REVIEW

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Correlations between morphological changes induced by curcumin and its biological activities

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Abstract

Curcumin is a phytochemical polyphenol extracted from turmeric rhizome, with multiple biological activities, intensively studied in various therapeutic areas. Its effects covers a wide range of specialties, from the neuroprotective to the antimetastatic properties, influencing pathologies from cardiovascular, neuronal and oncological fields, as a part of its broad spectrum of action. These effects are explained by antioxidant, anti-inflammatory and anticarcinogenic simultaneous roles of curcumin and its derivatives. In this review, we selected the information about morphological evidences correlated with the biological effects on the following organ systems: the central nervous system (including neurological pathology, such as Parkinson's and Alzheimer's disease), the cardiovascular system (including disorders like atherosclerosis, endothelial dysfunction and drug-induced myotoxicity), multiple forms of cancer, and metabolic syndromes including diabetes. The central point of this review was to target a variety of morphological changes at microscopic level induced by curcumin, using different microscopy techniques.

Keywords: curcumin, morphological changes, transmission electron microscopy.

Introduction

Curcumin, a natural compound extracted from *Curcuma longa* – a plant with Indian origins [1], known as turmeric –, is used for centuries as a spice (Indian turmeric), dye, beauty agent, but especially in ayurvedic medicine. Over the years, curcumin has proven its effectiveness in various pathologies, demonstrating anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, cholesterol-lowering treatment, having a benefic intervention in chronic diseases such as: diabetes, psoriasis, allergies, Parkinson's disease, Alzheimer's disease, etc. Some authors consider this natural product as a potential candidate for the plurifactorial therapy, due to the numerous uses in traditional Indian and Chinese medicine, being surnamed "spice for life" [2]. Curcumin is considered an ideal starting point in pharmaceutical research for the discovery of new medications. For example, in rheumatoid arthritis, the superiority of curcumin was already demonstrated in relation to new steroidal anti-inflammatory medication through a very low ulcerogenic potential, and the absence of antipyretic effect [3]. The inflammatory cascade plays an important role in the development of chronic illnesses, such as allergies, autoimmune, cardiovascular, endocrine, neurodegenerative and neoplastic diseases [4–6]. Curcumin is able to decrease inflammation by interacting with many inflammatory processes such as down-regulation for the activity of cyclooxygenase-2 (COX-2), lipoxygenase, and inducible nitric oxide synthase (iNOS) [7–10].

Curcuma longa rhizome has been traditionally used also as antimicrobial agent as well as an insect repellant [11]. Several studies have reported the broad-spectrum antimicrobial activity for curcumin, including antibacterial, antiviral, antifungal, and antimalarial activities. Despite its many biological activities, curcumin has a big disadvantage, which is the low bioavailability. This is the reason why, in the last five decades, the researchers are concerned to do various modifications in curcumin structure and its administration form, in order to improve the bioavailability and effectiveness. In many studies, curcumin is used as a structural sample to design new antimicrobial agents with modified and increased antimicrobial activities, through the synthesis of various derivatives related to curcumin [12–14].

Curcumin is a pigment that has good affinity for many organic substances. It was known, since the time of Paul Ehrlich and Hans Christian Gram, that organic pigments may exhibit different affinities for a wide variety of human tissues, cells or subcellular compartments, thus allowing to act selectively in terms of staining, resulting in an easier way to identify certain preparations. An illustrative example is the study which shows that a product containing pure curcumin exhibit special selectivity for pathological fibrils of amyloid from Alzheimer's disease [15]. This observation led to the conclusion that pure curcumin and its derivatives have considerable potential as inhibitors of different proteins or beta-amyloid aggregation. On the other hand, Ryu *et al.* [16] demonstrated that curcumin administered intravenously in mice was accumulated preferential in the liver, spleen, lungs and brain. These authors concluded that curcumin and some of its derivatives have specific affinity for some tissues. Other

prospects for the use of curcumin and its derivatives in medicinal therapies include arthritis, diabetes, cardiovascular diseases, wound healing, and the list continues with each experiment and published article [17].

Curcumin and neurodegenerative diseases

Parkinson's disease (PD) is a neurodegenerative disorder characterized by degeneration of dopaminergic neuron in *substantia nigra pars compacta*, resulting in a loss of dopamine (DA) in the striatum [18]. Although the etiology of PD is unknown, various chemical alterations initiated by oxidative stress and mitochondrial dysfunction are major factors which self-initiate neurodegeneration [19]. Neuroprotective mechanism of a novel pyrazol derivate of curcumin was investigated to a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (mitochondrial complex I inhibitor) rodent model. Pretreatment with 24 mg/kg/day curcumin derivate ameliorates the behavior of the rats and is correlated with ultrastructural changes showing normal nuclear morphology with increased mitochondrial number and structural stability. In MPTP-untreated group, mitochondria showed abnormal structure with damaged cristae and the total number was decreased [20].

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which ultimately affect all the cognitive functions. The progression of AD is multifactorial and includes aggregation of amyloid β-peptide (Aβ), oxidative stress and decreased levels of acetylcholine (AChE) [21–23]. Curcumin ameliorates the neuronal degeneration in hippocampi of AD rats (a recognition disorder model with 10-μL injection of Aβ1-40 in their hippocampi). In AD rats group, Hematoxylin and Eosin (HE) staining of specific area reveal loss of the majority of the neurons, the rest of them presents karyopyknosis and the number of the pyramidal cells was reduced compare with curcumin group. The neurons in curcumin treatment group (intraperitoneal injection of 300 mg/kg for seven days) were mildly impaired [24].

Curcumin and neuroprotection

Neurons are particularly vulnerable to oxidation damage, which is able to start alterations in their structure and function [25]. The presence of free radicals causes changes at mitochondrial level, influencing the activity of the respiratory chain complexes. Antioxidative effect of curcumin could intercept free radicals and minimize chemical modification of lipids and proteins, which can cause mitochondrial damage and initiation of neuronal apoptosis. The results of oxidative measurements for free radicals are correlated with morphological changes induced by curcumin administration in rat models of senescence, suggesting that antioxidants like curcumin can delay progression of neurodegradation and improve cognition. D-galactose administered for 56 days to Wistar rats disrupted structure of neurons (pyknotic cells), while association of curcumin restore the normal structure for hippocampal neurons [26].

Curcumin exhibit a therapeutic potential in hypoxia–hypercapnia brain damage in rat models, because of its antioxidant role. Curcumin is able to reduce expression of Fas (type I receptor, member of tumor necrosis factor family) and his ligand FasL (type II receptor) who mediate apoptosis and brain edema by producing reactive oxygen species (ROS) [27]. Transmission electron microscopy (TEM) reveals pathological changes in rat brain expose to hypoxia–hypercapnia environment, such as: swollen cell organelles, shrunken nucleus, chromatin condensation and formation of apoptotic bodies and marginalization. Curcumin association mitigates the changes, suggesting that this polyphenol may be a candidate agent in brain damage prevention [27].

Curcumin has neuroprotective action against arsenic induced cholinergic dysfunction in rat brain, probably explained by reducing alteration in expression of pro-apoptotic and anti-apoptotic proteins in brain [28, 29]. Ultrastructural micrographs reveal mitochondrial changes produced by arsenic [20 mg/kg body weight (b.w.) *p.o.*], such as: damaged and reduced cristae density, decreased number of synapses in corpus striatum [29], loss of myelin sheath in frontal cortex and hypocampus at rat model. All the mentioned alterations were improved in the rat group treated simultaneously with arsenic and curcumin (100 mg/kg b.w. p.o.).

Curcumin and diabetes

Diabetes mellitus (DM) is a metabolic disease with an unclearly defined etiopathology: oxidative stress, inflammatory and autoimmune reactions could contribute together to initiate this disease [30]. Excess of free radicals in DM is the result of mitochondrial superoxide overproduction [31] and can also be produced by inflammatory mechanisms [32]. Inflammation and oxidative stress are therefore "essential partners" in DM as both processes contain mechanisms for mutual amplification. Hence, a candidate antidiabetic drug should be one that possesses polypharmacological abilities, like curcumin. Long treatment with curcumin is capable to improve the histopathological aspects in diabetic liver of rats treated with streptozotocin. Areas of liver steatosis, microvascular vacuolization, focal necrosis, inflammation in portal area, observable in HE staining, are not found in curcumin treated group. Periodic acid–Schiff (PAS) staining showed marked depletion glycogen granules in liver section of diabetic rats, while in curcumin-treated animals, the number is increased [33]. Also, other authors [34] investigate the potential protective effect of curcumin on kidney and pancreas against damage from oxidative stress induced by DM and nicotine (NC). Kidney tissue from DM rats using HE staining presents an increased volume of glomerular tissue, glomerular sclerosis, lipid accumulation in cortical tubes, cellular infiltration and an increased fiber production. Pancreatic sections of DM rats present a decreased volume of nucleus, cytoplasmic vacuolization and damage islets, changes marked by nicotine and visibly improved after curcumin treatment.

Curcumin and cardiovascular diseases

Endothelial dysfunction can be a predictor of cardiovascular diseases; oxidative stress is one of the key factors for the initiation and evolution of cardiac pathology [22]. Cyclosporine, which is considered the "golden standard" therapy in various pathologies, is an immunosuppressant agent indicated in transplanted patients but can determine

in the same time undesirable serious side effects, such as nephrotoxicity [35], arterial hypertension [36], hepatotoxicity and cardiotoxicity [37]. Co-administration of curcumin 200 mg/day for 15 days reveals benefic effect against cyclosporine A (CsA)-induced endothelial dysfunction, which can be demonstrated with both light and electron microscopic examinations [37]. HE staining on aorta sections reveals a high degree of disorganization and separation of tunica media and intima and a decreased volume of endothelial cells in CsA group, while in curcumin group, histological appearance of aorta is improved. Transmission electron micrographs of aortic wall highlights the shape changes of endothelial cells (such as triangular or polygonal shapes), some cells presenting also a large area of intercellular space. A dilated endoplasmic reticulum, swollen mitochondria and vanished mitochondrial cristae were also observed. Curcumin treatment was effective in preventing the mitochondrial degeneration or dilatation of endoplasmic reticulum and intercellular spaces [37].

Myotoxicity, a frequent effect of statins was evaluated histopathologically and ultrastructural using albino rats treated with atorvastatin 50 mg/day/90 days, for different type of muscles [38]. Similar changes were observed on skeletal and smooth muscle (diaphragm), and cardiac muscle, such as: myofibrils degeneration, sarcoplasm fragmentation, excessive collagen fibers around the affected myofibrils and abnormal aggregation of mitochondria in the subsarcolemma and intermyofibrillar spaces. For the cardiac muscle, ultrastructural changes were significant visible with the presence of an important number of abnormal mitochondria, while curcumin association to model rats group evidence nearly to normal appearance of sarcomere, nucleus, with numerous mitochondria in the subsarcolemmal area.

Curcumin supplementation diet at 20 mg/kg/day in a hypercolesterolemic apolipoprotein E (apoE) knock-out mice model reduce the atherosclerotic lesions of aorta sections with 50% in size, compared to control group [39].

Curcumin is effective in preventing the negative changes in blood vessel morphology, which are associated with hypertensive disease. A significant example is the study curcumin effect, alone or associated with piperine, on remodeling the aorta sections of rat hypertension model induced by chronic N-ω-nitro-L-arginine methyl ester (L-NAME) administration [40]. Six weeks of L-NAME-induced hypertension (L-NAME is a non-specific inhibitor of all three NO synthases) caused a significant enlargement of the aorta and its cross sectional area (measured by digital morphometry) and also was associated with a decreased amount of elastin, actin and collagen fibers. Curcumin association diet (100 mg/day for six weeks) to a rat model with hypertension was able to reduce the aortic wall thickness (especially the tunica media), with an average measurement close to the control group values. Also, it seems that curcumin prevents the reduction of elastin fibers and piperin co-administration has no better results than curcumin alone.

Curcumin and morphological changes in malignancies

Curcumin and its derivatives have been extensively analyzed in terms of their cytostatic properties and many studies have used microscopic morphological changes as evidence of cytostatic efficacy. The anticarcinogenic properties have been demonstrated in curcumin since 1985, when Kuttan *et al.* reported the cell growth inhibition, using 0.4 mg/mL tumeric extract, for lymphoma cells and also a reduced development of animal tumors [41]. Since then, many studies have accumulated and covered most types of cancer, underlining both the advantages and shortcomings of these molecules.

A study from 2013 showed that curcumin has a strong anti-tumorigenic action in meningioma cells *in vitro* by arresting cell growth and initiating the apoptosis in dose-dependent manner [42]. The same effect was observed for a wide range of primary human meningioma cell cultures and 0.5% dimethyl sulfoxide (DMSO), which was used as a solubilizing agent, had no effect on the microscopic morphology and viability.

Human astrocytoma cell lines were another tumor model with significant morphological changes after curcumin treatment [43]. TEM revealed that curcumin 100 μM triggered a progressive increase in large vacuoles, with respect of nuclear integrity, suggesting a special type of cellular death.

Huang *et al.* observed that curcumin inhibited lipopolysaccharide (LPS)-induced cell morphological changes characteristic of epithelial–mesenchymal transition (EMT) in MCF-7 and MDA-MB-231 cells (human breast cancer cell lines) [44]. Since EMT is considered a major element in cancer cell invasion and metastasis, the study highlights another possible mechanism of action for curcumin. Both optical microscopy and TEM showed a strong inhibition of LPS-induced cell morphological changes characteristic of EMT in the curcumin group, like the spindle-shaped and fibroblast-like phenotype or the number of extracellular microvilli.

Many papers demonstrated the enhancing effect of curcumin for the cytostatic effect of various drugs. An illustrative example is the synergism between curcumin and oxaliplatin for colorectal carcinoma. The enhancing effect is accompanied by ultrastructural changes together with an increased rate of apoptosis and cell cycle arrest in S and G2/M phases [45]. Using TEM, the authors demonstrated the synergism curcumin–oxaliplatin, showed by a greater level of nucleus changes and mitochondria anomalies of apoptotic tumor cells.

Another synergistic effect was reported by Patial *et al.* for curcumin and piperine in suppression of diethylnitrosamine (DENA)-induced hepatocellular carcinoma in rats [46]. The combined treatment of this cancer model of rats showed a significant decrease of morphological, histopathological, apoptotic and proliferative changes in the liver. Using the classical HE staining, the authors observed a reduction for the intensity of degenerative changes in the group treated with curcumin and piperine. Microscopic changes like mitotic bodies, large fat vacuoles, different cell shape, various nucleus shape, prominent nuclei, karyomegaly, karyorrhexis, dissolution of nucleus, multiple and double nuclei are less obvious in the group treated with combined treatment.

Another complex mixture (containing quercetin, curcumin, green tea, cruciferex and resveratrol) was reported to have antineoplastic proprieties against head and neck squamous cell carcinomas [47]. Using cancer

cell cultures, this study showed an inhibition of cell migration (by scratch test) and a reduction of cell invasion through Matrigel.

The same apoptosis induced effect was observed by Ko *et al.* for a curcumin derivative, where the metoxy groups of natural curcumin were removed [48]. Demethoxycurcumin (DMC) induces the apoptosis of human lung cancer cells using the mitochondrial-dependent pathway. Assessment of the cells morphological changes was performed using the phase-contrast microscope. DMC produced important morphological changes in a concentration-dependent manner. More important, using fluorescent antibodies and confocal microscopy, this study showed that DMC is able to influence the translocation of apoptosis-associated proteins in the human lung cancer cells.

Among the various microscopy methods used for a better understanding of the antitumoral proprieties of curcumin and curcuminoids, one potent variant is immunohistochemistry. Using this technique, Lu *et al.* were able to perform an immunocytochemical assessment of c-Jun and c-Fos protein expression, showing that curcumin 10 μM decrease the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin tumorigenesis [49].

It is noteworthy that one of the most used techniques for assessing the type and degree of cell death is flow cytometry [42, 45, 50, 51]. Using this technique, around 50% of the papers that address the effects of curcumin in cancer were able to estimate the efficacy and the general mechanisms after curcumin-treated cancer cultured cells. The most common approach was Annexin V–fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining assay.

In some instances, fluorescence microscopy was able to differentiate the type of cellular death for cancer cells treated with curcumin [52]. Using lymphoblastoid (Jurkat) T-cells, Piwocka *et al.* observed that the type of cellular death induced by curcumin cannot be classified as necrosis but also differ from "classical" apoptosis. Unlike UV irradiation, microscopy failed to show typical apoptotic bodies in curcumin treated cells, suggesting a novel apoptosis-like pathway.

Cell mechanics is also influenced by curcumin and/or curcuminoids treatments. Using atomic force microscopy and fluorescence microscopy, Saab *et al.* detected different behaviors of the two similar cell lines, non-malignant human mammalian epithelial cells (HMECs) and cancerous breast epithelial cells (MCF-7) [53]. They discovered that curcumin changes HMECs morphology but not for MCF-7 cells: the cells became stiffer and microtubules formed ring-like structures. These morphological changes could explain the drug resistance and the microscopy techniques are powerful tools for the understanding of curcumin biological proprieties.

Conclusions

In this review, we summarize the changes observed with different microscopy techniques induced by curcumin and its derivatives in various pathologies. These morphological changes presented in optical microscopy images are correlated with ultrastructural details offered by TEM and are irreplaceable evidences of biological mechanisms pathways for different pathologies.

Conflict of interests

The authors declare that they have no conflict of interests.

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Received: October 28, 2017 *Accepted: May 5, 2018*