Berberine: A Fluorescent Alkaloid with a Variety of Applications from Medicine to Chemistry

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Abstract: Berberine, a fluorescent, aromatic, heterocyclic alkaloid, produced in a diversity of plants, was found to have curative properties in Chinese herbal medicine. Berberine is used as a treatment for gastric and inflammatory diseases and as an antimicrobial agent. More recently the potential of this alkaloid in diverse new medical application like cardiology, neurology and oncology is being investigated. As a result of its fluorescent properties, it has also been used in chemistry to study protein conformation and protein/protein interactions and to quantitate non-fluorescent compounds. All these applications will be summarized and discussed in this review.

Keywords: Berberine, fluorescent, alkaloid, analysis, medicine, biochemistry.

1. INTRODUCTION

Berberine (Fig. (**1**)) is an isoquinoline alkaloid with a bright yellow colour that under ultraviolet light provides a strong yellow fluorescence. It is extracted from the plant *Coptidis rhizoma*, which has been used for centuries as a traditional Chinese medicinal herb to treat inflammatory diseases. Berberine-containing plants are used medicinally in many traditional medical cultures, including Ayurvedic herbal and Chinese herbal medicine. Berberine appears in the roots, flowers, shoots and bark of a number of important medicinal plants like *Berberis vulgaris*, *Hydrastis canadensis*, *Coptis chinensis*, *Arcangelisia flava* and *Berberis aquifolium*. As other alkaloids it has been used as folk medicine for a long time in China and other countries [1]. This compound has a wide range of pharmacologic effects, including protective effects on some gastric ulcer [2], treatment of inflammatory [2], cardiovascular or lipid- and glucose- related [3-10] diseases. In addition, berberine possesses antimicrobial activity against some bacterial [11, 12] or fungal infections [13]. Recent evidence has indicated that berberine has also anticancer properties and is currently being used to treat a variety of different diseases or medical disorders like diarrhoea, high cholesterol levels, diabetes and microbial infections such a fungal (*Candida*) or bacterial (*Salmonella*).

In addition to its medicinal properties, berberine is also used as a fluorescent probe to study cells, DNA and other diverse macromolecules in analytical and biochemical research. It is thus the aim of this minireview to summarize the most important applications of berberine in medicine, analytical chemistry and biochemistry. In addition the chemical properties of this alkaloid will be discussed as a basis to understand its biological and chemical effects.

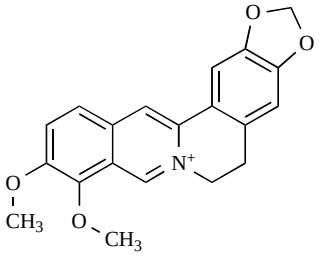

Fig. (1). Berberine cation.

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2. CHEMICAL PROPERTIES OF BERBERINE

Berberine is a quaternary amonium salt belonging to the family of isoquinoline alkaloids. It presents a dipolar structure that facilitates ion-induced dipole interactions with other molecules. This property explains the fact that virtually any compound assotiated with berberine produces a variation in the emission spectrum [14-16]. This variation is exclusively related to either an increase or decrease of berberine fluorescence intensity. Thus, this phenomenon has been exploited as a general detection method as emission changes are in proportion to the concentration of the compound. This type of detection, which can be carried out in either liquid or in solid phase, allows saturated hydrocarbons and other non-fluorescent, low-polarity molecules to be detected with high sensitivity. Fluorescence change induced by saturated hydrocarbons has been an unexpected and interesting phenomenon that has proven very useful to quantitate these types of compounds.

3. MEDICINAL APPLICATIONS

3.1. Diverse Applications of Berberine in Medicine (Table 1)

In the case of diabetes one study analyzed berberine's putative hypoglycemic activity by comparing its efficacy to that of other drugs used in treatment of these diseases like metformin [4]. This study indicated that berberine might be beneficial for the recovery of beta cell function (cells responsible for insulin secretion in the pancreas) during diabetes or prediabetes.

In the case of gastric ulcers, berberine has also been shown to have beneficial effects [2]. It was shown that berberine could significantly protect gastric mucosa from damage by ethanol in rats. This effect may be due to reduced expression of inducible nitric oxide synthase (iNOS). Thus, berberine may affect nitric oxide production and associated damage by inhibiting iNOS expression in damaged gastric tissue and, ultimately, improve in the healing of ulcers.

The anti-inflammatory properties of berberine have been exploited for a long time and a possible mechanism for this activity has been proposed *in vitro*. It was concluded that berberine inhibits the binding of AP1 transcription factor to DNA resulting in cyclooxygenase reduced expression [17]. Since cyclooxygenases have been implicated in inflammation and carcinogenesis, those *in vivo* observations may help to explain the anti-inflammatory and anti-cancer (see below) activity of berberine.

Berberine also functions as an antibacterial molecule [11]. It is a weak antimicrobial *per se* but its activity is strongly synergized by 5'-methoxyhydnocarpin, an inhibitor of the bacterial multidrug resistance pump (MDR). Since gram-positive bacterias are the main targets of plant derived MDR inhibitors, this may explain why so

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Table 1. Summary of Diverse Applications of Berberine in Medicine. Y= Tested; N= Not Tested

	Target	*In vitro*	*In vivo*	Mechanism of Action
Anti-microbial	Bacteria	Y	Y	Inhibition of multidrug resistance pump
	Fungi	Y	Y	
Anti-inflammatory	Carcinogenesis	Y	Y	Inhibition of AP1
	Microbial diahrrea	N	Y	Inhibition of intestinal secretion
Diabetes	Insulin production	Y	N	Recovery of beta-islet function
Hyperlipidemia	LDL absorption	Y	Y	↑↑ LDL receptors in liver
Gastric disease	Ulcer	Y	Y	↓↓ iNOS expression
Neuronal damage	Alzheimer	Y	N	↓↓ secretion of amyloid-β peptide
				Inhibition of neuronal apoptosis
	Ischemic stroke	Y	Y	
Cancer	Tumor cell survival	Y	Y	Cell death
				Tumor angiogenesis

few of these bacteria are plant pathogens. Plants produce compounds structurally similar to berberine that intercalate into the DNA helix. Since berberine also binds to DNA irrespective of its sequence, this compound fulfills the requirements for a highly effective anti-bacterial agent since it is unaffected by mutations.

There are other several examples in which berberine is combined with other substances to potentiate its antibacterial activity. Flavones, chrysosplenol-D and chrysoplenetin from *Artemisia annua L. (Asteraceae)*, which possess very weak antibacterial action by themselves, produce potent combinations with berberine resulting in very effective inhibition of *Staphylococcus Aureus* growth. As mentioned above, it appears that the mechanism also involves the inhibition of the multidrug resistance pump [18].

Beberine has also been employed as an analgesic for the treatment of neurological conditions. Berberine has been shown to ameliorate tolerance inducement to some narcotics like morphine [19].

For the treatment of neurodegenerative disorders, it has been shown *in vitro* that berberine reduces the secretion of amyloid-β peptide by human neuroglioma cells [20]. The effect of berberine *in vivo* has been studied in a rat model of Alzheimer disease. Intraperitoneal administration of berberine in rats increased the spatial memory of animals by increasing IL1beta and iNOS expression in hippocampus [21]. In addition, berberine inhibited neuronal damage during stroke in a mouse model of ischemic injury *in vivo* and *in vitro* [22]. This effect was attributed to inhibition of ROS production and subsequent blockade of the mitochondrial apoptotic pathway during ischemia.

Another application of berberine is the regulation of lipid metabolism. Berberine was found to reduce cholesterol in serum of human volunteers by a mechanism different to that of statins. They showed in a rat model that berberine up-regulated the low density lipoprotein receptor (LDLR) in the liver [3]. Recently Abidi *et al.* have shown that the root extract of goldenseal, a herbal supplement that contains the alkaloids hydrastine, berberine, berberastine, hydrastinine, tetrahydroberberastine, canadine and canalidine, had a similar effect in a hamster model [10]. In both studies, the effect of this increase in LDLR was the reduction of LDL associated cholesterol in blood.

As elaborated below, recent studies analysed the effectiveness of berberine in anti-cancer therapies [8, 23, 24] and suggested that the anti-tumoral effect of berberine could be due to its ability to reduce cholesterol levels in blood [8].

3.2. Anti-tumor Activity of Berberine

Although at present only a speculative medical application, we have included anti-tumor function of berberine as an independent section, mainly because of the large number of studies addressing this topic over the last ten years. Several compounds used in chemotherapy against diverse tumor types are alkaloids like Vincristine or the taxanes Paclitaxel and Docetaxel. Berberine also possesses anti-tumor activity against a number of tumor types. Most of the anti-tumor activity is based on the *in vitro* evidence on the ability of berberine to affect several steps of tumor development including cell proliferation, cell death and invasiveness [25, 26]. Cancer cells sensitive to berberine include hematological maligances like leukemia, lymphoma or myeloma or solid carcinomas like liver, stomach, lung, pancreas, tongue, skin, colon, breast, bone and brain [27].

Cell death induced by berberine *in vitro* mostly exhibits a typical apoptotic phenotype, including the activation of a family of proteases, the caspases, and cytochrome c release from mitochondria. Berberine has been shown to activate the two major pro-apoptotic mechanisms known as extrinsic and intrinsic pathways. Intrinsic (also known as mitochondrial) pathway is regulated by the members of the Bcl-2 familiy. This family comprises pro-apoptotic (Bak, Bax, Bid, Bim, Puma, etc) or anti-apoptotic (Bcl-2, Bcl-XL, Mcl-1, A1, etc) proteins [28]. Activation and/or inhibition of pro-apoptotic or anti-apoptotic members respectively, induce the permeablization of the mitochondrial outer membrane and, subsequently, the release of apoptogenic factors like cytochrome c or Apoptosis Inducing Factor (AIF). Cytochrome c together with Apaf-1 and caspase 9 forms a complex known as apoptosome that activates caspase 3 by proteolysis. Active caspase 3 is the ultimate effector molecule, responsible for cell dismantling including chromatin condensation and DNA fragmentation. The intrinsic pathway is activated at different levels like cell cycle arrest, disruption of anti-apoptotic/pro-apoptotic ratio of Bcl-2 family members, DNA damage and subsequent activation of the p53 repairing factor or proteasome inhibition. Berberine induces cell death *in vitro* by the intrinsic pathway in several mouse and human tumor cell lines. The mechanism by which this pathway is initiated is cell type dependent. A more detailed overview of these mechanisms has been recently published [26]. The extrinsic (also known as death receptor) pathway is activated after a specific ligand (Death ligand. i.e. FasL) binds to its receptor (Death receptor. i.e. Fas) on the cell membrane [29]. This binding activates a proteolytic signalling pathway initiated by caspase-8 that induces the direct or mitochondrial-mediated caspase-3 activation and the subsequent apoptotic cell death. Two studies have proposed that

Table 2. Summary of Diverse Applications of Berberine in Biochemistry

	Target	Mechanism of Action
Cell labelling	Mast cell membrane	Heparan sulphate binding
	Mitochondria	Electrochemical gradient accumulation
	Chromosomes	DNA binding
Macromolecule detection	DNA	Electrostatic interaction
	Albumin	Electrostatic interaction
	Alcohol dehydrogenase	Lipohilic interaction / Zn-SH complex
	Lipids	Electrostatic interaction
	Estrogens	?
	Vitamins	?
	Aminoacids	Electrostatic interaction

berberine induces cell death by this pathway in human colon carcinoma [30] and human hepatoma [31] cell lines, however direct evidences of this mechanism was lacking. In neither of those studies the role of Fas or FasL was tested if blocking the ligand or receptor could prevent cell death induction by berberine.

Apart from its cytotoxic potential, berberine is able to inhibit tumor cell motility and invasiveness *in vitro* in a number of human tumor cell lines like lung, breast, gastric, tongue or nasopharyngeal carcinoma cell lines [26, 32-35]. A small number of studies have addressed the anti-tumoral activity of berberine *in vivo*. Berberine alone or in combination with other compounds has been shown to reduce tumor development in mice inoculated with mouse leukemia (WEHI-3) [36], mouse melanoma (B16) [37] and mouse lung carcinoma (Lewis) [38] cell lines. It also reduced tumor proliferation in xenograft models of immunodeficient mice inoculated with human tumor cell lines like lung carcinoma (A549) [39], tongue carcinoma [40] or prostate carcinoma (LNCaP) [41]. Concerning inducible tumor models *in vivo*, it has been shown that berberine administration during methylcholantrene-induced tumorigenesis exerts an inhibitory effect [42]. It was also found to reduce the severity of the leukemia induced by the mouse retrovirus, Friend [43].

In vitro studies suggest that berberine is more targeted than other chemotherapeutic drugs. It has been reported that naive or activated T cells are resistant to berberine [44]. Furthermore, it has been shown that berberine is able to protect thymocytes (precursors of T cells) from cell death induced by dexamethasone, etoposide or camptothecin [45]. Finally it has been reported that macrophages are not only resistant to berberine induced cell death but also able to get activated and produce immune-stimulatory cytokines like IFN-gamma and IL12 *in vitro* [46] and *in vivo* [47].

3.3. Detrimental Effects of Berberine

Berberine toxicity is low in rat and mouse models when tested by histopathological criteria and with relatively high LD50 (dose at which 50% of animals die) values [26]. Although berberine has not been reported to be mutagenic, this compound is able to intercalate into the DNA double helix [48, 49]. Thus it is plausible that this binding may lead to mutations in the genome of exposed cells. Some studies have shown than non-transformed cells take up much lower levels of berberine than do tumor cells [50]. However, at present the basis of this phenomena is not understood and needs to be investigated.

4. ANALYTICAL APPLICATIONS

As outlined above, berberine can be used as dye to enhance detection of other types of compounds. In addition, if considered the analyte, it has also been detected by means of fluorescence increases or decreases ("quenching") of other fluorescent compounds. For example, low concentrations of berberine can be detected in the presence of halogenated solvents due to an increase in fluorescent emission in the spectrum of berberine by High Performance Liquid Chromatography (HPLC) [51]. Similar fluorescence increases have been found in the presence of cyclodextrines [52], Cucurbit-[7]-uril [53] or sodium lauryl sulfate [54]. In the later berberine was detected by fluorescence quenching using ethanol as solvent [55]. Additional examples where berberine is detected via its fluorescence "quenching" properties include optical fiber sensors based on immobilized 2-(4-diphenylyl)-6-phenylbenzoxazole [56] or 1,4-bis(naphth[2,1-d]oxazole-2-yl)benzene [57] In those examples sensing was based on the fluorescence quenching of the experimental molecules after their interaction with berberine. Fluorescence quenching of the tyrosine oxidation product by H_2O_2 (a reaction catalyzed by the enzyme horseradish peroxidase (HRP)) in the presence of berberine has also been used as a method of detection [58].

Hua *et al.* have developed an LC–MS method to quantify berberine in samples of human plasma after spiking with berberine chloride *in vitro* [59]. More importantly, Zuo *et al.* were able to quantify berberine in plasma of rats after oral administration of berberine chloride [60]. However, these methods generally lack the sensitivity required for simultaneous determination of berberine and other alkaloids in biological tissues, such as serum. Gupta *et al.* [61] have developed a bioanalytical method using protein precipitation with acetonitrile to clean up the serum samples prior to determination. Furthermore, this method has been validated for the simultaneous quantification of hydrastine and berberine in humans after oral administration of goldenseal supplement.

5. BIOCHEMICAL APPLICATIONS

It is generally accepted in the pharmaceutical industry that the overall distribution, metabolism, and efficacy of many drugs can be altered depending on their affinity to serum albumin [62]. Hu *et al.* [63] investigated the association of Human Serum Albumin (HSA) with berberine. It was found that an interaction occurred at the IIA domain of HSA. Since other alkaloid drugs may share with berberine binding sites in HSA, this finding may help to design therapeutic drugs with reduced binding constant to HSA and, thus, more efficient than current ones.

Berberine has also been widely used as a fluorescent dye in biochemical applications (Table **2**). Because of its bright fluorescence and affinity to the glycosaminoglycan heparin, it has been used in histology to mark mast cells in several tissues [64-68]. Furthermore, berberine is used to stain several cellular organelles and/or structures like mitochondria, cell nucleus, chromatin or single chromosomes and the cytosol of basophils [69-72]. Additional studies have shown berberine to interact with diverse macromolecules such as liver alcohol dehydrogenase [73], human serum albumin [74], acetylcholinesterase [75], lipids [76], estrogens [77],

bile salts [78] and DNA [79]. The interaction of berberine with DNA [80-84] has been elucidated in fine detail including the effect of other molecules such as different metal cations and cyclodextrins [85, 86].

Our group has separated and quantified different mixtures of neutral lipids and phospholipids by High Performance Thin Layer Chromatography (HPTLC) with fluorescence detection using pre- or post- impregnation with berberine [16]. Fig. (**2**) illustrates a TLC chromatogram showing the fluorescence corresponding to tocopherol using berberine-impregnated HPTLC silica gel plates.

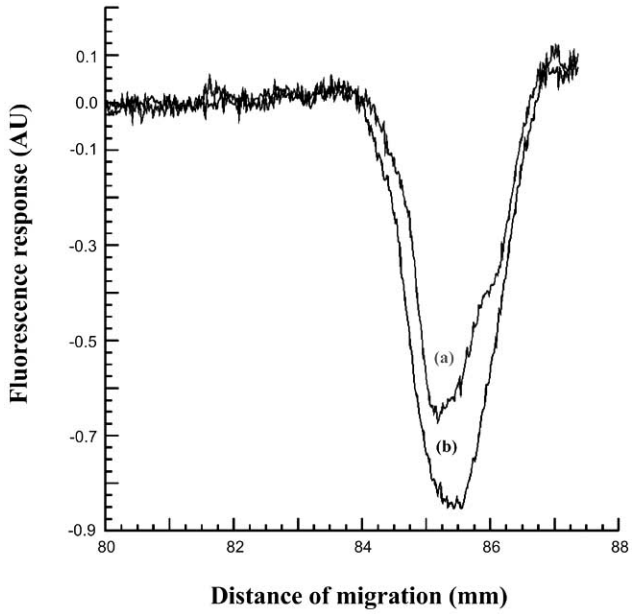

Fig. (2). TLC chromatogram of berberine-impregnated (6 mg/100 mL-1 methanol) HPTLC silica gel plates, tocopherol by fluorescence at λ=365 nm; (a) 1μg. (b) 5μg.

We have also used berberine to stain DNA and proteins after electrophoresis (Fig. (**3**)). Berberine is scarcely fluorescent in water, non-toxic, and as shown in Fig. (**4**) the fluorescence emission intensity in the presence of DNA increases. These properties suggest that berberine is a safer alternative than ethidium bromide or propidium iodide (both have been identified as a mutagen, carcinogen and teratogen [87, 88]) to analyse nucleic acids.

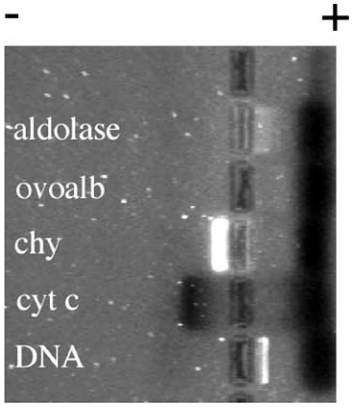

Fig. (3). Berberine can be used as detection dye for protein or DNA gel electrophoresis. Plasmid DNA or the proteins aldolase, ovoalabumin (ovoalb), chymotrypsinogen (chy) orcytochrome C (cyt c) were separated in a 2% agarose gel under constant voltage (negative and positive electric poles indicated above). After that the gel was embedded in a berberine solution and photographed under an UV light excitation lamp.

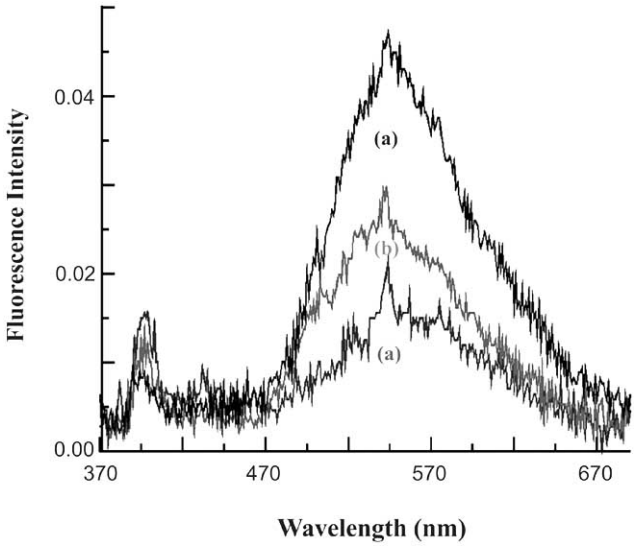

Fig. (4). Berberine fluorescence is increased in the presence of DNA. Emission spectrum (λ_{exc}= 350 nm) of a berberine solution (8mg/100mL^{-1} H_2O) in the absence (a) or presence (b, c) of increasing amounts of DNA: (b, 5 mg; c, 10 mg).

6. CONCLUSIONS AND FUTURE PERSPECTIVES

From ancient Chinese medicinal know-how it is clear that berberine possesses a wide range of medicinal properties including antimicrobial, anti-inflammatory and anti-tumor activities. Recent studies about the chemical behaviour of this fluorescent alkaloid have indicated that it can be used as an analytical probe to detect a great variety of fluorescent and more importantly non/fluorescent compounds in a reversible fashion. The later findings offer the possibility of using berberine as a dye for a variety of analytical and biochemical applications. Most importantly, it has been demonstrated that berberine can be utilized as a perfect detection dye to develop sensitive and reversible fluorescent biosensors.

More detailed studies of its chemical behaviour will allow us to understand why berberine presents all those beneficial activities and to reduce some of its adverse *in vivo* effects.

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