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Review

Molecular targets and anticancer potential of sanguinarine—a benzophenanthridine alkaloid

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ABSTRACT

Background: Cancer is an enormous global health burden, and should be effectively addressed with better therapeutic strategies. Currently, over 60% of the clinically approved anticancer agents are either directly isolated from natural sources or are modified from natural lead molecules. Sanguinarine (SNG), a quaternary benzophenanthridine alkaloid has gained increasing attention in recent years as a potential anticancer agent.
Purpose: There is a large untapped source of phytochemical-based anticancer agents remaining to be explored. This review article aims to recapitulate different anticancer properties of SNG, and describes some of the molecular targets involved in exerting its effect. It also depicts the pharmacokinetic and toxicological properties of SNG, two parameters important in determining the druggability of a molecule.
Methods: Numerous *in vivo* and *in vitro* published studies have signified the anticancer properties of SNG. In order to collate and decipher these properties, an extensive literature search was conducted in PubMed, ScienceDirect, and Scopus using keywords followed by the evaluation of the relevant articles where the relevant reports are integrated and analyzed.
Results: Apart from inducing cell death, SNG inhibits pro-tumorigenic processes such as invasion, angiogenesis, and metastasis in different cancers. Moreover, SNG has been shown to synergistically enhance the sensitivity of several chemotherapeutic agents and is effective against a variety of multi-drug resistant cancers.

Introduction

According to the National Cancer Institute (NCI) surveillance epidemiology and end result (SEER) program report, it was estimated that during 2015 approximately 1.6 million new cancer cases will be diagnosed, and more than 580 thousand people will die from cancer in the United States alone (Howlader et al., 2016). Indeed, the cancer burden and related deaths is predicted to increase alarmingly by 2020, with approximately 15 million new cases and 12 million deaths globally. This is further expected to witness a catastrophic increase of 21 million new cancer cases and 13 million deaths by 2030 (American Cancer Society, 2015; Kanavos, 2006). Clearly, cancer is presenting as an enormous global health burden, and should be effectively addressed with better therapeutic strategies. Currently, the most commonly used anticancer treatment involves chemotherapy, radiotherapy, and surgical resection.

Phytochemicals are non-nutritive components found in plants. They have long been used in the treatment of various ailments such as

Abbreviations: ABC, ATP-binding cassette; AIF, apoptosis-inducing factor; AP-1, activator protein 1; ATF4, activating transcription factor-4; Atg, autophagy-related gene; ATO, arsenic trioxide; Bad, Bcl-2-associated death promoter; Bax, Bcl-2-associated x protein; BCA, 3,4-benzacridine; Bcl-2, b-cell lymphoma-2; Bcl-xL, B-cell lymphoma-extra-large; Bid, BH3 interacting-domain death agonist; Caspase, cysteine-dependent aspartate-directed protease, CDK, cyclin-dependent kinase; C-FLIP, cellular flice-inhibitory protein; CHOP, CCAAT-enhancer-binding protein homologous protein; CKI, cyclin-dependent kinase inhibitors; COX-2, cyclooxygenase-2; Cyt-c, cytochrome-c; DHSNG, dihydrosanguinarine; DR5, death receptor-5 ; DUSP, dual specificity phosphatase; EGFR, epidermal growth factor receptor; Egr-1, early growth response gene-1; eIF2α, eukaryotic initiation factor-2α; ERK1/2, extracellular signal-regulated kinase 1/2; FasL, Fas ligand; GRP78, glucose regulated protein 78; GSH, glutathione; H_2O_2, hydrogen peroxide; HIF1α, hypoxia-inducible factor 1-alpha; IAP, inhibitor of apoptosis protein; IL, interleukin; Jak2, Janus kinase 2; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; Mcl-1, myeloid cell leukemia 1; MDR, multi-drug resistant; MMP, matrix metalloproteinase; NCI, National Cancer Institute; NF-κB, nuclear factor-κB; NO, nitric oxide; $O_2^{\bullet -}$, superoxide anion; PCNA, proliferating cell nuclear antigen; PERK, protein kinase RNA-like endoplasmic reticulum kinase; P-gp, P-glycoprotein, PKB, protein kinase B; PLC-gamma1, Phospholipase C-gamma1; ROS, reactive oxygen species; SEER, surveillance, epidemiology and end result; SGOP, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; SMAC/DIABLO, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI; SNG, sanguinarine; STAT3, signal transducer and activator of transcription 3; tBID, truncated BID; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor-α; TRAIL, TNF-related apoptosis-inducing ligand; UVB, ultraviolet B; VEGF, vascular endothelial growth factor; XIAP, X-linked inhibitor of apoptosis protein

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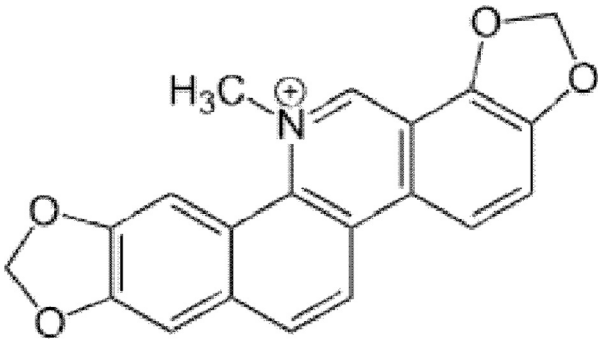

Fig. 1. Chemical structure of sanguinarine.

diabetes, cardiovascular disease, hypertension, and cancer. Currently, over 60% of the clinically approved anticancer agents are either directly isolated from natural sources or are modified from natural lead molecules (da Rocha et al., 2001). However, of the 250,000 plant species available, less than 10% have been tested for possible biological activities, and only few of these have gone through extensive high-throughput screening (Harvey, 2000). This underscores the fact that there is a large untapped source of phytochemical-based anticancer agents remaining to be explored.

Sanguinarine (SNG) (13-methyl-[1,3]benzodioxolo[5,6-c]- 1,3-dioxolo[4,5-i]phenanthridinium) ($C_{20}H_{14}NO_4$) (Fig. 1) is a quaternary benzophenanthridine alkaloid encountered in many plant species of *Papaveraceae* family. It is mainly extracted from the roots of bloodroot plant (*Sanguinaria canadensis*) (Deroussent et al., 2010), seeds of Mexican prickly poppy (*Argemone Mexicana*) (Deroussent et al., 2010), the roots and aerial parts of greater celandine (*Chelidonium majus*) (Colombo and Bosisio, 1996; Meng et al., 2009), and the fruits and leaves of plume poppy (*Macleaya cordata*) (Lee et al., 2013; Yao et al., 2010). It is also found in eastern horned poppies (*Dicranostigma lactucoides*) (Gregorová et al., 2010), opium poppy (*Papaver somniferum*) (Frick et al., 2005), and Kelway's coral plume (*Macleaya microcarpa*) (Wang et al., 2010). SNG and extracts of SNG containing plants have long been used in tooth pastes and other oral hygiene products due to its anti-plaque and anti-inflammatory properties (Hannah et al., 1989; Kuftinec et al., 1990). In addition, Sanguiritrin (a mixture of SNG and a structurally similar alkaloid, chelerythrine) has been used as a veterinary preparation for the treatment of mastoiditis in cows (Psotova et al., 2006a). It is also used in animal husbandry as an additive to animal feeds (Sangrovit) (Zdarilova et al., 2008). A wide range of pharmacological activities has been documented for SNG including antihypertensive (Mackraj et al., 2008; Singh et al., 2006), antimicrobial (Hamoud et al., 2014; Obiang-Obounou et al., 2011), and anti-inflammatory (Lenfeld et al., 1981; Niu et al., 2012) activities. The great interest in SNG over the past few decades is due to its anticancer properties (Choi et al., 2008; Dong et al., 2013; Gu et al., 2015; Hammerová et al., 2011; Jang et al., 2009; Kalogris et al., 2014; Kim et al., 2008; Rosen et al., 2015).

This review focuses on different anticancer properties of SNG and also describes some of the molecular targets involved in this. It also depicts the pharmacokinetic and toxicological properties of SNG, which are important in determining its druggability.

Pharmacokinetics of sanguinarine

Despite enhanced interest in the anti-cancer properties of SNG, information on the pharmacokinetic parameters of SNG is limited. The gastrointestinal absorption of SNG is poor, which may be due to its quaternary nitrogen atom (Becci et al., 1987; Hong et al., 2006). In an *in vivo* experiment conducted in rats, it was reported that only 2% of the sanguiritrin is absorbed from the gastric tract following its daily oral administration (10 mg/kg animal body weight) for 109 days. The remaining alkaloid was unabsorbed and excreted in the feces (Psotova et al., 2006a). This is further reflected in a short-term toxicity study in rats, where SNG was demonstrated to be considerably less toxic following acute oral administration (LD50 value 1658 mg/kg) compared to acute intravenous administration (LD50 value 29 mg/kg) (Becci et al., 1987). Following absorption, SNG was found to be distributed in plasma, liver, and kidney of pigs and rats (Kosina et al., 2004; Psotova et al., 2006a). Distribution of SNG was also observed in the milk of rabbits following its parenteral administration (Hakim et al., 1961; Mackraj et al., 2008). The initial reports on the metabolic transformation of SNG described 3,4-benzacridine (BCA) as its only metabolite (Hakim et al., 1961; Tandon et al., 1993). However, some of the later experiments contradicted these findings. Using more advanced and highly sensitive analytical tools, Psotova et al. and Vecera et al. demonstrated that BCA is not present in the urine, plasma, and liver of rats following single dose administration of SNG. Nevertheless, they identified dihydrosanguinarine (DHSNG), a less toxic benzophenanthridine, as the principal metabolite of SNG (Psotova et al., 2006b; Vecera et al., 2007). The conversion of SNG into DHSNG was also observed in experiments with rat hepatocyte cell cultures (Psotova et al., 2006a). This is consistent with similar findings previously reported in plants, where SNG was shown to be metabolized into DHSNG by the enzyme SNG reductase (Weiss et al., 2006). Nonetheless, the metabolic enzyme responsible for SNG biodegradation in mammals is as yet unknown. Recently, Wu et al. demonstrated that carbonyl reductase and/or quinone oxidoreductases may be involved in the reduction of SNG (Wu et al., 2013). In addition, an *in silico* analysis conducted by Vogel et al. demonstrated that plant SNG reductase exhibits close structural homologies with domains of human biliverdin IX β reductase and type 8 17 β-hydroxysteroid dehydrogenase (Vogel et al., 2010). However, further research incorporating more advanced recombinant molecular biology techniques is required to identify the mammalian enzyme involved in the SNG reduction.

As mentioned earlier, studies on the pharmacokinetic parameters of SNG and DHSNG are limited. Vecera et al. determined the T_{max}, and C_{max} of SNG and DHSNG. Oral administration of single dose of SNG (10 mg/kg) resulted in a T_{max} of 2 h (for both SNG and DHSNG) and C_{max} of 192.3 ng/ml for SNG and 545.9 ng/ml for DHSNG (Mackraj et al., 2008; Vecera et al., 2007). The study also concluded that complete elimination of both compounds from the plasma and liver occurred within 24 h, and they were not detected in the urine (Mackraj et al., 2008; Vecera et al., 2007). Furthermore, a pilot study conducted in rats by repeated dosing of DHSNG (14 or 58 mg/kg/day for 3 months) did not demonstrate any detectable amount of DHSNG in urine (Vrublova et al., 2008). The conversion of DHSNG into undetermined polar conjugates might be the reason for its nonappearance in the urine (Dvorák and Simánek, 2007; Mackraj et al., 2008; Psotova et al., 2006b). However, further studies are required for a detailed understanding of SNG pharmacokinetics.

Toxicity profile of sanguinarine

Epidemic dropsy is an acute toxic disease caused by ingestion of edible oil adulterated with *Argemone mexicana*. Both SNG and DHSNG have been ascribed as the etiological agent responsible for epidemic dropsy (Babu et al., 2006; Babu et al., 2007; Das et al., 2005b). Considering its poor gastrointestinal absorption, it is highly unlikely that SNG is solely responsible for the toxic effect of argemone oil, rather, some other oil component may well be the key player involved in this toxicity (Psotova et al., 2006a). In line with this, some of the previous studies examining the safety of SNG and DHSNG manifested no signs of argemone oil toxicity, despite being present at concentrations as high as those reported in argemone oil intoxicated patients (Banerjee et al., 2000; Kosina et al., 2004; Psotova et al., 2006a; Vrublova et al., 2008). Moreover, Kosina et al. conducted an *in vivo* safety assessment of sanguiritrin (5 mg/kg body weight) in pigs following its oral