PLANT-DERIVED ANTICANCER AGENTS - CURCUMIN IN CANCER PREVENTION AND TREATMENT

Elena Creţu¹, Adriana Trifan², Al. Vasincu¹, Anca Miron²
University of Medicine and Pharmacy “Grigore T. Popa” - Iasi
1. Ph.D. student
Faculty of Pharmacy
2. Discipline of Pharmacognosy

PLANT-DERIVED ANTICANCER AGENTS. CURCUMIN IN CANCER PREVENTION AND TREATMENT (Abstract): Nowadays cancer is still a major public health issue. Despite all the progresses made in cancer prevention, diagnosis and treatment, mortality by cancer is on the second place after the one caused by cardiovascular diseases. The high mortality and the increasing incidence of certain cancers (lung, prostate, colorectal) justify a growing interest for the identification of new pharmacological agents efficient in cancer prevention and treatment. In the last fifty years many plant-derived agents (vinblastine, vincristine, vindesine, paclitaxel, docetaxel, topotecan, irinotecan, elliptinium) played a major role in cancer treatment. Other very promising plant-derived anticancer agents (combrestatins, betulinic acid, roscovitine, purvalanols, indirubins) are in clinical or preclinical trials. Curcumin, a liposoluble polyphenolic pigment isolated from the rhizomes of Curcuma longa L. (Zingiberaceae), is another potential candidate for new anticancer drug development. Curcumin has been reported to influence many cell-signaling pathways involved in tumor initiation and proliferation. Curcumin inhibits COX-2 activity, cyclin D1 and MMPs overexpression, NF-kB, STAT and TNF-alpha signaling pathways and regulates the expression of p53 tumor suppressing gene. Curcumin is well-tolerated but has a reduced systemic bioavailability. Polycurcumins (PCurc 8) and curcumin encapsulated in biodegradable polymeric nanoparticles (NanoCurc) showed higher bioavailability than curcumin together with a significant tumor growth inhibition in both in vitro and in vivo studies. Keywords: ANTICANCER AGENTS, PHYTOCHEMICALS, CURCUMIN, MOLECULAR TARGETS, BIOAVAILABILITY.

At the beginning of the 21st century, cancer is still a major global public health issue. Recent statistics show that cancer is the second-leading cause of death after cardiovascular diseases. But it is the first most frequent cause of death in 45-65 years old people including a high percentage of working population. The most common cancers in term of incidence are the lung, breast and colorectal cancers. Cancer incidence is supposed to increase dramatically in the next years. In these circumstances, cancer is a research priority in understanding its basic mechanisms, identification of early diagnostic methods and especially in development of efficient treatment methods. In recent years, interest for anticancer drugs with high efficiency and good tolera-
bility has increased and it will probably continue to increase in the near future (1, 2).

**PHYTOCHEMICALS IN CANCER TREATMENT**

In the last fifty years many plant-derived agents played a significant role in cancer chemotherapy. Numerous phytochemicals with antitumor activity have been isolated from plants. Only few advanced into clinical use; most of them served as prototype chemical structures for semisynthesis and synthesis of more potent analogues (3).

The first plant-derived anticancer agents in clinical use were vinblastine and vincristine, bisindole alkaloids isolated from *Catharanthus roseus* G. Don. sin. *Vinca rosea* L. (*Apocynaceae*). Vinblastine, vincristine, semisynthetic derivatives of vinblastine (vinorelbine, vindesine) block mitosis in metaphase leading to apoptosis. They bind to microtubule ends suppressing their dynamics which is essential for chromosomes movements to the two poles of the mitotic spindle. Vinblastine (Velbe®), vincristine (Oncovin®), vinorelbine (Navelbine®) and vindesine (Eldisine®) are used in the treatment of leukemias, lymphomas, advanced testicular, breast and lung cancers, Kaposi’s sarcoma (3, 4, 5).

Podophyllotoxin, an aryltetralin lignan, was first isolated from *Podophyllum peltatum* L. (*Berberidaceae*). Later on, it was isolated from other *Podophyllum* species (*P. emodi* Wall sin. *P. hexandrum* Royle, *P. pleianthum* Hance).

Podophyllotoxin blocks polymerization of tubulin and mitotic spindle assembly arresting mitosis in metaphase. All trials regarding clinical uses of podophyllotoxin failed because its severe side effects (nausea, vomiting, damage of normal tissues). Therefore, extensive studies were carried out in order to obtain more potent and less toxic semisynthetic derivatives. These studies led to etoposide and teniposide, cyclic acetals of epipodophyllotoxin-glucoside.

Unlike podophyllotoxin, these derivatives irreversibly inhibit catalytic activity of DNA topoisomerase II. Etoposide and teniposide form stable complexes with DNA and topoisomerase II. These complexes induce irreversible breaks in single- and double-stranded DNA leading to cell death. Etoposide (Vepesid®, Etopophos®, Eposin®) and teniposide (Vumon®) are used in the treatment of lymphomas, acute leukaemia, small cell lung, testicular, ovarian and bladder cancers (3, 4, 6, 7).

Paclitaxel (taxol) is a polyoxygenated diterpenoid isolated from *Taxus brevifolia* Nutt. (*Taxaceae*, Pacific Yew) and other *Taxus* species (*T. wallichiana* Zucc.). Like other plant-derived antitumor agents, paclitaxel is a spindle poison. But its mechanism of action is completely different and unique. Paclitaxel stabilizes the microtubules suppressing their depolymerization to tubulin. In addition, paclitaxel induces apoptosis by inactivating apoptosis stopping protein Bcl-2. Paclitaxel (Taxol®, Abraxane®) is used in refractory ovarian cancer, metastatic breast and lung cancer, Kaposi’s sarcoma. Docetaxel, a semisynthetic analogue of paclitaxel, is more active than the latter. Docetaxel (Taxotere®) is used for the treatment of metastatic breast cancer and non-small cell lung cancer (3, 4, 6, 8).

Camptothecin is a quinoline alkaloid isolated from *Camptotheca acuminata* Decne (*Nyssaceae*), *Ophiopogon pumila* Champ. ex Benth. (*Rubiaceae*), *Mapia foetida* Miers. (*Acanthaceae*). Camptothecin selectively inhibits DNA topoisomerase I but it was
Plant-derived anticancer agents. curcumin in cancer prevention and treatment

withdrawn from clinical trials due to its severe toxicity (nephrotoxicity, myelosuppression). Topotecan and irinotecan are the only semisynthetic analogues of camptothecin in clinical use. Both compounds are soluble in water and have the same mechanism of action as camptothecin: they bind covalently to topoisomerase I blocking the enzyme activity. Topotecan (Hycamtin®) is used for the treatment of ovarian cancer and small cell lung cancer while the major therapeutic indication for irinotecan (Camptosar®) is the treatment of colorectal cancer (3, 4, 6, 8).

Other plant-derived anticancer agents in clinical use are homoharribontine and elliptinium. Homoharribontine, an indole alkaloid from Cephalotaxus harringtonia var. drupacea (Sieb and Zucc.) (Cephalotaxaceae), induces apoptosis, tumor growth and metastasis by inhibiting tumor neangiogenesis. Homoharribontine (Ceflatocin®) is used in various leukemias, including those resistant to standard treatment. There have been reported cases of complete remission in patients with chronic myelogenous leukemia. Elliptinium is an ellipticine derivative, an indole alkaloid isolated from several genera of the Apocynaceae family, especially from Bleekeria genera (Bleekeria vitensis A. C. Sm.). It acts similar to alkylating agents by forming covalent linkages with DNA. Elliptinium (Celiptium®) is used in the treatment of breast cancer (3).

Numerous plant-derived anticancer agents are in the clinical and preclinical development; some of them proved to be very potent and might become anticancer drugs in the near future.

Combrestatins are compounds with different structures (bibenzyl, stilbene, phenanthrene skeleton) isolated from Combretum cajfrum (Eckl. & Zeyh.) Kuntze (Combretaceae).

The most active combrestatin, combrestatin A-4, is a stilbene derivative; it binds to beta-tubulin blocking microtubule and mitotic spindle assembly. Its phosphate, hydrosoluble, is now in phase II clinical trials in USA and Great Britain. Magnetic resonance imaging experiments showed that combrestatin A-4 phosphate reduces blood flow to tumor cells. In bioassays based on animal models a sodium phosphate derivative of combrestatin A-4 blocked blood supply in metastatic tumors leading to tumor necrosis. Numerous structural analogues of combrestatin A-4 have been synthetized; some of them are in clinical and preclinical trials (3, 4, 6).

A promising case is that of betulinic acid, a lupane-type pentacyclic triterpene, very common in many plants. Betulinic acid has selective cytotoxicity against several human melanoma cancer cell lines (MEL-1, MEL-2, MEL-4, B16). Betulinic acid induces apoptosis through multiple mechanisms: rapid increase in intracellular reactive oxygen species production, disruption of mitochondrial membrane potential, DNA fragmentation. Studies on its possible clinical use in topical and systemic formulations are in progress (3, 4, 9).

Many plant-derived anticancer agents, investigated in preclinical and clinical trials, are potent inhibitors of cyclin-dependent kinases. These enzymes play a key role in cell cycle regulation and their overexpression is associated with tumorigenesis. Important examples are roscovitine and purvalanols (synthetic analogues of olomucine, purinic derivative from cotyledons of radish, Raphanus sativus L., Brassicaceae), indirubins (bis-indoles from Indigofera tinctoria L., Leguminosae and their substituted derivatives) (3, 10).
CURCUMIN AND MOLECULAR TARGETS IN CANCER PREVENTION AND TREATMENT

In the last years, numerous studies reported that curcumin has a huge potential in cancer prevention and treatment. Curcumin, a polyphenol with a diferuloylmethane skeleton, was first isolated from the rhizome of Curcuma longa L. sin. C. domestica Valeton (Zingiberaceae), an Indian species commonly known as turmeric. The therapeutic potential of curcumin was investigated in numerous inflammatory, cardiovascular, neurodegenerative, immunologic and metabolic disorders. Besides, there are many reports on the capacity of curcumin to induce cell cycle arrest and apoptosis and reduce tumor invasion and angiogenesis. The mechanisms are numerous and complex involving modulation of both cell signaling pathways and enzymes activity (11-16).

The overexpression of COX-2 in different types of tumors has already been reported. In the hormone-dependent breast cancer, COX-2 stimulates aromatase activity increasing estrogen secretion. Curcumin inhibits COX-2 activity and consequently reduces estradiol secretion in patients with hormone-dependent breast cancer (12, 13).

In the malignant cells, the aberrant activity of the transcription factors induces not only the tumor progression but also the resistance of the tumor cells to chemotherapy and radiotherapy. Many of the tumor cells protect themselves against apoptosis via nuclear factor-kappa B (NF-kB), one of the main transcription factors that regulate the expression of many genes involved in inflammation, cell proliferation and survival. Curcumin blocks the degradation of the unit alpha of NF-kB inhibitor (IκB); thus NK-κB remains in a latent, inactive form in the cytoplasm being unable to enter the nucleus, bind to DNA and activate transcription (12, 13). In multiple myeloma, lymphomas, leukemias, certain solid tumors there has been reported an increased activity of the signal transducer and activator of transcription (STAT). STAT induces cell growth and proliferation by increasing the expression of Bcl-2 and Bcl-x1 thus inhibiting the apoptosis. It is obvious that STAT modulation might be a possible approach in cancer therapy. Curcumin inhibits the phosphorylation of STAT3 thus blocking its translocation to the nucleus. Curcumin also inhibits STAT1 phosphorylation but not STAT5 phosphorylation (12, 14).

The p53 tumor suppressing gene acts as a transcription factor which activates the genes involved in the regulation of the cell cycle. In normal cells, it blocks the cell cycle in the G1/S phase until the DNA lesions are repaired. If the lesions are extensive and cannot be repaired, p53 initiates apoptosis. The mutations in the p53 gene represent the most frequent genetic modification seen in human cancers. In head and neck squamous cell carcinoma curcumin selectively induces apoptosis in the G2 phase of the cell cycle mainly by regulating the expression of p53 (13, 14).

Cyclin D1 is a nuclear protein involved in the cell cycle regulation. Cyclin D1 and the cyclin-dependent kinases Cdk4 and Cdk6 are responsible for the transition from the G1 phase to the S phase of the cell cycle. Cyclin D1 overexpression leads to the abnormal proliferation of the cells by shortening G1 phase. In tumor cells curcumin regulates the aberrant expression of Cyclin D1 and the activity of Cdk4 (13, 14).

The tumor transformation is often correlated with an abnormal activity of the growth factors, their receptors and signal-
Plant-derived anticancer agents. curcumin in cancer prevention and treatment

The aberrant expression of the epidermal growth factor receptors (EGFR) was reported in numerous solid tumors, including colorectal cancer. By blocking the receptors of the epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), curcumin significantly inhibits the neoangiogenesis which allows tumor growth and metastasis (12).

Curcumin inhibits the tumor invasion and angiogenesis by inhibiting the matrix metalloproteinases (MMPs) overexpression. A significant growth in the synthesis of MMPs is correlated with an aggressive tumor development and a high metastatic potential. MMP-2 and MMP-9 have recorded a significant decrease in the prostate and breast cancer cells treated with curcumin. In the cell line Colo-205 curcumin reduced the activity of MMP-2. There was also reported a reduced degradation of the extracellular matrix that is strongly involved in the tumor dynamics (12, 14, 15).

The tumor necrosis factor (TNF) represents a family of proinflammatory cytokines playing a major role not only in inflammation but also in tumor initiation and proliferation. The proinflammatory effects of TNF are mainly due to its ability to activate NF-kB. TNF-alpha, a vital member of the TNF family, induces the phosphorylation and degradation of IkB; NF-kB is thus released from its cytoplasmic location being able to enter the nucleus and act as a transcription factor. Curcumin suppresses TNF-alpha signaling by inhibiting NF-kB translocation into the nucleus (12, 14).

The mitogen-activated protein kinases (MAPKs) signaling pathway is intensively studied as a possible target in cancer prevention and treatment. In human ovarian cancer cells resistant to cisplatin, curcumin induced apoptosis by modulating the activity of p38 MAPK, kinases involved in NF-kB activation (1).

**CURCUMIN IN HUMAN STUDIES**

The promising results in the phase I clinical studies regarding the effects of oral administration of curcumin in patients with pancreatic, cervical and cutaneous cancer supported the initiation of phase II studies in which curcumin proved high efficacy in patients with colorectal and pancreatic cancers (12, 15, 16).

In a phase II study, curcumin (8 g per day for 8 weeks) significantly reduced NF-kB, COX-2 and STAT3 expression in patients with pancreatic cancer. At the beginning of the study, most of the patients had significant higher levels of these markers than those found in healthy volunteers. No toxic effects were recorded. Despite its limited absorption, curcumin was active in patients with pancreatic cancer (17).

In another study, 62 patients with cancerous lesions on some external organs (vulva, breast, skin) were treated topically with curcumin. There were reported a reduction of itching in almost all patients, a reduction of lesions in 10% of the patients and a reduction of exudates in 70% of the patients. An increase of local itching was reported in a single patient with scalp melanoma (12).

A prospective phase I clinical study on 23 patients with high-grade prostatic intraepithelial neoplasia revealed the safety and tolerance of a new plant-based supplement, Zyflamend®, one of its ingredients being curcumin. Zyflamend® was administered orally (780 mg x 3/day for 18 months) both as monotherapy and in combination with nutritional supplements (probiotics, multivitamins, green and white tea extracts, docosahexaenoic acid). The stand-
ard biomarkers and the prostate-specific antigen (PSA) were monitored every three days. At the end of the treatment, reduced values for the C reactive protein and NF-kB were determined in the serum. 40% of the patients showed a 25-50% reduction of PSA. Zyflamend® was well-tolerated by all the patients (17).

CURCUMIN TOLERANCE AND BIOAVAILABILITY

Clinical studies showed that the administration of high doses of curcumin (2-12 g/day) generated few side effects, mild nausea or diarrhea (15). However, curcumin has a reduced systemic bioavailability at oral administration (11). This reduced bioavailability is reflected in its inability to inhibit the pulmonary and mammary tumors in patients treated *per os* and intravenously. Curcumin is practically insoluble in water whereas in neutral and alkaline solutions its degradation occurs very quickly. Several potential transport systems were tested in order to increase the bioavailability of curcumin such as: nanoparticles, liposomes or conjugates with polyethilenglycol.

PCurc 8 is a polyacetal-based polycurcumin, soluble in water and stable under physiological conditions. *In vitro* studies on several cancer cell lines (SKOV-3, MCF-7, OVCAR-3) showed that PCurc 8 blocks the cell cycle in the G0/G1 transition phase or in the G1 phase mainly through a caspase-3 dependent pathway. *In vivo* studies also showed a remarkable tumor-inhibiting capacity of this polymer (18).

NanoCurc, curcumin encapsulated in biodegradable polymeric nanoparticles, has a high systemic bioavailability when compared to pure curcumin. Administration of NanoCurc in association with Gemcitabine determined a full regression of the metastases in orthotopic pancreatic cancer models. The inhibition of the tumor growth was accompanied by a reduction in the NF-kB activity and MMP-9 and cyclin D1 expression (19).

CONCLUSIONS

The capacity of curcumin to modulate numerous cell signaling pathways and to interact with different molecular targets proves the real therapeutic potential of this compound. Both *in vitro* and *in vivo* studies support the therapeutic value of curcumin. Further studies are needed to identify the analogues with high bioavailability and efficiency in cancer prevention and treatment.

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Plant-derived anticancer agents. curcumin in cancer prevention and treatment