikowska, E. Skrzypczak-Jankin, Determining whether curcumin degradation/condensation is actually bioactivation, Int. J. Mol. Med. 37 (2016) 1151–1158, https://doi.org/10.3892/ijmm.2016.2524.

[77] G.K. Markantes, M.A. Michalaki, G.A.B. Vagenakis, F.N. Lamari, E. Pitsi, M. Eliopoulou, N.G. Beratis, K.B. Markou, Plasma beta-glucuronidase activity: a novel tool to distinguish type 1 from type 2 amiodarone-induced thyrotoxicosis? Eur. Thyroid J. 8 (2019) 215–220, https://doi.org/10.1159/000500355.

[78] H. Kuhl, Pharmacology of estrogens and progestogens: influence of different routes of administration, Climacteric 8 (Suppl. 1) (2005) 3–63, https://doi.org/10.1080/13697130500148875.

[79] B. Sperker, U. Werner, T.E. Mürdter, C. Tekkaya, P. Fritz, R. Wacke, U. Adam, M. Gerken, B. Drewelow, H.K. Kroemer, Expression and function of beta-glucuronidase in pancreatic cancer: potential role in drug targeting, Naunyn-Schmiedebergs Arch. Pharmacol. 362 (2000) 110–115, doi: 10.1007/s002100000260.

[80] M.A. Tomeh, R. Hadianamrei, X. Zhao, A review of curcumin and its derivatives as anticancer agents, Int. J. Mol. Sci. 20 (2019) 1–26, https://doi.org/10.3390/ijms20051033.

[81] M.-H. Pan, T.-M. Huang, J.-K. Lin, Biotransformation of curcumin through reduction and glucuronidation in mice, Drug Metab. Dispos. 27 (1999) 486–494.

[82] T. Dorai, Y.-C. Cao, B. Dorai, R. Buttyan, A.E. Katz, Therapeutic potential of curcumin in human prostate cancer. III. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo, Prostate 47 (2001) 293–303, https://doi.org/10.1002/pros.1074.

[83] A.B. Kunnumakkara, P. Diagaradjane, S. Guha, A. Deorukhkar, S. Shentu, B.B. Aggarwal, S. Krishnan, Curcumin sensitizes human colorectal cancer xenografts in nude mice to γ-radiation by targeting nuclear factor-κB regulated gene products, Clin. Cancer Res. 14 (2008) 2128–2136, https://doi.org/10.1158/1078-0432.CCR-07-4722.

[84] B.E. Bachmeier, A.G. Nerlich, C.M. Iancu, M. Cilli, E. Schleicher, R. Vené, R. Dell'Eva, M. Jochum, A. Albini, U. Pfeffer, The chemopreventive polyphenol curcumin prevents hematogenous breast cancer metastases in immunodeficient mice, Cell Physiol. Biochem. 19 (2007) 137–152, https://doi.org/10.1159/000099202.

[85] H. Moghtaderi, H. Sepehri, F. Attari, Combination of arabinogalactan and curcumin induces apoptosis in breast cancer cells in vitro and inhibits tumor growth via overexpression of p53 level in vivo, Biomed. Pharmacother. 88 (2017) 582–594, https://doi.org/10.1016/j.biopha.2017.01.072.

[86] P. Yoysungnoen-Chintana, P.S. Bhattarakosol, S. Patumraj, Antitumor and antiangiogenic activities of curcumin in cervical cancer xenografts in nude mice, BioMed. Res. Int. (2014) Published On-Line ID 817972, doi: 10.1155/2014/817972.

[87] S. Bimonte, A. Barbieri, G. Palma, D. Rea, A. Luciano, M. D'Aiuto, C. Arra, F. Izzo, Dissecting the role of curcumin in tumour growth and angiogenesis in mouse model of human breast cancer, BioMed. Res. Int. (2015) 878134, doi: 10.1155/2015/878134.

[88] P. Yoysungnoen, P. Wirachwong, C. Changtam, A. Suksamrarn, S. Patumraj, Anti-cancer and anti-angiogenic effects of curcumin and tetrahydrocurcumin on implanted hepatocellular carcinoma in nude mice, World J. Gastroenterol. 14 (2008) 2003–2009, doi: 10.3748/wjg.14.2003.

[89] J.-H. Hong, G. Lee, H.-Y. Choi, Effect of curcumin on the interaction between androgen receptor and Wnt/β-catenin in LNCaP xenografts, Korean J. Urol. 56 (2015) 656–665, https://doi.org/10.4111/kju.2015.56.9.656.

[90] H. Inano, M. Onoda, N. Inafuku, M. Kubota, Y. Kamada, T. Osawa, H. Kobayashi, K. Wakabayashi, Chemoprevention by curcumin during the promotion stage of tumorigenesis of mammary gland in rats irradiated with γ-rays, Carcinogenesis 20 (1999) 1011–1018, https://doi.org/10.1093/carcin/20.6.1011.

[91] S.S. Bansal, H. Kausar, M.V. Vadhanam, S. Ravoori, J. Pan, S.N. Rai, R.C. Gupta, Curcumin implants, not curcumin diet, inhibit estrogen-induced mammary carcinogenesis in ACI rats, Cancer Prev. Res. 7 (2014) 456–465, https://doi.org/10.1158/1940-6207.CAPR-13-0248.

[92] J. García-Quiroz, R. García-Becerra, C. Santos-Cuevas, G.J. Ramírez-Nava, G. Morales-Guadarrama, N. Cárdenas-Ochoa, M. Segovia-Mendoza, H. Prado-Garcia, D. Ordaz-Rosado, E. Avila, A. Olmos-Ortiz, S. López-Cisneros, F. Larrea, L. Díaz, Synergistic antitumorigenic activity of calcitriol with curcumin or resveratrol is mediated by angiogenesis inhibition in triple negative breast cancer xenografts, Cancers 11 (2019) 1739, https://doi.org/10.3390/cancers11111739.

[93] J.H. Hong, K.S. Ahn, E. Bae, S.S. Jeon, H.Y. Choi, The effects of curcumin on the invasiveness of prostate cancer *in vitro* and *in vivo*, Prostate Cancer Prostatic Dis. 9 (2006) 147–152, https://doi.org/10.1038/sj.pcan.4500856.

[94] M. Li, Z. Zhang, D.L. Hill, H. Wang, H.R. Zhang, Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3/mTOR/ETS2 pathway, Cancer Res. 67 (2007) 1988–1996, doi: 10.1158/0008-5472.CAN-06-3066.

[95] M.C. Perry, M. Demeule, A. Regina, R. Moumdjian, R. Beliveau, Curcumin inhibits tumor growth and angiogenesis in glioblastoma xenografts, Mol. Nutr. Food Res. 54 (2010) 1192–1201, https://doi.org/10.1002/mnfr.200900277.

[96] G.G. Zhu, Q. Shena, H. Jianga, O. Jia, L. Zhu, L. Zhanga, Curcumin inhibited the growth and invasion of human monocytic leukaemia SHI-1 cells in vivo by altering MAPK and MMP signalling, Pharmaceut. Biol. 58 (2020) 25–34, https://doi.org/10.1080/13880209.2019.1701042.

[97] R.R. Falah, W.H. Talib, S.J. Shbailat, Combination of metformin and curcumin targets breast cancer in mice by angiogenesis inhibition, immune system modulation and induction of p53 independent apoptosis, Ther. Adv. Med. Oncol. 9 (2017) 235–252, https://doi.org/10.1177/1758834016687482.

[98] A.P. Ranjan, A. Mukerjee, L. Helson, R. Gupta, J.K. Vishwanatha, Efficacy of liposomal curcumin in a human pancreatic tumor xenograph model: inhibition of tumor growth and angiogenesis, Anticancer Res. 33 (2013) 3603–3610.

[99] J.-R. Tsai, P.-L. Liu, Y.H. Chen, S.H. Shah-Hwa Chou, Y.J. Cheng, Y.-J. Hwang, I.W. Chong, Curcumin inhibits non-small cell lung cancer cells metastasis through the adiponectin/NF-κb/MMPs signaling pathway, PLoS ONE 10 (2015) e0144462, https://doi.org/10.1371/journal.pone.0144462.

[100] S. Yılmaz, H. Ülger, T. Ertekin, A.H. Yay, M. Nisari, Ş. Alpa, N. Acer, Investigating the anti-tumoral effect of curcumin on the mice in which ehrlich ascites and solid tumor is created, Iran J. Basic Med. Sci. 22 (2019) 418–425, https://doi.org/10.22038/ijbms.2019.33623.8019.

[101] T.O. Khor, Y.-S. Keum, W. Lin, J.-H. Kim, R. Hu, G. Shen, C. Xu, A. Gopalakrishnan, B. Reddy, X. Zheng, A.H. Conney, A.-N.-T. Kong, Combined inhibitory effects of curcumin and phenethyl isothiocyanate on the growth of human PC-3 prostate xenografts in immunodeficient mice, Cancer Res. 66 (2006) 613–621, https://doi.org/10.1158/0008-5472.CAN-05-2708.

[102] W. Zhang, T. Cui, L. Liu, Q. Wu, L. Sun, L. Li, N. Wang, C. Gong, Improving anti-tumor activity of curcumin by polymeric micelles in thermosensitive hydrogel system in colorectal peritoneal carcinomatosis model, J. Biomed. Nanotechnol. 11 (2015) 1173–1182, https://doi.org/10.1166/jbn.2015.2073.

[103] Q. Dai, D. Zhou, L. Xu, X. Song, Curcumin alleviates rheumatoid arthritis-induced inflammation and synovial hyperplasia by targeting mTOR pathway in rats, Drug Design Devel. Ther. 12 (2018) 4095–4105, https://doi.org/10.2147/DDDT.S175763.

[104] D.-O. Dong-Oh Moon, M.-O. Mun-Ok Kim, H.Y. Yung Hyun Choi, Y.-M. Yung-Min Park, G.-Y. Kim, Curcumin attenuates inflammatory response in IL-1β-induced human synovial fibroblasts and collagen-induced arthritis in mouse model, Int. Immunopharmacol. 10 (2010) 605–610, doi: 10.1016/j.intimp.2010.02.011.

[105] E.-S.-M. Ammar, N.M. Gameil, N.A. Shawky, M.A. Nader, Comparative evaluation of anti-inflammatory properties of thymoquinone and curcumin using an asthmatic murine model, Int. Immunopharmacol. 11 (2011) 2232–2236, https://doi.org/10.1016/j.intimp.2011.10.013.

[106] C. Ma, Z. Ma, Q. Fu, S. Ma, Curcumin attenuates allergic inflammation by regulation of CD4 + CD25 + regulatory T cells (Tregs)/Th17 balance in ovalbumin-sensitized mice, Fitoterapia 87 (2013) 57–64, https://doi.org/10.1016/j.fitote.2013.02.014.

[107] F.E. Parodi, D. Mao, T.L. Ennis, M.B. Pagano, R.W. Thompson, Oral administration of diferuloylmethane (curcumin) suppresses proinflammatory cytokines and destructive connective tissue remodeling in experimental abdominal aortic aneurysms, Ann. Vascular Surg. 20 (2006) 360–368, https://doi.org/10.1007/s10016-006-9054-7.

[108] T.-H. Chen, Y.C. Yang, J.C. Wang, J.-J. Wang, Curcumin treatment protects against renal ischemia and reperfusion injury-induced cardiac dysfunction and myocardial injury, Transpl. Proc. 45 (2013) 3546–3549, https://doi.org/10.1016/j.transproceed.2013.09.006.

[109] S. Khadka, S. Omura, F. Sato, K. Nishio, H. Kakeya, I. Tsunoda, Curcumin β-D-glucuronide modulates an autoimmune model of multiple sclerosis with altered gut microbiota in the ileum and feces, Front. Cell. Infect. Microbiol. 11 (2021) 772962, doi: 10.3389/fcimb.2021.772962.

[110] R.R. Schulte, R.H. Ho, Organic anion transporting polypeptides: emerging roles in cancer pharmacology, Mol. Pharmacol. 95 (2019) 490–506, https://doi.org/10.1124/mol.118.114314.

[111] I.F. Antunes, H.J. Haisma, P.H. Elsinga, V. Di Gialleonardo, A. van Waarde, A.T.M. Willemsen, R.A. Dierckx, E.F.J. de Vries, Induction of β-glucuronidase release by cytostatic agents in small tumors, Mol. Pharm. 9 (2012) 3277–3285, https://doi.org/10.1021/mp300327w.

[112] Q. Shi, G.R. Haenen, L. Maas, V.M. Arlt, D. Spina, Y.R. Vasquez, E. Moonen, C. Veith, F.J. Van Schooten, R.W.L. Godschalk, Inflammation-associated extracellular β-glucuronidase alters cellular responses to the chemical carcinogen benzo[a]pyrene, Arch. Toxicol. 90 (2016) 2261–2273, https://doi.org/10.1007/s00204-015-1593-7.

[113] R.L. Edwards, P.B. Luis, P.V. Varuzza, A.I. Joseph, S.H. Presley, R. Chaturvedi, C. Schneider, The anti-inflammatory activity of curcumin is mediated by its oxidative metabolites, J. Biol. Chem. 292 (2017) 21243–2125, doi: 10.1074/jbc.RA117.000123.

[114] A. Karthikeyan, N. Senthil, T. Min, Nanocurcumin: a promising candidate for therapeutic applications, Front. Pharmacol. 11 (2020) 487, doi: 10.3389/fphar.2020.00487.

[115] K. Mansouri, S. Rasoulpoor, A. Daneshkhah, S. Abolfathi, N. Salari, M. Mohammadi, S. Rasoulpoor, S. Shabani, Clinical effects of curcumin in enhancing cancer therapy: a systematic review, BMC Cancer 20 (2020) 791, https://doi.org/10.1186/s12885-020-07256-8.

[116] M.L. Aggarwal, K.M. Chacko, B.T. Kuruvilla, Systematic and comprehensive investigation of the toxicity of curcuminoid-essential oil complex: a bioavailable turmeric formulation, Mol. Med. Rep. 13 (2016) 592–604, doi: 10.3892/mmr.2015.4579.

[117] E. Burgos-Moron, J.M. Calderon-Montano, J. Salvador, A. Robles, M. Lopez-Lazaro, The dark side of curcumin, Int. J. Cancer 126 (2010) 1771–1775, doi: 10.1002/ijc.24967.