Review

Search for a novel antioxidant, anti-inflammatory/analgesic or anti-proliferative drug: Cucurbitacins hold the ace

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Cucurbitacins are triterpenoid steroids reported to have several biological activities and are predominantly isolated from Cucurbitaceae family. They are efficient anti-oxidant and this property lies in their ability to scavenge free-radicals such as hydroxyl radical, superoxide anions and singlet oxygen. This broad spectrum radical-scavenging capacity surpasses what had been reported for other natural antioxidants such as grape-seed extract, wheat, alfalfa and ginkgo biloba extracts. Reports also show that cucurbitacins adequately inhibit lipid peroxidation and oxidation. Two cucurbitacins, 23, 24 dihydrocucurbitacin and cucurbitacin R isolated from the root of *Cayaponia tayuya* exhibit the anti-inflammatory and analgesic properties typical of cucurbitacins. The mechanism lies in their ability to inhibit the expression of TNF in macrophages and lymphocytes and the expression of such pro-inflammatory mediators such as nitric-oxide synthase-2 and cyclooxygenase-2. Cucurbitacins display strong anti-tumorigenic activity. Abnormal activation of STAT3 is prevalent in breast, pancreatic, ovarian, head and neck, brain, and prostate carcinomas, as well as in melanomas, leukemias, and lymphomas. In those tumors investigated, aberrant STAT3 activation is required for growth and survival. Antiproliferative effect of cucurbitacins is mediated through suppression of phosphotyrosine STAT3 levels which results in the inhibition of STAT3 DNA binding. Studies showed that cucurbitacins induce dramatic changes in the cytoskeleton, inhibit proliferation and induce significant S-phase cell cycle arrest and apoptosis. It is a general knowledge among researchers working with natural medicinal products that any of the cucurbitacins have the attributes or potential to become fully patented as anti-inflammatory or anti-cancer drug.

Key words: Antioxidant, anti-inflammatory, analgesic, anti-proliferative, cucurbitacins.

INTRODUCTION

The worldwide population has used herbs and plants products for decreasing the risk of diseases since immemorial times. In the early stages, the science of medicine developed around those plants which had curative properties (Agrawal and Paridhavi, 2007). A continued search for medicinal plants during the last several centuries has given rise to a long list of plants which are of great use in the treatment of diseases, and for promoting health.

The World Health Organization (WHO) is now actively encouraging developing countries to use herbal medicine which they have traditionally used for centuries. For example, WHO has identified 3,000 plants from the forest of India and other tropical countries which can be used as medicine (Agrawal and Paridhavi, 2007). With such a huge section of an ever-increasing population relying on herbal remedies, it is imperative that the plant products which have been in use for such a long time be scientifically supported for their efficacy, and possibly translating some of the discoveries to the level of drug development.

Cucurbitacins are a group of diverse highly oxygenated triterpenoid molecules containing skeleton characterized

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by a 19-(10-->9beta)-abeo-10alpha-lanost-5-ene. They were originally isolated from Cucurbitaceae plants such as *Ecballium elaterium*, *Cayaponia tayuya*, *Trichosanthes kirilowii*, *Citrillus colocynthis* or *Cucurbita pepo* (Tannin-Spitz, 2007; Haritunians, 2008; Wakimoto et al., 2008) but have been found in other plant families such as Scrophulariaceae, Brassicaceae or Polemoniaceae, among others (Sturm and Stuppner, 1998; Greige-Gerges et al., 2007). Isolation of 10alpha-cucurbita-5, 24-dien-3beta-ol, the simplest tetracyclic triterpene with a cucurbitane skeleton from germinating seeds of *Bryonia dioica* (Cucurbitaceae) authenticated the view that cucurbitacins are biosynthesized by plants (Cattel et al. 1981). Che et al. (1985) classified the naturally occurring cucurbitacins into cucurbitacin A, B, C, D, E, F, I, L, 23, 24-dihydrocucurbitacin F, and hexanorcucurbitacin F, as well as the three acetylated derivatives. They differ from each other by hydroxylation at C-2, -3,-19, -24, the presence of ketone function at C-3, double bond between C-23 and C-24, and by the acetylation of the C-26 hydroxy group. A large number of biological activities have been attributed to cucurbitacins and to their glycosylated derivatives (Greige-Gerges et al., 2007). Apart from acting as kairomones for a large group of diabroticite beetles (Chrysomelidae, Galerucinae, Luperini) whereby they act as feeding stimulants for these insects and can be added to bait containing an insecticide thus reducing the levels of other insecticide treatments needed to control these pests (Behle, 2002; Martin et al., 2002; Martin and Blackburn, 2003). Cucurbitacins are well known for their cytotoxic behaviour and broad range of bioactivities such as antioxidant and free radical-scavenging activities, antiproliferative, anti-inflammatory and analgesic, antimicrobial, antihelminthic, hepatoprotective and cardiovascular properties *in vitro* and *in vivo* (Agil et al., 1999., Peters et al., 1999, 2003; Blaskovich et al., 2003; Jayaprakasam et al., 2003).

This review is intended to bring to the fore in a single medium, some of the outstanding studies and discoveries that have conspicuously placed cucurbitacins as targets for development as potent anti-oxidant, anti-inflammatory or anti-proliferative drug.

THE ANTIOXIDANT AND FREE RADICALS SCAVENGING EFFECTS OF CUCURBITACINS

In living systems, oxygen species such as hydroxyl radicals, superoxide anion radicals, and singlet oxygen and other carbon/nitrogen reactive species, may attack proteins, polyunsaturated fatty acids in cell membrane (giving rise to lipid peroxidation) or DNA, causing the alteration of gene expression and cellular metabolism. Although, the amount of free-radicals can be minimized by cellular defense systems, endogenous antioxidant defenses are inadequate in scavenging them completely (Halliwell and Gutteridge, 1990). On-going oxidative

damage to lipids, proteins, DNA, and the other molecules may contribute to the development of cancer, cardiovascular disease, and, possibly, to neurodegenerative diseases (Zang et al., 2003). The availability of antioxidant compounds as phytochemical constituents has in recent times become a major research focus (Gill et al., 2010). Cucurbitacins have been widely reported for their anti-inflammatory and anti-proliferative properties and quite often the antioxidant activity underlie these biological properties.

Naguchi et al. (1994) categorized anti-oxidants into four main types based on their mechanism of action: preventive anti-oxidants, radical scavenging anti-oxidants, repair and *de novo* anti-oxidants, and adaptive anti-oxidants. Existing reports show that cucurbitacins exhibit preventive and radical scavenging antioxidant properties. Tannin-Spitz et al. (2007) evaluated the antioxidant capacity of combination of glucosides of cucurbitacins B and E (CGC) by its ability to reduce preformed ABTS into its native form through electron donation. CGC was shown to inhibit linoleic oxidation dose dependently, as shown by reduced levels of MDA (malonaldehyde) and 4-HNE (4-hydoxynonenal). MDA and 4-HNE are lipid-oxidation products and are considered to be more stable than free radicals and are therefore considered to be more harmful to cell systems (Esterbauer, 1993). This finding raises the hope of therapeutic potential of cucurbitacins for prevention or treatment of artherosclerosis, a condition suspected to arise from lipoprotein modification by MDA and 4-HNE (Esterbauer, 1993). Tannin-Spitz et al. (2007) further demonstrated that CGC has a high capacity to scavenge free-radicals such as hydroxyl radical, superoxide anion, and singlet oxygen. This broad spectrum radical scavenging activity of CGC surpasses what had been reported for other natural antioxidants such as grape-seed extract (Yamaguchi et al., 1999), wheat, alfalfa and ginkgo biloba extracts (Boveris et al., 1998).

THE ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF CUCURBITACINS

Many researchers have paid attention to the anti-inflammatory and analgesic potentials of members of Cucurbitaceae, a plant family widely known to synthesize cucurbitacins predominantly (Tannin-Spitz, 2007; Haritunians, 2008; Wakimoto et al., 2008). The seeds and fruits of various plants of the family have been evaluated for their anti inflammatory and analgesic activities (Vouldoukis et al., 2004., Semiz and Sen, 2007; Kumar et al., 2008; Gill et al., 2009, 2010; Marzouka et al., 2010). The mechanisms of anti-inflammatory and analgesic activities of cucurbitacins have been widely investigated. Recio et al. (2004) isolated two cucurbitacins; 23, 24-dihydrocucurbitacins B (DHCB) and cucurbitacin R (CCR) from the roots of *C. tayuya*. They

demonstrated that the anti-inflammatory, anti-allergic and anti-arthritic activity of DHCB and CCR *in vitro* and *in vivo* was due to their ability to inhibit the expression of TNFα in lymphocytes and in macrophages, and their interference with the activity of the nuclear factor NF-AT. The *in vivo* study of the expression of proinflammatory enzymes (nitric-oxide synthase-2 and cyclooxygenase-2) with the aid of the Western blot technique, and that of tumor necrosis factor-α (TNF-α) and prostaglandin E_2 by means of enzyme-linked immunosorbent assays demonstrated a clear decrease in both the enzymes and the mediators in paw homogenates. The analysis for prostaglandin E_2, nitric oxide, and TNF-α production in RAW 264.7 macrophages, as well as that for TNF-α in human lymphocytes, indicated a reduction of all mediators. Cucurbitacin R was also found to inhibit signal transducer and activator of transcription 3 activation in the lymphocytes of both healthy and arthritic men (Escandell et al., 2007). *Wilbrandia ebracteata*, of Cucurbitaceae; is a plant from South America used in folk medicine as an antiulcer and analgesic and for the treatment of chronic rheumatic diseases. The ethanolic extract of roots of *W. ebracteata* Cogn, and the semi-purified fraction (F-III) was investigated for its anti-inflammatory and antinociceptive actions in rats and mice. A dose-dependent inhibition of carrageenan-induced paw edema and acetic acid-induced pain in Swiss mice was observed. The formalin test showed inhibition of the neurogenic (first phase) and inflammatory phase (second phase) of formalin-induced pain. The F-III isolated was identified as cucurbitacins B and E, which accounted for the analgesic and anti-inflammatory actions of the HPLC fractions (Peters et al., 1997). Furthermore, Peters et al. (1999) studied the anti-inflammatory action of dichloromethane fraction, a purified fraction and cucurbitacin B extracted from this plant on carrageenan-induced pleurisy in mice. They observed that cucurbitacin B was responsible for the anti-inflammatory activity of *W. ebracteata* and this was by inhibition of the production of PGE_2 and activity of cyclooxygenase-2 (COX-2). A study on WEDC showed its analgesic effects by investigating its actions using the hot plate test and zymosan-induced writhing test in mice, as well as zymosan-induced arthritis in rats to evaluate articular inflammatory pain, cell migration and determination of nitrous oxide release into the joint exudate. A dose-dependent reduction of articular incapacitation and abdominal contortions in the writhing test was observed accompanied by inhibition of COX-2 and nitric oxide release. WEDC selectively inhibited COX-2 but not COX-1 activity in COS-7 cells, and no gastrointestinal toxicity was shown (Peters et al., 2003). Jayaprakasam et al. (2003) likewise showed that cucurbitacins B, D, E and I isolated from the extract of *Cucurbita andreana* fruits inhibited COX-2 enzymes and had no effect on COX-1 enzymes. Latest findings by Siqueira et al. (2007) corroborated the previous reports on the anti-inflammatory effect of cucurbitacin B isolated from *W. ebracteata*. The various studies serve to show that cucurbitacins have a wide spectrum of anti-oxidant, anti-inflammatory and analgesic activities and are relevant not only for the acute forms but also for chronic type inflammatory conditions such as arthritis, ulcers, gouts and hypersensitivity reactions.

THE ANTI-PROLIFERATIVE EFFECTS OF CUCURBITACINS

Cucurbitacins were primarily found to inhibit DNA, RNA and protein synthesis in HeLa cells (a cervical carcinoma cell line with fibroblastic growth properties), and the proliferation of HeLa cells (Witkowski et al., 1984), endothelial cells (Duncan and Duncan, 1997), and T lymphocytes (Smit et al., 2000). Cucurbitacins were shown to suppress skin carcinogenesis (Konoshima et al., 1995) and inhibit cell adhesion (Musza et al., 1994). However, several recent reports now clearly indicate that cucurbitacins have anti-tumorigenic activity due to its inhibition of Janus kinase/Signal Transducer Activator of Transcription 3 (JAK/STAT3) signaling pathway. Several lines of evidence have implicated some STAT family members in malignant transformation and tumor cell survival (Bowman et al., 1999; Turkson et al., 2000). STAT3 involvement in oncogenesis is the most thoroughly characterized, having been found constitutively tyrosine phosphorylated and activated in many human cancers (Bowman et al., 1999; Turkson et al., 2000; Bowman et al., 2000). This abnormal activation of STAT3 is prevalent in breast, pancreatic, ovarian, head and neck, brain, and prostate carcinomas, as well as in melanomas, leukemias, and lymphomas. In those tumors investigated, aberrant STAT3 activation is required for growth and survival (Bowman et al., 1999; Turkson et al., 2000; Bowman et al., 2000). Evidence show that many known oncogenes especially those belonging to the non-RTK family such as src, induce constitutive activation of STAT3 (Yu et al., 1995) and expression of a constitutively activated mutant of STAT3 was shown to be sufficient to induce cell transformation and tumor growth in nude mice (Bromberg et al., 1999). The most compelling evidence for the requirement of STAT3 for oncogenesis and its validation as an anticancer drug target comes from experiments in which a dominant negative form of STAT3 was used in cultured cells and in gene therapy animal experiments to show that blocking aberrant activation of STAT3 results in inhibition of tumor growth and survival and induction of apoptosis with few side effects to normal cells (Niu et al., 1999; Catlett-Falcone, 1999). Base on these previous findings, Blaskovich et al. (2003) reported that cucurbitacin I (Cuc I) suppressed the levels of phosphotyrosine STAT3 in v-Src-transformed NIH 3T3 cells and human cancer cells potently (IC_{50} value of 500 nm in the human lung adenocarcinoma A549) and rapidly

(complete inhibition within 1–2 h). The suppression of phosphotyrosine STAT3 levels resulted in the inhibition of STAT3 DNA binding and STAT3-mediated but not serum response element-mediated gene transcription. Cuc I also decreased the levels of tyrosine-phosphorylated JAK but not those of Src. and was highly selective for JAK/STAT3 but did not inhibit other oncogenic and tumor survival pathways such as those mediated by Akt, extracellular signal-regulated kinase 1/2, or c-Jun NH_2-terminal kinase. The compound was found to potently inhibit growth in nude mice of A549 tumors, v-Src-transformed NIH 3T3 tumors, and the human breast carcinoma MDA-MB-468, all of which expressed high levels of constitutively activated STAT3, but it did not affect the growth of oncogenic Ras-transformed NIH 3T3 tumors that are STAT3 independent or of the human lung adenocarcinoma Calu-1, which has barely detectable levels of phosphotyrosine. It also inhibited tumor growth and significantly increased survival of immunologically competent mice bearing murine melanoma with constitutively activated STAT3. Ability of cucurbitacins to target the JAK/STAT3 signaling pathway conveniently place cucurbitacins as potential target for anticancer drug development.

A structure-activity relationship was reported by Sun et al. (2005) among five cucurbitacin (Cuc) analogs, A, B, E, I, and Q as regards their inhibition of the JAK/STAT3 signaling pathway. Cuc Q inhibits the activation of STAT3 but not JAK2; Cuc A inhibits JAK2 but not STAT3 while Cucs B, E, and I, inhibit the activation of both. The study demonstrated that conversion of the C3 carbonyl of the cucurbitacins to a hydroxyl results in loss of anti-JAK2 activity, whereas addition of a hydroxyl group to C11 of the cucurbitacins results in loss of anti-STAT3 activity. Cuc Q inhibits selectively the activation of STAT3 and induces apoptosis without inhibition of JAK2, Src, Akt, Erk, or JNK activation. Also, Cuc Q induces apoptosis more potently in human and murine tumors that contain constitutively activated STAT3 that is (A549, MDA-MB-435, and v-Src/NIH 3T3) as compared to those that do not (that is, H-Ras/NIH 3T3, MDA-MB-453, and NIH 3T3 cells).

Quite interestingly, cucurbitacins have shown anti-proliferative effect in other cancer cell lines that are independent of JAK/STAT 3 signalling pathways. Escandell et al. (2008) sought to establish the role of activated kRas in the responsiveness of cells to cucurbitacins. Isogenic colon cancer cell lines, HCT116 and Hke-3, which differ only by the presence of an activated kRas allele were used for the study. They compared the activity of 23, 24-dihydrocucurbitacin B (DHCB) and cucurbitacin R (CCR), two recently isolated cucurbitacins (Recio et al., 2004); with cucurbitacin I (CCI), a cucurbitacin with established anti-tumorigenic activity. They showed that cucurbitacins induced dramatic changes in the cytoskeleton (collapse of actin and bundling of tubulin microfilaments), inhibited proliferation

and finally induced apoptosis of both HCT116 and Hke-3 cells. However, the presence of oncogenic kRas significantly decreased the sensitivity of cells to the three cucurbitacins tested, CCR, DHCB and CCI. It was confirmed that mutational activation of kRas protects cells from cucurbitacin-induced apoptosis when non-transformed intestinal epithelial cells with inducible expression of kRasV12 was used. Cucurbitacins induced the expression of p53 and p21 predominantly in HCT116 cells that harbor mutant Ras and using HCT116 cells with targeted deletion of p53 or p21 Escandell et al. (2008) confirmed that p53 and p21 protect cells from apoptosis induced by cucurbitacins. The obvious conclusion was that sensitivity of human colon cancer cell lines to cucurbitacins depends on the kRas and p53/p21 status, and that cucurbitacins potently exert antitumorigenic activity in the absence of activated STAT3.

Measurable effect of cucurbitacins on cancer cells have been vividly described by Liu et al. (2008) as significant efficacy in growth inhibition, cell cycle arrest at G_2/M phase, and apoptosis induction in a dose- and time-dependent manner. Measurement of the modulation of regulators in the cell cycle, apoptosis and signal transductions by Western blot analysis showed that the effect of cucurbitacin B was due to suppression of the expression of p-STAT3, Bcl-2, and cyclin B1. *In vivo* studies performed in a mouse xenograft model further confirmed that cucurbitacin B inhibited tumor growth in a dose-dependent manner and the antitumor effect of cucurbitacin B on Hep-2 cells was due to the induction of cell cycle arrest as well as apoptosis. The possible mechanism underlying the action was attributed to the suppression of STAT3 phosphorylation. Anti-proliferative effects of cucurbitacin B on various leukemia and lymphoma cell lines, as well as on primary mononuclear bone marrow cells derived from patients with acute myeloid leukemia or myelodysplastic syndrome have been demonstrated by Haritunians *et al.* (2008). Myeloid leukemic cells treated with cucurbitacin B exhibited significant S-phase cell cycle arrest, enlarged cell size, multinucleation, and enhanced expression of a monocytic- and granulocytic-specific CD11b. The results obtained demonstrate that cucurbitacin B prominently alters the cytoskeletal network of leukemic cells, inducing rapid and improper polymerization of the F-actin network. These encouraging results suggest the appropriateness of clinical trials of cucurbitacins for the treatment of hematopoietic malignancies. The antiproliferative effects of cucurbitacin B against human breast cancer cells were tested by Wakimoto et al. (2008). Six human breast cancer cell lines which represent a diverse mix of breast cancer subtypes varying in expression of estrogen receptor (ER), Her2/neu, and p53 mutant were tested. *In vivo* morphologic changes associated with disruption of the microtubules and F-actin was observed. Human MDA-MB-231 (ER-, p53 mutated) breast cancer cells was orthotopically implanted into the breasts of nude mice

receiving either cucurbitacin B 1.0 mg/kg or vehicle intraperitoneally. Tumor volume was reduced by 55% in the group treated with cucurbitacin B for 6 weeks compared with vehicle controls. No apparent organ tissue damage was observed by pathological assessment and the experimental mice had relatively lower serum glucose levels. Cucurbitacin B was tipped after taxanes and vincristine in a family of drugs targeting the microtubules. The *in vitro* and *in vivo* results suggest that cucurbitacin B may be an effective, new approach for the treatment of ER-, Her2/neu amplified, and p53 mutant breast cancers (Wakimoto et al., 2008). Bioassay-guided fractions of the crude extract of *Elaeocarpus hainanensis* (Elaeocarpaceae) were tested on the proliferation of non-small cell lung cancer A549/ATCC and human hepatocellular carcinoma BEL-7402 cells. The two cucurbitacins D and I were found to exhibit the strongest cytotoxicity against these cell lines *in vitro* with IC_{50} values of less than 1 microM (Meng et al., 2008). Cucurbitacin E was identified by Duncan et al. (1996) as having potent growth inhibitory activity *in vitro* against prostate carcinoma explants. It was observed that cucurbitacin E also caused marked disruption of the actin cytoskeleton. The anti-proliferative activity correlated directly with the disruption of the F-actin cytoskeleton.

CONCLUSION

The various reports point towards the fact that cucurbitacins are highly potent anti-oxidant, anti-inflammatory and anti-proliferative. These activities are comparable to standard or reference drugs and most importantly were found to be safe without any deleterious effect on the body cells. These are the strong evidence on ground that raises the hope that one of the cucurbitacins will scale the clinical trials.

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