Introduction

Biochanin A (BCA), is a naturally occurring isoflavone present in legumes of many clover species, most notably red clover, and in many herbal dietary supplements. In zigzag clover (Trifolium medium) it occurs in high concentration, and in red clover (Trifolium pratense), crimson clover (Trifolium incarnatum), haresfoot clover (Trifolium arvense), hungarian clover (Trifolium pannonicum) and red-feather clover (Trifolium rubens) its concentration is lower. It is also present in other plants such as soy, alfalfa, peanuts, and chickpea (Breikaa, 2013). This isoflavone is also reported in Cassia fistula and Dalbergia odorifera (Sartorelli, 2009; Zhang, 2011).

Pharmacokinetics of Biochanin A in rats

It is known that isoflavones undergo enterohepatic recirculation following the formation of glucuronide conjugates (Jia et al., 2004). BCA can be regarded as a prodrug of GEN and is rapidly converted into the demethylated metabolite GEN in vitro and in vivo (Tolleson et al., 2002), probably under the catalysis of cytochrome P450 (CYP) enzymes (Hu et al., 2003; Zhang et al., 2004), its biological effects observed in vivo are not identical to those of GEN. For example, BCA can significantly suppress the tumor growth of the human gastrointestinal cancer cells HSC-45M2 and HSC-41E6 transplanted in athymic nude mice, but GEN cannot, Yanagihara et al., (1993) suggesting that BCA or its metabolites, other than those derived from GEN, also exert significant in vivo effects.

The metabolism of BCA is summarized in Figure 2. In addition to demethylation, which converts BCA into GEN, BCA and the metabolite GEN undergo rapid glucuronidation and sulfation (Sfakianos et al., 1997; Peterson et al., 1998; Jia et al., 2004). The resultant conjugative metabolites have been shown to possess some biological activity (Zhang et al., 1999; Wong and Keung, 1997) and may serve as an important source of cellular aglycones upon enzymatic hydrolysis at the target site. The oxidative metabolism of BCA and GEN by cytochrome P450 enzymes has been observed when BCA and GEN are incubated with human or rat liver microsomes. The
metabolites are mainly hydroxylated products such as 3′-, 6-, or 8-hydroxy BCA or GEN (Kulling et al., 2002; Roberts et al., 2004). However, the in vivo significance of these oxidative metabolites is unknown.

**Bioavailability of Biochanin A**

Bioavailability of nutrients is defined as the “the proportion of a nutrient capable of being absorbed and available for use or storage” (Srinivasan, 2001) and depends upon the factors affecting its absorption, distribution, metabolism and elimination kinetics. Generally, after consumption, glycosylated isoflavones are rapidly deglycosylated, absorbed and metabolized in intestinal enterocytes and liver, entering the systemic circulation predominantly as conjugates with limited bioavailability (Patisaul and Jefferson, 2010).

BCA is a 4′-O-methyl derivative of genistein (GEN). BCA was extensively metabolized to GEN in human subjects after ingestion of herbal products containing BCA (Setchel et al., 2001). Although O-demethylation of BCA has been attributed to metabolism by gut microflora (Mizunuma et al., 2002), hepatic microsomal enzymes can perform the same transformation (Tolleson et al., 2002; Chen et al., 2004). Conversion of BCA to GEN in rat liver microsomes was found to be rapid and saturable (V_{max} of 490 pmol/min/mg, K_{m} of 64.5 µM). Conversion of BCA into GEN also occurs in rat intestinal microsomes (Jia et al., 2004).

The plasma concentration-time profiles of oral BCA exhibited a rapid absorption phase followed by the biexponential disappearance of BCA. BCA was rapidly converted into GEN, and GEN, as well as conjugates of BCA and GEN, was detected in rat plasma. It is noteworthy that the concentration of BCA was higher than that of metabolite GEN for a long time after its IV and oral administration, especially after the 50 mg/kg oral dose of BCA, when BCA plasma concentrations were higher than those of GEN for more than 20 hours (Moon et al., 2006).

**Pharmacological benefits of Biochanin A**

Biochanin A is a natural isoflavone with diverse biological actions, most notably as a phytoestrogen. Biochanin A has been associated with a variety of human health benefits. Most beneficial health effects linked to isoflavones such as biochanin A are believed to be mediated by the estrogenic and antioxidative properties of these compounds. Firstly, due to the similar structure to estrogens, BCA can combine with estrogen receptor α (ERα) and β (ERβ) which be called estrogenic activity (Dornstauder et al., 2001). Secondly, BCA has various other biological activities, such as anti-proliferative, anti-inflammatory (Kole et al., 2011), protection of dopaminergic neurons (Chen et al., 2007), stimulation of osteoblastic differentiation, (Lee and Choi, 2005) and inhibition of melanogenesis (Lin et al., 2011). Especially to deserve to be mentioned, data from the animals and the in vitro studies provided that Biochanin A, which are possible through the path of inhabiting the enzyme activity and inducting apoptosis, can reverse, inhabit, or prevent cancers or tumor development, such as prostate cancer (Szliszka et al., 2013), breast cancer (Moon et al., 2008), lung tumor (Lee et al., 1991) and liver cancer (Mansoor et al., 2011).

**Anti-microbial activity**

Biochanin A is a potent inhibitor of the intracellular gram-negative bacteria *Chlamydia pneumoniae* and *C. trachomatis*. It was also established that Biochanin A has good buccal mucosal penetration without any stability or metabolism issues during the buccal permeation. Due to the inherent bioavailability issues associated with Biochanin A for oral formulations, a buccal product strategy has been
proposed to evaluate the anti-chlamydial potential of Biochanin A (Hanski et al., 2014).

**Chemo preventive properties**

Biochanin A, an isoflavone, is known to exert an anticancer effect on various cancer types: (a) Breast cancer: Biochanin A is selectively targets HER-2+ SK-BR-3 breast cancer cells and inhibits multiple deregulated mechanisms associated with malignant transformation. Biochanin A drastically reduced cell invasion, inhibited multiple signaling pathways and lowered the cell viability in a dose dependent manner (Sehdev et al., 2009). (b) Brain tumor: Malignant gliomas like glioblastoma multiform are the most lethal form of adult brain tumor. Jain et al (2015) have demonstrated that Biochanin A inhibits invasion in human glioblastoma cells. Biochanin A inhibited endothelial cell functions observed in gliomas such as migration, invasion and cell viability. The activation of several proangiogenic proteins such as ERK, AKT, and mTOR was significantly inhibited. Overall, Biochanin A appears to provide dual targeted agent that inhibits two processes, invasion and angiogenesis. (c) Pancreatic cancer: The highly aggressive nature of pancreatic cancer cells is attributed to the mutations of tumor-promoting and tumor suppressor genes. The levels of EGFR and EGF mRNA are elevated in pancreatic cancer cells compared with the normal pancreas, creating an autocrine stimulation of cellular proliferation, associated with shorter postoperative survival in pancreatic cancer patients (Yamanaka et al., 1993). Szliszka et al (2013) reported that Biochanin A effectively reduced pancreatic cancer cell survival (MTT and annexin V staining), proliferation (colony formation and mitogenic signaling), and progression (inhibition of migration and invasion). Mechanistically, it was confirmed that Biochanin A inhibited the activation of AKT and MAPK pathways in pancreatic cancer. (d) Colon cancer: It was demonstrated that Biochanin A potentiated the effectiveness of gamma radiation in inhibiting the growth of radio resistant HT29 colon cancer cell line. In combination with radiation, Biochanin A enhanced lipid peroxidation, promoted an increased formation of reactive-oxygen species and enhanced mitochondrial membrane potential. Also, Biochanin A led to increased caspase-3 activity in the cells and enhanced apoptosis causing DNA damage in the radio resistant HT29 colon cancer cells (Puthli et al., 2013).

Sulfotransferases are a family of phase II drug metabolizing enzymes, which are important for xenobiotic detoxification and regulation of biological signaling molecule biological activities. Improper regulation of sulfotransferases leads to improper functions of biological signaling molecules, which in turn can cause cancer or other diseases. Biochanin A can significantly induce sulfotransferases enzyme activities and gene expressions in rat liver and intestines. These results may also provide information on the anticancer activities of Biochanin A (Chen et al., 2010).

**Antidiabetic activity**

Biochanin A was shown to have significant antidiabetic activity against streptozocin-induced diabetic rats has suggested that Biochanin A can modulate glucose metabolism effectively. In diabetic rats Biochanin A was lowered HBA1c level on continuous dosing. Moreover, it also normalized the liver enzymes and the body weight upon long-term administration. The mechanism of BioA's observed activity was related to the improvement in visfatin expression (Azizi et al., 2014). Further credence to the anti-diabetic potential was provided by the agonistic properties on both PPAR-α and PPAR-β exhibited by natural products including BioA (Wang et al., 2014).

**Osteoarthritis**

A recent study indicated that biochanin A exerted antiproliferative and anti-inflammatory effects through the inhibition of iNOS expression, p38-MAPK and ATF-2 phosphorylation, and blocking of NF-κB nuclear translocation (Kole et al., 2011). A previous study indicated that biochanin A inhibited tumor invasion in human glioblastoma (U87MG) cells by suppressing the enzymatic activities of MMP-2 and MMP-9 (Puli et al., 2006). As MMPs are regarded as major factors in the pathophysiology of osteoarthritis, this close link between biochanin A and MMPs prompted us to explore protective effect biochanin A in osteoarthritis by regulating MMPs (Wu et al., 2014).

**Antiparasitic activity**

Biochanin A was isolated from the methanol extract of the fruits of Cassia fistula (Leguminosae) by Sartorelli et al (2009) and was tested on antiparasitic activity in protozoans. It was found that Biochanin A shows antiparasitic activity against T. cruzi trypomastigotes, L. chagasi promastigotes and L. chagasi amastigotes. Hence, Biochanin A can be used as toll for drug design studies in the development of new therapeutics especially against Chagas’ disease.

**Summary and conclusion**

Isoflavones are the most important type of phytoestrogen found in legume plant, and are week estrogenist receptor ligands with mixed agonist-antagonist activity. Biochanin A one of the predominant isoflavones, existing in red clover, cabbage, alfalfa and Trifolium lucanicum Gasp, has been associated with a variety of human health benefits. BCA has various other biological activities, such as anti-proliferative, anti-inflammatory, protection of dopaminergic neurons, stimulation of osteoblastic...
differentiation, and inhibition of melanogenesis. In conclusion, based on the retiture reviewed BCA seems to be a potential candidate for the human health benefits.

Acknowledgment

The financial support to Arjunan Sundaresan as Post Doctoral Fellow (F./PDFSS-2015-17-TAM-12124 dated 01.04.2017) from University Grants Commission (UGC), New Delhi, is gratefully acknowledged.

Conflict of interest

There is no conflict of interest in the present study.

References


