OPEN ACCESS

PROGRESS IN PREVENTIVE MEDICINE

ORIGINAL RESEARCH

The 3 Curcuminoid Analogs Comprising the Curcumin Extract Comparably Inhibit Nuclear Factor kappa-light-chain-enhancer Activation

Franco Cavaleri[a,b]

[a]Experimental Medicine Program, Department of Medicine, Faculty of Medicine, Center for Brain Research, UBC Hospital, Vancouver, British Columbia, Canada; and [b]Biologic Pharmamedical Research, Surrey, British Columbia, Canada.

Address reprint requests to Franco Cavaleri, Biologic Pharmamedical Research 688-2397 King George Hwy, Surrey, BC, Canada V4A 7E9. E-mail address: franco.c@biologic-med.com (F. Cavaleri)

ABSTRACT

Introduction: **The curcumin extract, although relatively isolated from the rest of the plant's constituents, still exhibits an expansive polypharmacology. The extract is made up of 3 main curcuminoid analogs: diferuloylmethane (curcumin I), desmethoxycurcumin (curcumin II), and bisdesmethoxycurcumin (curcumin III). Each curcuminoid analog displays homologous structure with slight differences that should contribute to differential pharmacology. The study of these curcuminoids in isolation using different subcellular targets and cell lines may help us better understand the mechanisms involved in the curcumin extract's total polypharmacology. This research can also help us determine how the pharmacology of these curcuminoid analogs might be used with greater drug-target selectivity.**

Methods/Results: **As a start to this lengthy process, process a human embryonic kidney cell line containing the SV40 T-antigen (HEK293T) cell line is chosen for transfection with a basic Nuclear Factor kappa-light-chain-enhancer (NF-kB) reporter plasmid to study, by luciferase assay, the inhibitive potential of the curcuminoids in isolation. All 3 curcuminoids are shown here to inhibit p65–p50 (one of the NF-kB family protein complexes) activation in tumor necrosis factor (TNFα)–stimulated HEK293T cells with a comparable level of inhibitive activity. Each of the 3 curcuminoids exhibits the same IC50 (concentration of an inhibitor to half the activity) in 2 different curcuminoid contexts studied with regard to NF-kB inhibition.**

Conclusion: **We will continue to study these curcuminoid analogs with a cautious expectation that they will exhibit differential pharmacology with respect to alternative targets we will study. However, with regards to NF-kB inhibition, the three structurally different curcuminoids exhibit similar pharmacology.**

Keywords: **anti-inflammatory, curcumin, natural medicine, turmeric**

Introduction

Curcuma longa (turmeric) has been long studied for its anti-inflammatory activity, the main mechanism of which centers on the inhibition of Nuclear Factor kappa-light-chain-enhancer (NF-kB) signaling.[1–4] Nevertheless, modulation of NF-kB is a precarious initiative because the transcription factor plays a central role in healthy basal activity. The proportion of the curcuminoids within the curcumin extract can change from one lot or batch to another even from one supplier. Knowing how each of the curcuminoids factors into the modulation of its subcellular targets, including NF-kB, can help us generate more precise therapeutic agents from the extract. This precision might be important in the context of NF-kB modulation.

NF-kB regulates the expression of as many as 150 genes that orchestrate inflammatory and immune system responses.[5–8] Nevertheless, it is also an important factor in basal health of the cell. NF-kB is constitutively activated in glutamatergic neurons playing a central basal role in cell development and synaptic transmission.[9,10] Complete abrogation of NF-kB in healthy cells can result in apoptosis because the transcription factor is essential for cell survival.[11,12] Compromised activity of the transcription factor short of apoptosis in neurons can result in cognitive and other health impediments.[13,14] It is understood that constitutively dysregulated NF-kB plays a central role in cancer cell survival, the inhibition of which represents a central target in cancer treatment.[15,16] NF-kB is also a major player in inflammatory diseases where downregulation of the transcription factor can play a role in relief from inflammation. Controlling NF-kB is a delicate balancing act in the context of disease management and using inhibitors that are not completely characterized makes it even more difficult for treating practitioners to rely on the treatment. A more pointed understanding of the curcumin-based natural medicine may provide the basis for treatment with the natural medicine to be more reliable.

PROGREVMED 2019; 4: e0023

Published online 16 July 2019

10.1097/pp9.0000000000000023

As is common for transcription factor regulation, many factors play a role in regulation. Phosphorylation of the protein plays a paramount regulatory role on transcriptional activity. The key NF-kB Family protein (p65) serine^{276} site, in the transactivation domain of p65, is a critical one.[17,18] This phosphorylative coding serves as a control switch for the transcription factor's regulation of the 150 or more genes it targets.[19,20] The kappa-B nucleotide motif of the NF-kB gene target is a requisite feature for p65–p50 docking, but it does not ensure docking and transactivation.[21,22] The kappa-B nucleotide motif (GGG ACT TTC C) is situated in the first intron of the target genes.

This distinct nucleotide motif is also a requisite in the expression of genes regulated by transcription factors other than NF-kB where NF-kB serves as a collaborative transactivation element. An example of the expansive influence by the transcription factor beyond cytokines directly associated with NF-kB transactivation is that of human proto-oncogene (c-fos) transcription. NF-kB enhances c-fos transactivation via direct binding to the response element at the first intron of the gene and as such facilitates c-fos transactivation.[23,24] The status of these p65 phosphorylation sites can prevent the heterodimer docking on gene promoters,[21,22,25,26] even of those equipped with the kappa-B nucleotide motif.[27,28]

In this initial stage of the curcuminoid research, the inhibitive potential on NF-kB signaling activity was studied and the relative inhibitive force by each of the curcuminoids in isolation was quantified. This inhibitive activity was found to be quite similar for each of the curcuminoids.

Materials and methods

Cell culture

Human embryonic kidney cell line containing the SV40 T-antigen (HEK293T) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Oakville, Ontario, Canada) complete medium (DMEM + 1% ampicillin + 10% fetal bovine serum). Approximately 2×10^6 HEK293T cells per well were seeded in each well of a 6-well plate with 2.0 ml complete medium and cultured overnight at 37°C and 5% carbon dioxide.

Plasmids and cells preparation of NF-kB/reporter plasmid

NF-kB luciferase reporter plasmid was a gift of Dr. Weihong Song, PhD, University of British Columbia Faculty of Medicine Professor. The plasmid is a transfection-ready vector containing a NF-kB–responsive element upstream of the promoter.

Luciferase assay of transiently transfected HEK293T cells

Preparation of HEK293T cells for tumor necrosis factor-α–stimulated luciferase assay

HEK293T cells were cotransfected with our NF-kB plasmid-luciferase reporter construct and LacZ (β-Galactosidase Enzyme Assay System) purchased from Promega (product E2000; Madison, Wis.) to serve as an internal control. The objective was to measure inhibition of NF-kB by various curcumin/curcuminoid-based drug preparations and compare them against the inhibitive potential of other drugs such as Bay-11, acetylsalicylic acid, dexamethasone, ibuprofen, and various curcuminoid-based drugs using the luciferase assay to produce a measurable result.

Chemicals/drug preparation

The drugs were procured as follows: Bay 11–7082 NF-kB Inhibitor (Santa Cruz, Calif.); Dexamethasone (Sigma-Aldrich); Ibuprofen (Sigma-Aldrich); Commercial Curcuminoid Preparation from Biologic Nutrigenomic Health Research Corp (Surrey, BC, Canada) ;Curcumin I research standard (ChromaDex, Irvine, Calif. [certificate of analysis (CoA) 97.7% Purity water excluded]); Synthetic Curcumin I (ChromaDex); Curcumin II research standard [ChromaDex (CoA 97.3% Purity water excluded)]; Curcumin III research standard [Sigma-Aldrich (CoA 97.7% Purity)]; Curcuminoids/Curcumin Extract (curcumin I, 77.7%; curcumin II, 16.9%; curcumin III, 0.9%) research standard [ChromaDex (CoA 95.3% Purity water excluded)]; and Tumor necrosis factor-α (TNFα) (Sigma-Aldrich). Curcumin/curcuminoids and other drugs were all prepared to various concentrations in DMEM containing 0.2% dimethyl sulfoxide (DMSO).

MTT assay

MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Sigma-Aldrich) was performed on HEK293T to study the cytotoxicity of curcuminoid analogs. For MTT assay 3×10^4, cells were seeded on each well of a 96 well plates. The drugs used were the same ones described above: Commercial Curcumin Preparation, Curcumin I, Curcumin II, Curcumin III, and Synthetic Curcumin I. Each curcumin preparation was tested at the following concentrations on each cell line to generate the corresponding graph: 5.0, 10.0, 20.0, 40.0, and 80.0 µg/ml. Absorbance was measured at 570 nm using a Perkin Elmer Envision 2103 Multilabel Reader (Shelton, Conn.). Results were expressed as percent of the absorbance found in control cells (n = 4).

Drug treatment and diagnostic

After an optimization period, the selected drug concentration was chosen: 22.0 µg/ml. Curcumin drug pretreatment of the wells is executed as described in the following order: Bay 11(30 µM); no treatment/cells ONLY; DMSO only (0.2%)—Control; Curcumin Extract 95% (research standard) (22.0 µg/ml); Curcumin Extract 95% (research standard) (22.0 µg/ml); Commercial Curcumin Extract (Off-the-shelf) (22.0 µg/ml); Curcumin I (research standard) (22.0 µg/ml), Curcumin II (research standard) (22.0 µg/ml); and Curcumin III (research standard) (22.0 µg/ml). Upon successful transfection, HEK293T cells were treated with the drugs for 30 minutes followed by 6 hours stimulation with TNFα (1.0 µL per well of the 5.0 ng/µl stock TNFα solution). After stimulation, drug pretreated cells were washed with 100–150 µL phosphate-buffered saline, and treated with lysis buffer. The lysates were harvested and processed for luciferase assay (Sigma-Aldrich,

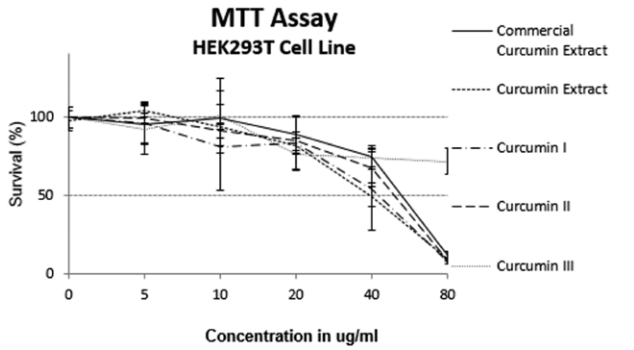

Fig 1. Graphed MTT assay results showing the relative toxicity of the different curcuminoid-based preparations tested. Results for each curcuminoid/extract tested in this HEK293T cell line are similar in pattern with 75%–80% survival with treatment concentrations as high as 80 µg/ml. Curcumin III is an outlier in the graphed results potentially demonstrating lower toxicity, or a possible increased resilience to toxicity by these cell lines to curcumin III. MTT, 3-[4, 5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; HEK293T, human embryonic kidney cell line containing the SV40 T-antigen.

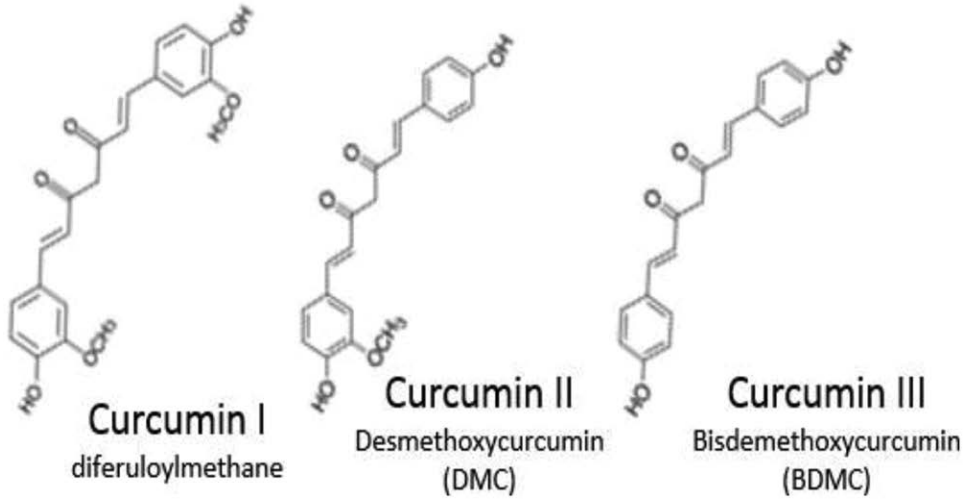

Fig 2. Curcuminoid structural differences contribute to differing pharmacologic features although their homology affords these molecules common biological activities. BDMC, bisdesmethoxycurcumin; DMC, desmethoxycurcumin.

Oakville, ON) according to the manufacturer's instruction using a Perkin Elmer Envision 2103 Multilabel Reader. Seven luciferase assay iterations (biologic repeats) were executed.

Concentration curve for IC50 using luciferase assay

HEK293T cells were cotransfected with NF-kB plasmid-luciferase reporter construct (gift from Dr. Weihong Song; University of British Columbia, Vancouver, British Columbia, Canada) and LacZ (Promega, Madison, Wis.). The IC50 was generated for each of the curcuminoid analog test drugs (curcumin I, curcumin II, curcumin III, and commercial curcumin/curcuminoid preparation). Transfected HEK293T cells were used as described above with TNFα solution stimulation. Three iterations, each as new biologic repeats, were generated and averaged. Each iteration was designed with three internal repeats.

Results

Cytotoxicity of curcumin

The MTT assay results show that the relative toxicity of the different curcumin- and curcuminoid-based preparations tested in HEK293T cells is similar but not the same. Nevertheless, the results shown at Fig 1 demonstrate that curcumin extract and curcuminoid concentrations used for treatment (22.0 µg/ml) in the experimental series are not cytotoxic.

There is a clear concentration-dependent cytotoxicity by all curcumin compounds. Cell survival dropped to approximately 50% of the total cell count in the concentration range between 40 and 60 µg/ml (Fig 1). Each compound induced a consistently steep drop in cell survival after 40 µg/ml, and 100% cell killing was seen at 80 µg/ml. The IC50 for the curcumin-based drugs is approximately 40 µg/ml in the HEK293T cell line (Fig 1). Fig 2 presents the 3 curcum-

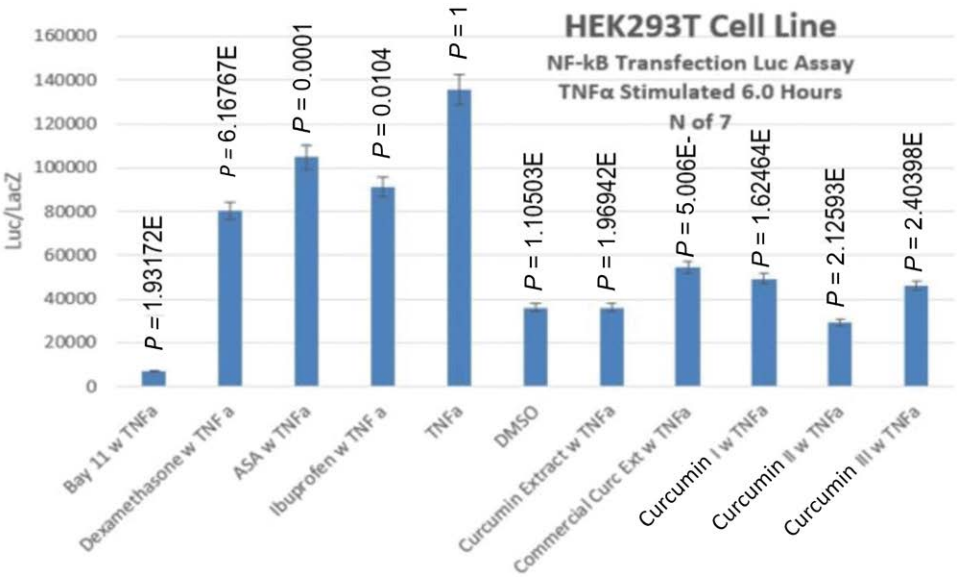

Fig 3. Results displaying relative inhibition of NF-kB using the firefly luciferase/luciferin assay as a reporter. TNFα is applied to stimulate the transfected HEK293T mammalian cell line after pretreatment with the various drugs. Drug concentrations used are as follows and shown as final well concentrations: Bay 11 (30 µM), dexamethasone (27 µM), ASA (5.67 mM), ibuprofen (3.49 mM), TNFα only, control: dimethyl sulfoxide only (0.2%), curcumin extract 95% purity (research standard) (43 M), commercially available curcumin off the retail shelf (43 µM), curcumin I (research standard) (43 µM), curcumin II (research standard) (43 µM), and curcumin III (research standard) (43 µM). Luc/LacZ indicates the ratio of Luciferase Assay reading divided by β-galactosidase (LacZ) reading. ASA, acetylsalicylic acid; HEK293T, human embryonic kidney cell line containing the SV40 T-antigen; NF-kB, Nuclear Factor kappa-light-chain-enhancer; TNFα, tumor necrosis factor-α.

TABLE 1.

***P* Values for Luciferase Assay of Transiently Transfected HEK293T Cells**

Drug tested	*P* value
Bay 11 with TNFα	1.93172 E-08
Dexamethasone with TNFα	6.16767 E-06
ASA with TNFα	0.000149
Ibuprofen with TNFα	0.010441
TNFα only	1
DMSO only	1.10503 E-07
Curcumin extract with TNFα	1.96942 E-07
Commercial curcumin extract with TNFα	5.006 E-07
Curcumin I with TNFα	1.62464 E-06
Curcumin II with TNFα	2.12593 E-07
Curcumin III with TNFα	2.40398 E-07

ASA, acetylsalicylic acid; DMSO, dimethyl sulfoxide; HEK203T, human embryonic kidney cell line containing the SV40 T-antigen; TNFα, tumor necrosis factor-α.

inoids to highlight their homologues structures. Although there are mild molecular features differentiating the curcuminoid analogs, they perform comparably in the context of cytotoxicity and the following experimental models.

Curcumin and curcuminoids inhibit TNFα-induced NF-kB activation

Based on the above toxicity study, our curcumin-related experimentation was carried out at drug concentrations of 22.0 μg/ml across the curcumin-based drug testing. This is well under the MTT-demonstrated toxicity range. Fig 3 displays the quantified results from 7 biologic repeats (with 3 internal repeats) using a NF-kB–luciferase vector as a transiently transfected reporter construct in the HEK293T cell model. One commercially available curcumin extract was used to represent an off-the-shelf leading brand to consumers. Other common anti-inflammatory drugs were used as comparatives such as dexamethasone, acetylsalicylic acid, and ibuprofen. Bay 11 and DMSO ONLY were used as research controls.

Results from this experimental model demonstrate that NF-kB activation by TNFα stimulation of the transfected HEK293T cell line is inhibited by the curcumin extract ($P = 2\times10^{-7}$) and its isolated curcuminoid analogs I, II, and III ($P < 1.0\times10^{-7}$) comparably. The isolated curcuminoid analogs each performed with similar efficacy and with greater inhibitory potential on NF-kB p65–p50 than common nonsteroidal anti-inflammatory drugs including a commonly prescribed corticosteroid drug, dexamethasone ($P = 6.2\times10^{-6}$).

The "Curcumin Extract" (research standard) effectively abolished ($P = 2.0\times10^{-7}$) NF-kB activation to baseline (DMSO ONLY). The off-the-shelf commercial preparation presented significant inhibitory activity as well ($P = 5.0\times10^{-7}$). The *P* values are also more clearly presented at Table 1.

Concentration curve for IC50

Each curcumin drug displayed very similar patterns of inhibitive pharmacology on NF-kB activity in both the TNFα-stimulated cells and the nonstimulated cells as shown in Fig 4. All research standards, Curcumin Extract, Curcumin I, Curcumin II, and Curcumin III, performed comparably as did the commercial preparation with regard to NF-kB inhibition. Each displayed an IC50 of approximately 22.0 μg/ml for cells treated with TNFα. It was very clear, however, that at approximately

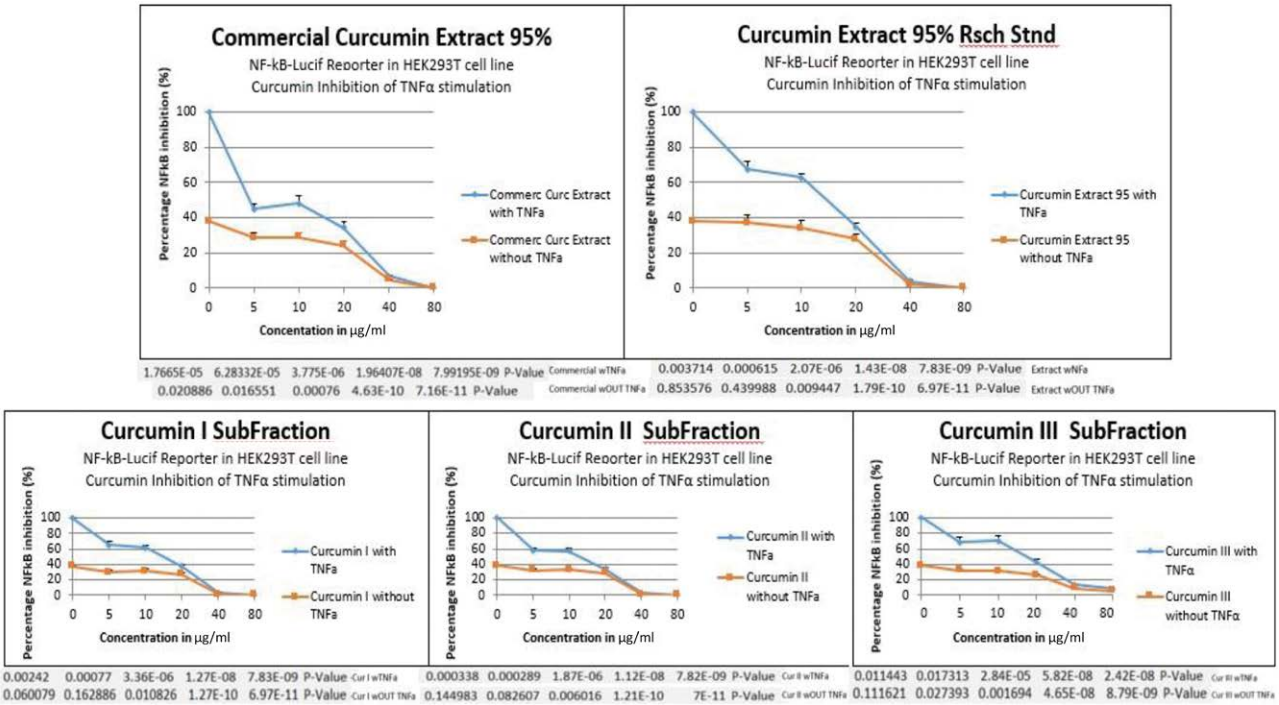

Fig 4. Concentration curves measured and calculate based on chemiluminescence data. These cells are pretreated with the various concentrations of the curcumin-based drug preparations before stimulation with a TNFα solution to quantify the drugs' inhibitive potential on drug-treated samples. Cells NOT treated with TNFα as a stimulant are also evaluated in the same drug concentrations to determine inhibitive activity of the drugs on basal activity. *P* values are included in the diagram but are also presented in Table 2 for clarity. HEK203T, human embryonic kidney cell line containing the SV40 T-antigen; NF-kB, Nuclear Factor kappa-light-chain-enhancer; TNFα, tumor necrosis factor-α.

TABLE 2.

***P* Values for Curcumin Concentration Curves Studied to Compare Each Drug in Equal Concentrations**

		Concentration (μ/ml)				
	P Values for Concentration Curve Context 1	5	10	20	40	80
P values	Commercial curcumin with TNFα	1.7665E-05	6.283E-05	3.775E-06	1.9641E-08	7.99195E-09
	Commercial curcumin without TNFα curcumin	0.02088591	0.0165508	0.00076	4.6274E-10	7.15644E-11
	Extract Rsch Stand with TNFα	0.003714161	0.0006148	2.067E-06	1.4265E-08	7.82787E-09
	Curcumin extract Rsch Stand without TNFα	0.853575999	0.439988	0.0094466	1.786E-10	6.96731E-11
	Curcumin I with TNFα	0.002419673	0.0007701	3.357E-06	1.2714E-08	7.82992E-09
	Curcumin I without TNFα	0.060078693	0.1628864	0.0108259	1.2728E-10	6.96806E-11
	Curcumin II with TNFα	0.000338187	0.0002892	1.869E-06	1.12E-08	7.81959E-09
	Curcumin II without TNFα	0.144983451	0.082607	0.0060161	1.2058E-10	7.00194E-11
	Curcumin III with TNFα	0.011443472	0.0173131	2.844E-05	5.8193E-08	2.42372E-08
	Curcumin III without TNFα	0.111620813	0.0273932	0.001694	4.6526E-08	8.78614E-09

Rsch Stand, research standard; TNFα, tumor necrosis factor-α.

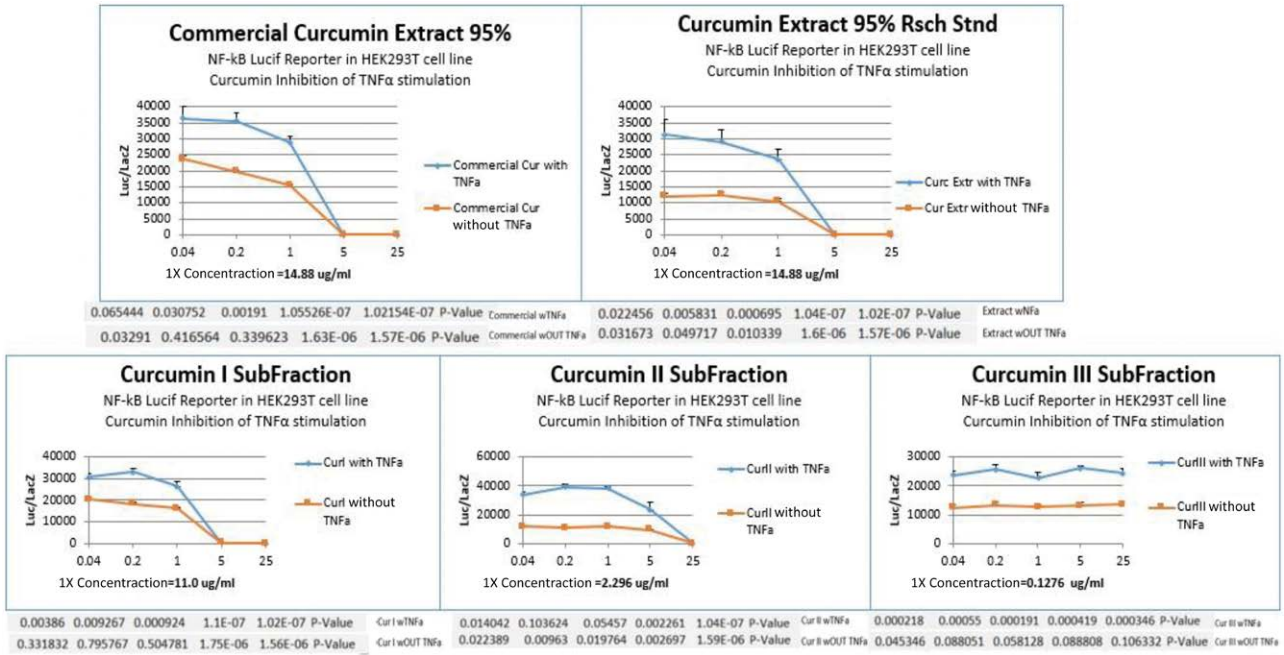

Fig 5. Concentration curves measured and calculated based on chemiluminescence data. These cells are pretreated with the various concentrations of the curcumin-based drug preparations before stimulation with a TNFα solution to quantify the drugs' inhibitive potential on drug-treated samples "in the context of their contributions when in their natural proportions" in the curcumin extract. Cells NOT treated with TNFα as a stimulant are also evaluated in the same drug concentrations to determine inhibitive activity of the drugs on basal activity. *P* values are included in the diagram but are also presented in Table 3 for clarity. Luc/LacZ indicates the ratio of Luciferase Assay reading divided by β-galactosidase (LacZ) reading. HEK203T, human embryonic kidney cell line containing the SV40 T-antigen; NF-kB = Nuclear Factor kappa-light-chain-enhancer; TNFα, tumor necrosis factor-α.

20.0–22.0 μg/ml concentration, each of these curcumin-based drugs begin to steeply reduce basal NF-kB activity toward zero where zero is approached for all but curcumin III by a final concentration of 40 μg/ml. The *P* values are also more clearly presented at Table 2.

We also measured the relative contribution by each curcuminoid to the curcumin extract's inhibitory activity on NF-kB (p65–p50) (Fig 5). The proportion of curcuminoids in a common curcumin extract approximate: curcumin I, 77.7%; curcumin II, 16.9%; and curcumin III, 0.9%. These inherent proportions were used to establish the tested drug concentrations for each of the curcuminoids: I, II, and III in this experiment. Curcumin I was used at the highest concentration to produce a prominent inhibitive activity with respect to NF-kB inhibition, and it did so in both basal nonstimulated cells and the TNFα-induced cells. A 1.0× concentration was established based on these inherent proportions as described in the Methods, and from this number, the various concentrations were chosen to establish relative curves. Each 1.0× value was treated as follows: 0.04×, 0.20×, 1.0×, 5.0×, and 25× to generate a relative curve for each curcuminoid analog, again, in the context of their inherent proportions in the mother extract. Results are posted in Fig 5. These *P* values are also more clearly presented at Table 3.

The lower inhibitive force displayed by curcumin II [demethoxycurcumin (DMC)] drug treatment using this strategy was a function of its lower concentration in the mother curcumin extract (Fig 5). If concentration equivalents were calculated back into the graph, it is evident that curcumins I and II perform relatively similarly on a gram-per-gram basis, as seen in Fig 4, where NF-kB activity was inhibited in both basal and TNFα-induced cells. Curcumin

TABLE 3.

***P* Values for Curcumin Concentration Curves Studied With Curcuminoid Concentrations Relative to that of the Curcuminoids in the Curcumin Extract**

		Concentration by Dilution Factor				
	P Values for Concentration Curve Context 2	0.04×	0.2×	1×	5×	25×
P values	Commercial curcumin with TNFα	0.065443889	0.030751823	0.001910065	1.05526E-07	1.02154E-07
	Commercial curcumin without TNFα	0.032910287	0.416563724	0.339622609	1.63026E-06	1.57047E-06
	Curcumin extract Rsch Stand with TNFα	0.022455688	0.005830787	0.000695393	1.03646E-07	1.02101E-07
	Curcumin extract Rsch Stand without TNFα	0.031673473	0.049717	0.010338615	1.60216E-06	1.56804E-06
	Curcumin I with TNFα	0.003859821	0.009266932	0.000923934	1.10277E-07	1.01633E-07
	Curcumin I without TNFα	0.331831839	0.795767202	0.504780607	1.75084E-06	1.56379E-06
	Curcumin II with TNFα	0.014042365	0.103623699	0.054570368	0.002260963	1.04045E-07
	Curcumin II without TNFα	0.022389471	0.009630463	0.019764173	0.002697382	1.59129E-06
	Curcumin III with TNFα	0.000218128	0.000549707	0.000190573	0.000419291	0.000345583
	Curcumin III without TNFα	0.045346392	0.088050715	0.058127682	0.088808331	0.106332229

Rsch Stand, research standard; TNFα, tumor necrosis factor-α.

III did not reach concentration thresholds high enough to induce efficacious inhibition in either basal or TNFα-induced cells (Fig 5). Unless extremely high doses of the curcumin extract are used to push curcumin III levels up, curcumin III [bisdemethoxycurcumin (BDMC)] rarely contributes pharmacology in the context of NF-kB inhibition when a typical curcumin extract is studied.

Discussion

Studying each of the curcuminoid analogs in isolation may help unravel some of the mystery surrounding this medicinal agent. Synthetic curcumin analogs, for example, can display unique pharmacologic characteristics associated with structure[29,30]; structural differences that are rather miniscule. The naturally occurring curcuminoid analogs display similar structural characteristics, but as shown in Fig 2, their unique features may also contribute distinct pharmacologic characteristics that are unique to each analog. However, the naturally occurring curcuminoid analogs have not been studied expansively in their isolated forms in the past.

It is postulated that a reevaluation of each of the curcuminoid analogs' pharmacology in isolation may provide more insight on the full spectrum of curcumin activity and the source of the curcumin extract's polypharmacology. It may also help us define a more accurate standardization process for the extract.

The first steps of this initiative to map the pharmacology of each curcuminoid analog show us that the curcuminoids each have very similar activity in the context of NF-kB activation inhibition. Nevertheless, this, in itself, validates the need to better understand the pharmacology for each of the curcuminoids. In the natural product industry, the principle curcuminoid, diferuloylmethane, also known as curcumin I, is known to be the main active in the curcumin fraction of the turmeric herb; the standardization process for the natural medicinal agent is based on quantification of this curcuminoid.

However, natural curcumin preparations that are standardized to a precise concentration, often as high as 95% curcumin, have within them underlying variances of the curcuminoid analog proportions that may be contributing to inconsistent outcomes in the context of some targets and unexpected compounding activity on other targets like the one studied here. Standardization testing for the curcumin extract in the natural product industry is centered on curcumin I testing to quantify the active. However, studies do point to the likelihood that the curcuminoids do not produce the same pharmacology on all targets. For example, BDMC (curcumin III aka BDMC) is shown to deliver cytotoxicity to inhibit growth of the K562 cell line and this inhibitory activity is significantly greater than that of curcumin (curcumin I aka diferuloylmethane) and DMC (curcumin II aka DMC).[31]

On the other end of the spectrum, studies showed that curcumin I and DMC (curcumin II) have equally potent inhibitory activity on tetradecanoylphorbol acetate-induced tumorigenesis, but BDMC (curcumin III) was less active.[32] The mechanisms are undefined and seemingly conflictive; nevertheless, indicative of different activity by the different curcuminoid analogs. The research results reported here tell us that each of the curcuminoid analogs inhibits NF-kB activation in these nonimmune cells with similar inhibitive activity. As such, the anti-inflammatory activity delivered by the curcumin extract in this context is a function of all 3 curcuminoids additively and is quantified more accurately by a measure that quantifies the amount of each curcuminoid in the extract and not just curcumin I. This information provides us with the scientific basis to justify the need for disclosure of each curcuminoid quantity on labels to gain a full understanding of the medicinal agent's potency.

Disclosure

The authors have no financial interest to declare in relation to the content of this article. The author is the owner, CEO, and a primary investigator of a research corporation (Biologic Pharmamedical Research) that funds and executes research on nutraceutical and pharmaceutical pharmacology including research of curcuminoids and development of curcumin-based therapeutic agents. The Article Processing Charge was paid for by the author.

References

[1] Payton F, Sandusky P, Alworth WL. NMR study of the solution structure of curcumin. J Nat Prod. 2007;70:143–146.
[2] Xu YX, Pindolia KR, Janakiraman N, et al. Curcumin inhibits IL1 alpha and TNF-alpha induction of AP-1 and NF-kB DNA-binding activity in bone marrow stromal cells. Hematopathol Mol Hematol. 1997;11:49–62.
[3] Leclercq IA, Farrell GC, Sempoux C, et al. Curcumin inhibits NF-kappaB activation and reduces the severity of experimental steatohepatitis in mice. J Hepatol. 2004;41:926–934.
[4] Tomita M, Kawakami H, Uchihara JN, et al. Curcumin inhibits constitutive active NF-kappaB, leading to suppression of cell growth of human T-cell leukemia virus type I-infected T-cell lines and primary adult T-cell leukemia cells. Int J Cancer. 2006;118:765–772.
[5] Baeuerle PA, Baichwal VR. NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. Adv Immunol. 1997;65:111–137.
[6] Barnes PJ. Nuclear factor-kappa B. Int J Biochem Cell Biol. 1997;29:867–870.

[7] Giuliani C, Napolitano G, Bucci I, et al. Nf-kB transcription factor: role in the pathogenesis of inflammatory, autoimmune, and neoplastic diseases and therapy implications. Clin Ter. 2001;152:249–253.
[8] Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. J Clin Invest. 2001;107:7–11.
[9] Franco DG, Markus RP. The cellular state determines the effect of melatonin on the survival of mixed cerebellar cell culture. PLoS One. 2014;9:e106332.
[10] Grilli M, Memo M. Nuclear factor-kappaB/Rel proteins: a point of convergence of signalling pathways relevant in neuronal function and dysfunction. Biochem Pharmacol. 1999;57:1–7.
[11] Beg AA, Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. Science. 1996;274:782–784.
[12] O'Neill LA, Kaltschmidt C. NF-kappa B: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci. 1997;20:252–258.
[13] Pickering M, O'Connor JJ. Pro-inflammatory cytokines and their effects in the dentate gyrus. Prog Brain Res. 2007;163:339–354.
[14] Meffert MK, Chang JM, Wiltgen BJ, et al. NF-kappa B functions in synaptic signaling and behavior. Nat Neurosci. 2003;6:1072–1078.
[15] Karin M, Cao Y, Greten FR, et al. NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer. 2002;2:301–310.
[16] Grandage VL, Gale RE, Linch DC, et al. PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kappaB, MAPkinase and p53 pathways. Leukemia. 2005;19:586–594.
[17] Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell. 1998;1:661–671.
[18] Arun P, Brown MS, Ehsanian R, et al. Nuclear NF-kappaB p65 phosphorylation at serine 276 by protein kinase A contributes to the malignant phenotype of head and neck cancer. Clin Cancer Res. 2009;15:5974–5984.
[19] Yasumoto K, Okamoto S, Mukaida N, et al. Tumor necrosis factor alpha and interferon gamma synergistically induce interleukin 8 production in a human gastric cancer cell line through acting concurrently on AP-1 and NF-kB-like binding sites of the interleukin 8 gene. J Biol Chem. 1992;267:22506–22511.
[20] Abou-Samra AB, Pugeat M, Dechaud H, et al. Increased plasma concentration of N-terminal β-lipotrophin and unbound cortisol during pregnancy. Clin Endocrinol. 1984;20:221–228.
[21] Kunsch C, Ruben SM, Rosen CA. Selection of optimal kappa B/Rel DNA-binding motifs: interaction of both subunits of NF-kappa B with DNA is required for transcriptional activation. Mol Cell Biol. 1992;12:4412–4421.
[22] Fujita T, Nolan GP, Ghosh S, et al. Independent modes of transcriptional activation by the p50 and p65 subunits of NF-kappa B. Genes Dev. 1992;6:775–787.
[23] Charital YM, van Haasteren G, Massiha A, et al. A functional NF-κB enhancer element in the first intron contributes to the control of c-fos transcription. Gene. 2009;430:116–122.
[24] Collart MA, Baeuerle P, Vassalli P. Regulation of tumor necrosis factor alpha transcription in macrophages: involvement of four kappa B-like motifs and of constitutive and inducible forms of NF-kappa B. Mol Cell Biol. 1990;10:1498–1506.
[25] Sasaki CY, Barberi TJ, Ghosh P, et al. Phosphorylation of RelA/p65 on serine 536 defines an I{kappa}B{alpha}-independent NF-{kappa}B pathway. J Biol Chem. 2005;280:34538–34547.
[26] Anrather J, Racchumi G, Iadecola C. Cis-acting, element-specific transcriptional activity of differentially phosphorylated nuclear factor-kappa B. J Biol Chem. 2005;280:244–252.
[27] Bren GD, Solan NJ, Miyoshi H, et al. Transcription of the RelB gene is regulated by NF-kappaB. Oncogene. 2001;20:7722–7733.
[28] Lavrovsky Y, Chatterjee B, Clark RA, et al. Role of redox-regulated transcription factors in inflammation, aging and age-related diseases. Exp Gerontol. 2000;35:521–532.
[29] Venkatesan P, Rao MN. Structure-activity relationships for the inhibition of lipid peroxidation and the scavenging of free radicals by synthetic symmetrical curcumin analogues. J Pharm Pharmacol. 2000;52:1123–1128.
[30] Anand P, Thomas SG, Kunnumakkara AB, et al. Biological activities of curcumin and its analogues made by man and Mother Nature. Biochem Pharmacol. 2008;76:1590–1611.
[31] Anuchapreeda S, Sadjapong W, Duangrat C, et al. The cytotoxic effect of curumin, demethoxycurcumin and bisdemethoxycurcumin purified from turmeric powder on leukemic cell lines. Bull Chiang Mai Assoc Med Sci. 2006;39:60.
[32] Huang MT, Ma W, Lu YP, et al. Effects of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion. Carcinogenesis. 1995;16:2493–2497.