Metabolism of Sanguinarine: The Facts and The Myths

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Abstract: Sanguinarine, a quaternary benzo[c]phenanthridine alkaloid, exhibits antimicrobial and anti-inflammatory activities and for this reason it is used in dental hygiene products and feed additives. Its metabolism and disposition is the subject of constant scientific discourse. In this paper we summarize current knowledge on sanguinarine metabolism. We show in particular that: (i) Sanguinarine is not transformed to 3,4-benzacridine and that the literature reporting this compound as a metabolite of sanguinarine is based on artifacts and misinterpretations that in course of time have created a dogma; (ii) Sanguinarine is converted to dihydrosanguinarine *in vivo*, the conversion being tentatively a detoxication pathway; (iii) Aryl hydrocarbon receptor metabolic signaling pathways modulate sanguinarine biological activity.

Key Words: Sanguinarine, Biotransformation, Metabolites, Cytochrome P450, Aryl hydrocarbon receptor.

INTRODUCTION

Sanguinarine, a quaternary benzo[c]phenanthridine alkaloid, is present in the *Papaveraceae*, *Fumariaceae*, and *Rutaceae* families of plants. The main sources of sanguinarine are the plant species *Chelidonium majus*, *Macleaya cordata*, and *Sanguinaria canadensis*. Sanguinarine exhibits multiple biological effects, in particular antimicrobial and anti-inflammatory activities for which it is used in dental hygiene products and feed additives [1]. Case report studies have shown a possible association between sanguinarine containing teeth cream use and oral pre-malignant lesions [2, 3]. The biological activity of sanguinarine is linked to an equilibrium between its quaternary cation and alkanolamine forms [4] Fig. (**1**). The conversion of the iminium ion to alkanolamine improves lipophilicity of sanguinarine and this may result in an increase in the alkaloid´s bioavailability [5]. Due to the iminium bond, polycyclic and planar structure sanguinarine can react with nucleophilic and anionic moieties of amino acids in biomacromolecules [6]. Further, the formation of molecular complex of sanguinarine with DNA by intercalation has been described [7]. Recently, Bartak *et al*. described non-covalent interaction of alkanolamine form with SH-proteins [8, 9].

SANGUINARINE METABOLISM *IN VIVO*

The first report on the metabolic transformation of sanguinarine in animals was published in 1957. It was found that sanguinarine is converted to a "*green-fluorescent compound*" in isolated rat liver [10]. Another research team injected lactating rabbits subcutaneously with sanguinarine. They observed transmission of sanguinarine to the milk of lactating rabbits (also cats, rats and monkeys), where it was bound to albumin. They performed thin layer chromatography (TLC) separation and they also reported the presence of a green-fluorescing metabolic product in the urine of injected rabbits. Based on comparative UV spectroscopy they concluded that this putative metabolite might be 3,4-benzacridine [11]. However, direct evidence for the presence of 3,4-benzacridine was not provided, as these authors did not analyze a standard solution of 3,4-benzacridine by TLC. Another drawback of this study was also the isolation procedure; drastic hydrolysis using hydrochloric acid could have been cause for artifact. In addition, both articles only comment on the presence of a green-fluorescent compound without presenting chromatography data [10, 11].

The biometabolic elimination and organ retention of sanguinarine were again studied in 1992 [12]. Rats and guinea pigs were administered a single oral dose of a mixture of sanguinarine and dihydrosanguinarine. The composition of this mixture was not defined as it was obtained by precipitation of the alkaloids from argemone oil by hydrochloric acid. TLC analyses of collected urine and feces samples were performed. The authors describe that after 72 h of treatment, sanguinarine (reported as an "*orange-fluorescence spot*") and dihydrosanguinarine (reported as a "*blue-fluorescence spot*") disappeared and a new "*green-fluorescence spot*" appeared in the chromatogram. The retardation factor of this green spot was between those of sanguinarine and dihydrosanguinarine and the authors interpreted it as 3,4-benzacridine [12]. They also showed that sanguinarine and dihydrosanguinarine were excreted in the bile of rats and guinea pigs. However, no green-fluorescence metabolite was detected in the bile [12, 13]. This study also suffers from several weaknesses: (i) The chromatograms were not shown in the paper; only schematic drawings with commentaries were supplied. (ii) The standard solution of 3,4-benzacridine was not analyzed by TLC. (iii) The interpretation of the green-fluorescence spot as 3,4-benzacridine was not supported by any direct analytical/structural evidence.

Recently, it has been demonstrated that the formation of dihydrosanguinarine might be the first step in sanguinarine detoxification in rats and its subsequent elimination in phase II reactions [14]. In the same study, 3,4-benzacridine was

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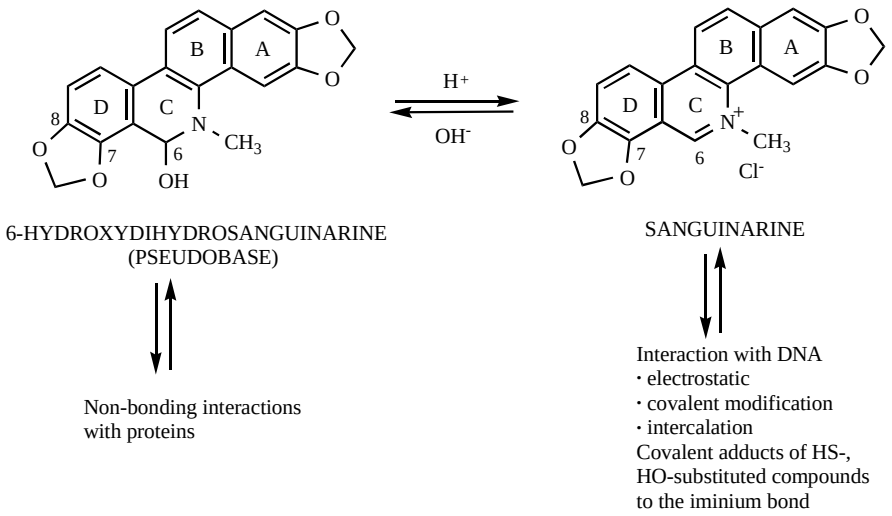

Fig. (1). Equilibrium between alkanolamine form and iminium ion form of sanguinarine.

found neither in urine nor in the plasma or liver of rats - Fig. (**2**). The follow-up pharmacokinetic study further confirmed this hypothesis [15]. This is in conformity with finding that sanguinarine is converted to dihydrosanguinarine in plants. This reaction is catalyzed by sanguinarine reductase and represents a sanguinarine detoxication pathway in plants [16].

In vivo safety assessment of sanguiritrin (a mixture of sanguinarine and chelerythrine, 6:2) in animals was performed. In a 90 day feeding experiment in swine, a daily oral dose of alkaloids up to 5 mg per 1 kg animal body weight was found to be safe [17]. The follow-up study investigated the subchronic safety of sanguiritrin in rats. Animals were fed a diet containing 120 ppm sanguiritrin for 109 days (daily oral dose of alkaloids up to 10 mg per 1 kg animal body weight) . It was reported that 2% of QBA was absorbed through the GIT while 98% was excreted in the faeces. No adverse effects were observed in rats [18].

A possible interaction between sanguinarine and the cytochrome P450 system is interesting. Single intraperitoneal administration of argemone oil to rats caused destruction of hepatic cytochrome P450 and consequent inhibition of monooxygenases activity [19]. Sanguinarine cytotoxicity may be associated with the aryl hydrocarbon receptor (AhR) signaling pathways, tentatively with CYP1A enzymes. Pretreatment of mice with 3-methylcholantrene (3-MC), an inducer of CYP1A enzymes, mitigated the sanguinarine toxic effects [20].

SANGUINARINE METABOLISM *IN VITRO*

There is an increasing body of evidence from the literature that the biological activity of sanguinarine may be modulated by the AhR-CYP1A metabolic pathway and *vice-versa*; sanguinarine may affect AhR-CYP1A system.

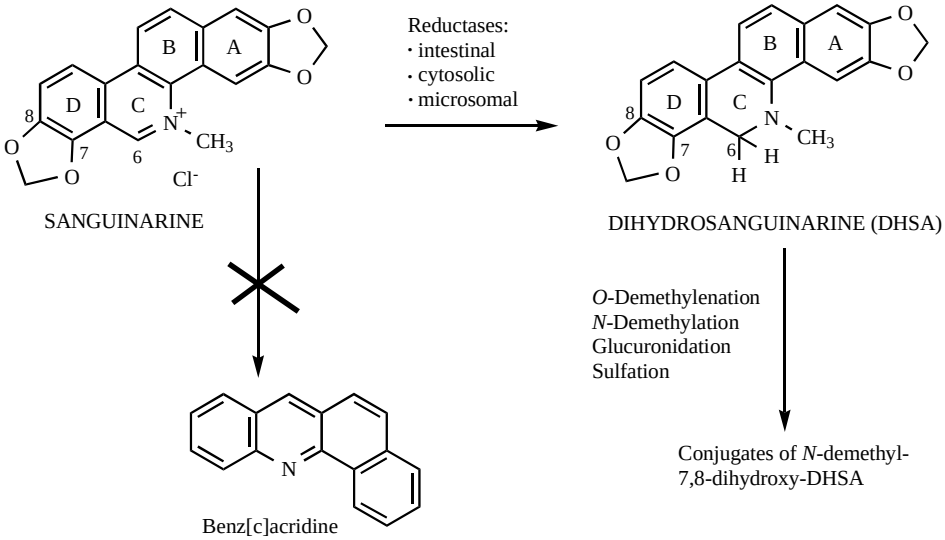

Fig. (2). Conversion of sanguinarine to dihydrosanguinarine. Sanguinarine is reduced to dihydrosanguinarine by not yet identified reductase. Resulting dihydrosanguinarine is not cytotoxic and is subjected to further biotransformation.

It has been found that sanguinarine inhibits the catalytic activity of CYP1A enzymes. Interestingly, enzyme inhibition proceeded by non-competitive and competitive mechanisms in the case of CYP1A1 and CYP1A2, respectively [21]. Inhibitory effects of sanguinarine against CYP1A enzymes were confirmed in two additional studies [22, 23]. A little controversial are the effects of sanguinarine on dioxin-inducible AhR-dependent expression of CYP1A genes. The suppression of AhR transcriptional activity by chelerythrine, a substance structurally close to sanguinarine, has been reported. This suppression occurred only in supramicromolar concentrations (5 μM) and it involved inhibition of protein kinase C catalytic activity, the essential enzyme for AhR transcriptional activation [24]. In contrast, sanguinarine in submicromolar concentrations had no effects on AhR-dependent CYP1A1 expression in human hepatoma HepG2 cells [23] and on AhR transcriptional activity in rat hepatoma H4IIE.luc cells [25]. Albeit it has been suggested that sanguinarine activates polycyclic aromatic hydrocarbon associated metabolic pathways in human oral keratinocytes and tissues [22], the data have been criticized and disputed [26].

A second series of papers deals with the effects of the AhR/CYP1A metabolic pathway on the biological activity of sanguinarine. Initially, it was reported that the AhR activator 3-MC mitigates sanguinarine toxic effects in mice (vide supra) [20]. Experiments *in vitro* have shown that pretreatment of HepG2 cells and/or primary rat hepatocytes with various AhR activators (3-MC, BNF, TCDD) diminish sanguinarine cytotoxicity [21]. The same study refuted the formation of 3,4-benzoacridine from sanguinarine in HepG2 cells and rat hepatocytes. In an ongoing study we have confirmed this phenomenon; sanguinarine cytotoxicity against primary rat hepatocytes is attenuated in cultures pretreated 48 h with TCDD and/or phenobarbital (PB) [27]. The primary explanation for these effects is the involvement of TCDD- and PB-inducible cytochromes P450 in sanguinarine metabolism/cytotoxicity. We have assumed the role of CYP1A1/2, CYP2Bs and CYP2C/3A as TCDD- and PB-inducible enzymes, respectively. The attention was then focused on the CYP1A subfamily, and we tested the hypothesis that these enzymes convert sanguinarine to less toxic metabolites. Surprisingly, co-treatment with sanguinarine and furafylline, an inhibitor of CYP1A enzymes, further attenuated sanguinarine cytotoxicity instead of the expected reversal of this effect. Hence, CYP1A enzymes do not attenuate but rather augment the cytotoxicity of sanguinarine. One plausible explanation is induction of cytosolic reductases by TCDD and/or PB [28, 29]. These enzymes may convert toxic sanguinarine to non-toxic dihydrosanguinarine, as observed in rats [14, 30]. Another mechanism could be induction of phase II enzymes by TCDD and/or PB [31]. Sanguinarine contains a poly-aromatic skeleton in its structure and the hydroxylation of this compound by CYP1A enzymes is likely. Such a product would be reactive, and this is consistent with the finding that furafylline decreases sanguinarine toxicity even in non-induced hepatocytes [27]. Induction of conjugation enzymes by TCDD and/or PB would accelerate elimination of reactive metabolites. In addition, it has been shown that incubation of sanguinarine with rat hepatic microsomes leads to the production of reactive species capable of forming DNA adducts [32]. Reactive species were formed in the presence of microsomes isolated from BNF-treated but not in vehicle-treated animals [32]. Unfortunately, the direct proof that CYP1A enzymes are responsible for the formation of DNA adducts is missing, since the approach of CYP1A inhibition by furafylline was not employed in this study.

CONCLUDING REMARKS

Sanguinarine-containing products are extensively used in practice. Sanguinarine exerts a plethora of biological activities. This aside, consistent study on sanguinarine metabolism and disposition has not been performed until this year. The main conclusions based on literary search and our own data are - Fig. (**3**):

(i) Sanguinarine is not converted to 3,4-benzacridine either *in vivo* or *in vitro*. The literature reporting this compound as a metabolite of sanguinarine is based on artifacts and misinterpretations that over the course of time has created a dogma. Nobody, has demonstrated in any publication a "green-fluorescence compound" which is reliably described and interpreted as 3,4-benzacridine.

(ii) Sanguinarine is converted to dihydrosanguinarine in rats. This conversion may be an important detoxication pathway in animals and tentatively in man.

(iii) There exists a reciprocal interaction between sanguinarine and the AhR/CYP1A metabolic pathway. Exogenous activation of AhR results in attenuation of sanguinarine cytotoxicity *in vivo* and *in vitro*. Detailed study of this phenomenon has revealed that AhR-mediated induction of CYP1A enzymes increases sanguinarine cytotoxicity and genotoxicity, probably *via* hydroxylation of sanguinarine by CYP1A enzymes and formation of reactive products. This effect is neutralized by three mechanisms occurring in parallel: 1) Sanguinarine inhibits catalytic activity of CYP1A enzymes and the formation of reactive metabolites *via* these enzymes; 2) Activated AhR up-regulates cytosolic dehydrogenases that detoxify sanguinarine *via* formation of dihydrosanguinarine; 3) Activated AhR up-regulates phase II enzymes that detoxify sanguinarine reactive metabolites *via* formation of conjugates.

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ABBREVIATIONS

3-MC	=	3-Methylcholanthrene
AhR	=	Aryl hydrocarbon receptor
BNF	=	Beta-naphtoflavone
CYP	=	Cytochrome P450
H4IIE.luc	=	Rat hepatoma cells stably transfected with DRE-LUC reporter
HepG2	=	Human hepatoma cells
PB	=	Phenobarbital
PKC	=	Protein kinase C

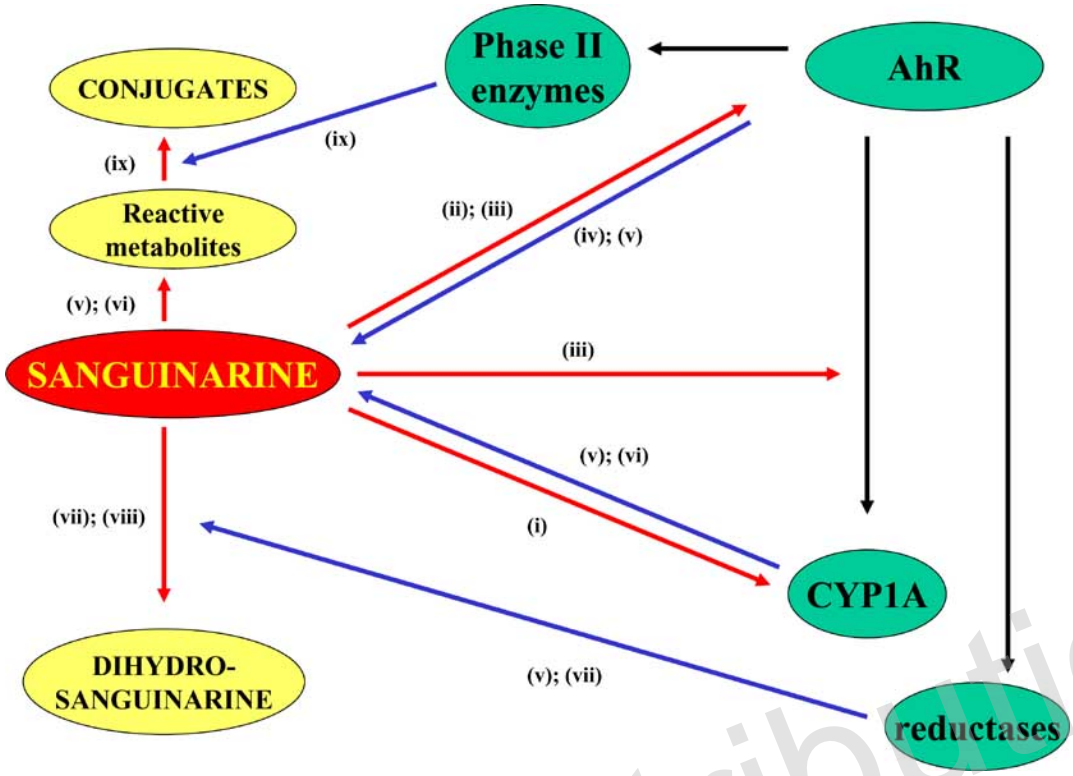

Fig. (3). Reciprocal interaction between sanguinarine and AhR/CYP1A metabolic pathway. (i) Sanguinarine inhibits CYP1A enzymes [21-23]; **(ii)** Sanguinarine in supramicromolar concentration inhibits transcriptional activity of AhR [24]; **(iii)** Sanguinarine in submicromolar concentration has no effect on transcriptional activity of AhR [23, 25, 26]; **(iv)** Cytotoxicity of sanguinarine is mitigated by 3-MC pretreatment in mice [20]; **(v)** Cytotoxicity of sanguinarine is attenuated by TCDD and PB in primary cultures of rat hepatocytes – the role of CYP1A is negative [27]; **(vi)** Genotoxicity of sanguinarine is elicited by pretreatment with AhR activator [32]; **(vii)** Sanguinarine is converted to dihydrosanguinarine in rats [14, 15]; **(viii)** Sanguinarine is not converted to 3,4-benzacridine in rats [14], rat hepatocytes and HepG2 cells [21].; **(ix)** Sanguinarine reactive metabolites are eliminated by conjugation enzymes.

QBA = Quaternary benzo[c]phenantridine alkaloids

TCDD = 2,3,7,8- Tetrachlorodibenzo-p-dioxin

TLC = Thin layer chromatography

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