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Research article

Potential antimutagenic activity of berberine, a constituent of *Mahonia aquifolium*

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Abstract

Background: As part of a study aimed at developing new pharmaceutical products from natural resources, the purpose of this research was twofold: (1) to fractionate crude extracts from the bark of *Mahonia aquifolium* and (2) to evaluate the strength of the antimutagenic activity of the separate components against one of the common direct-acting chemical mutagens.

Methods: The antimutagenic potency was evaluated against acridine orange (AO) by using *Euglena gracilis* as an eukaryotic test model, based on the ability of the test compound/fraction to prevent the mutagen-induced damage of chloroplast DNA.

Results: It was found that the antimutagenicity of the crude *Mahonia* extract resides in both bis-benzylisoquinoline (BBI) and protoberberine alkaloid fractions but only the protoberberine derivatives, jatrorrhizine and berberine, showed significant concentration-dependent inhibitory effect against the AO-induced chloroplast mutagenesis of *E. gracilis*. Especially berberine elicited, at a very low dose, remarkable suppression of the AO-induced mutagenicity, its antimutagenic potency being almost three orders of magnitude higher when compared to its close analogue, jatrorrhizine. Possible mechanisms of the antimutagenic action are discussed in terms of recent literature data. While the potent antimutagenic activity of the protoberberines most likely results from the inhibition of DNA topoisomerase I, the actual mechanism(s) for the BBI alkaloids is hard to be identified.

Conclusions: Taken together, the results indicate that berberine possesses promising antimutagenic/anticarcinogenic potential that is worth to be investigated further.

Background

Traditional Chinese medicine has been practised by Chinese communities world-wide for many generations, and there is a wealth of literature information available related

to the therapeutic use of this type of medicine. In recent years, there has been a global surge in the popularity of herbal/traditional medicine, and currently there is enormous interest in developing new pharmaceutical products from such resources.

Over the last decade, bis-benzylisoquinoline (BBI) and especially protoberberine alkaloids (e.g., berberine and jatrorrhizine; Fig. 1) have attracted considerable attention in this respect; protoberberines represent a structural class of organic cations and have been found to be predominantly distributed in several genera of the families *Ranunculaceae* and *Berberidaceae* (e.g., *Berberis, Mahonia, Coptis*). Berberine, a major representative of the protoberberine alkaloids, displays diverse biochemical and pharmacological actions while being relatively non-toxic to man; its antimicrobial activity has been demonstrated against many bacterial and fungal species [1–4]. The drug was subsequently screened for anti-cancer activity following evidence of antineoplastic properties [5–7]. It has also been shown that berberine exhibits the ability to induce apoptosis in promyelocytic leukemia HL-60 and 3T3 fibroblast cells [5,8]. In addition, some protoberberines are highly effective as cytotoxic agents against several carcinoma such as HeLa, SVKO(3), Hep-2, primary culture from mouse embryo and human fibroblast cells [9,10]; berberine showed consistently the highest cytotoxicity among the alkaloids tested. Only recently it has been reported that berberine possesses a dual topoisomerase I and II poisoning activity [11–13] and binds to double helical DNA with a high affinity [14]. Furthermore, computer modeling studies on protoberberine-DNA complexes suggest that these alkaloids are able to bind to the host DNA by both intercalative (through rings C and D; Fig. 1) and minor groove (rings A and B) binding modes [12,13].

All the above findings raise the possibility that protoberberines may be effective in deactivation of carcinogens and tumour promoters. For in vitro screening of antimutagens/anticarcinogens a variety of prokaryotic and eukaryotic micro-organisms have been used. In the present study, a new eukaryotic model, viz. green unicellular flagellate *Euglena gracilis* was chosen [15]. This micro-organism possesses a multigenomic system with nuclear, mitochondrial and chloroplast DNAs. The detection ability of this model is based on the preferential and selective sensitivity of the chloroplast genetic apparatus to xenobiotics resulting in elimination of the functional chloroplast from the cells. Antichloroplastic activity of mutagens is macroscopically manifested by an irreversible loss of the capability of cells to form green colonies (bleaching effect). Compounds with antimutagenic properties prevent such a bleaching of *E. gracilis* cells by mutagens / carcinogens. Previous studies on several standard mutagens and antimutagens revealed a high sensitivity and reliability of the model [16–19].

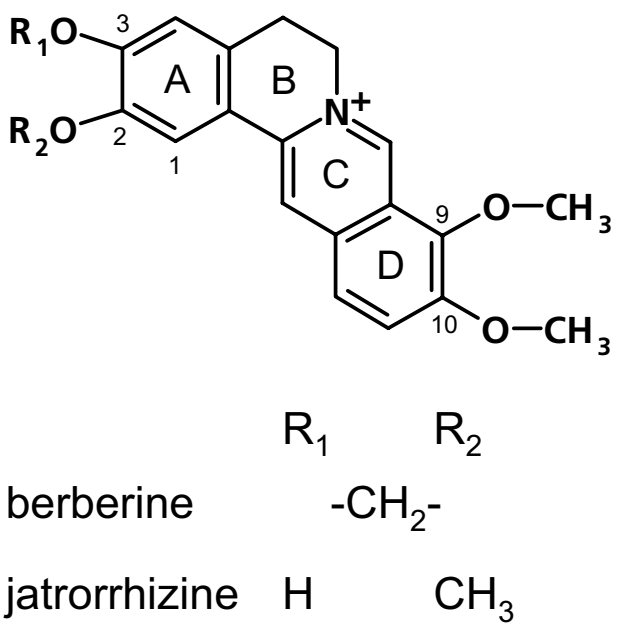

Figure 1
Chemical structures of berberine and jatrorrhizine.

Thus, the aim of the present work was to study the possible antimutagenic effect of berberine and jatrorrhizine as well as of the BBI alkaloids fraction and of the overall *M. aquifolium* crude extract, on acridine orange (AO) – induced mutagenicity in the *E. gracilis* assay.

Materials and methods

Plant material

The stem bark of *Mahonia aquifolium* (Pursh) Nutt. (*Berberidaceae*) was collected in October 1998 in the Arboretum Tesárske Mlyòany, Slovakia. Voucher specimens are deposited at the Herbarium of the Faculty of Pharmacy, Comenius University, Bratislava (No. Ma-108/8). Dried and powdered stem bark was macerated at room temperature with 62% aqueous EtOH (1:10 w/v) for 5 days and then filtered. The filtrate (crude *M. aquifolium* extract) was stored in a refrigerator until use in the biological assay. The BBI and the protoberberine alkaloid fractions were separated and purified using standard procedures as described previously [20].

Berberine [CAS 2086-83-1] and jatrorrhizine [CAS 3621-38-3], the main quaternary protoberberine alkaloids were isolated as iodides from the protoberberine fraction and identified by direct comparison of their physical and spectral properties with the literature data [20].

HPLC analysis of the BBI alkaloids was performed according to the procedure reported earlier [20]. Totally 6 tertiary BBI alkaloids (oxyacanthine, aromoline, baluchistine, berbamine, obamegine and aquifoline) were positively identified by referring to authentic compounds described earlier [20]. The identification was made on the basis of their UV absorption spectra and retention time.

Microorganism

Euglena gracilis (strain Z) was obtained from S.H. Hutner, Haskins Laboratory, Pace University, New York, NY, USA and maintained on Cramer-Myers (CM) medium [21] under static conditions at 27°C and with permanent lighting (16.4 W/m^2).

Chemicals

Acridine orange [CAS 65-61-2] was purchased from Merck, Darmstadt, Germany. Stock solutions of AO, berberine, jatrorrhizine, and BBI alkaloids were prepared by dissolving them in distilled water.

Mutagenicity assay

E. gracilis cells diluted to concentration 8×10^5 cells/ml CM medium were used in the experiments. Aliquots (0.2 ml) of the cell suspension were dispensed into test tubes, along with 2.3, 11.4 or 22.8 μM AO, indicated concentrations of berberine (0.03, 0.06, 0.15, 0.30, 0.45 μg/ml), jatrorrhizine (0.72, 1.44, 14.4, 28.8, 43.2 μg/ml), BBI alkaloids (0.72, 1.44, 14.4, 28.8,43.2 μg/ml) or the *M. aquifolium* extract (5.5, 11.0, 110,220,330 μg/ml), and at last the content of the incubation mixture was completed to a final volume (5 ml) by the addition of CM medium. Following a 24-h co-treatment, the cells were centrifuged at 3000 rpm for 20 min, the resultant pellet suspended in fresh CM medium and again centrifuged. After the pellet was resuspended in fresh CM medium, the cells were finally cultivated 14 days at 27°C under permanent illumination (16.4 W/m^2). The experiments were repeated in three independent series. Just before the counting, the movement of *E. gracilis* was stopped by adding a drop of EtOH and the counting of green and white (mutant) colonies was carried out in a Bürker chamber under a microscope. The viability of the *Euglena* cells was estimated by counting the total white mutants in the presence of mutagen (positive control) compared to the number of the spontaneous white mutants in the absence of mutagen (negative control). We defined the relative decrease of the bleaching as the antimutagenic potency (AP) which was calculated by the formula

$$AP(\%) = \frac{B_0 - B_r}{B_0} \times 100$$

where B_0 is the AO-induced *E. gracilis* bleaching (%) and B_r is the AO-induced and antimutagen-reduced *E. gracilis* bleaching (%).

The statistical significance of all the calculated values were determined by paired Student's t-test. The values represent the means ± standard deviation (SD).

Results and discussion

The mutagenic effect of AO on *E. gracilis* was tested in three concentrations, 2.3, 11.4 and 22.8 μM, which induced, respectively, 43 ± 2 %, 58 ± 2 % and 65 ± 3 % of white mutant cells. The AO concentrations were chosen so as to ensure no significant change in the viability of cells compared to the negative controls (*Euglena* cells in the absence of the mutagen). Similarly, no spontaneous white mutants were found in any sets of positive controls.

The dose-dependent inhibitory effect of the crude *Mahonia* extract, BBI alkaloids fraction, berberine and jatrorrhizine on the mutagenicity of AO applied at the above 3 concentrations is displayed in Figs. 2,3,4,5. As shown in Figs. 2 and 3, the plot of the percentage of bleached mutants vs. concentration for the *Mahonia* extract and the BBI fraction respectively displays typical curving even though over different concentration ranges. The lowest concentration that causes statistically significant ($p < 0.05$) reduction in the percentage of white colonies (as compared to the positive control) is 0.7 and 11.0 μg/ml for the *Mahonia* extract and the BBI alkaloids, respectively. For both samples, the decrease of the percent proportion of mutant colonies gradually continued with further increasing the concentration; however, the antimutagenic efficiency was much higher for the BBI alkaloids sample, for which the bleaching percentage (corresponding to the AO 2.3 μM curve) reaches ~5% level for the highest concentration tested.

As to the protoberberines berberine and jatrorrhizine, which are of prime interest here, the concentration dependence of the bleaching effect of jatrorrhizine (Fig. 5) is markedly similar to those observed for the above two samples. In contrast, the behaviour of berberine is completely different; as shown in Fig. 4, berberine chloride suddenly reduced the number of AO-induced mutant cells (to ca 5% for AO 2.3 μM) at concentration 0.03 μg/ml but further increase of the concentration did not cause progressive reduction of the bleaching activity or even, in the case of the AO 22.8 μM series, the number of white colonies increased slightly but significantly with respect to that observed for the most effective concentration ($p < 0.05$). For berberine, the antimutagenic potency (AP) was also calculated using the formula given at the end of the Material and methods section and the results are summarised in Table 1. As can be seen, berberine exhibited significant

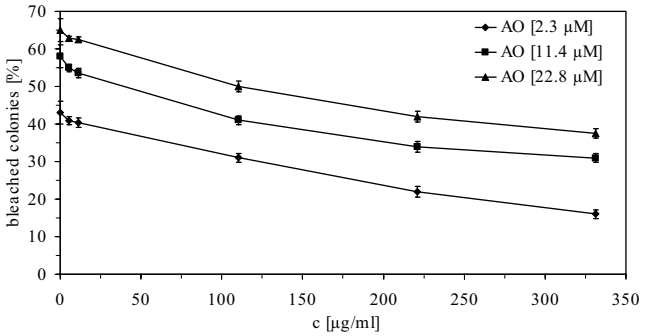

Figure 2
A plot of percentage of bleached colonies of *E. gracilis* vs. concentration of *M. aquifolium* extract at 3 concentrations of acridine orange (AO). Symbols and brackets denote means ± standard deviation of 3 independent determinations.

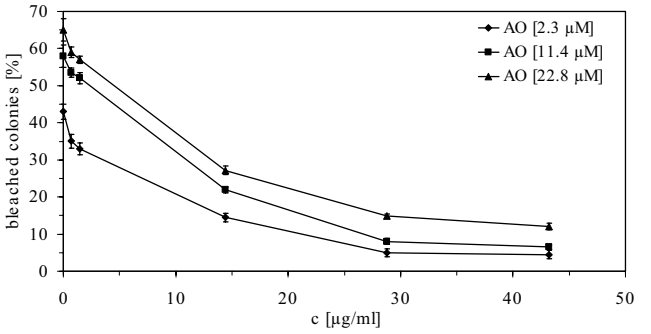

Figure 3
A plot of percentage of bleached colonies of *E. gracilis* vs. concentration of BBI alkaloid fraction at 3 concentrations of acridine orange (AO). Symbols and brackets have the same meaning as in Fig. 2.

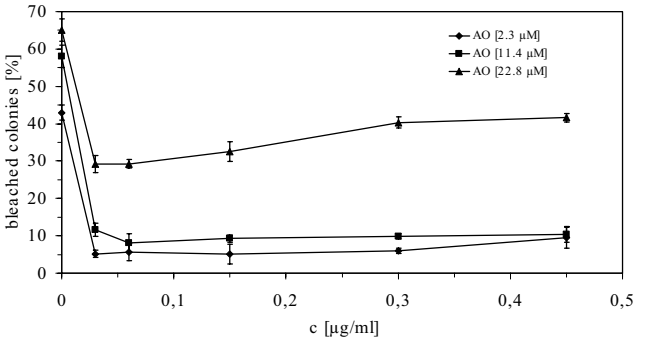

Figure 4
A plot of percentage of bleached colonies of *E. gracilis* vs. concentration of berberine at 3 concentrations of acridine orange (AO). Symbols and brackets have the same meaning as in Fig. 2.

antimutagenic potency (>80%) almost over the whole concentration range tested when the concentration of the mutagen (AO) was 2.3 and 11.4 µM while for the increased AO concentration (22.8 µM) the antimutagenic potential of berberine was much less effective (AP < 55%).

Although the BBI and protoberberine alkaloids studied in this laboratory exhibit a broad spectrum of biological activities, for most of them the molecular mechanism of action is still unclear. In the case of the BBI alkaloids, one possible mechanism of their antimutagenic activity could be based on the reports that tetrandrine and other BBI alkaloids are excellent scavangers of reactive oxygen species (ROS) such as singlet oxygen and/or superoxide anion radical [22] since it is well documented that that ROS play a central role in multistage mutagenesis and carcinogenesis [23,24]. However, the potential mechanism based on the ability of BBIs to prevent oxidative damage of DNA by ROS is unlikely since the ability of AO to produce ROS in a redox system has never been published. Instead, the antimutagenic potency of the BBI fraction is more likely linked to modulation of DNA at the transcriptional level. Recently, the role of eukaryotic transcription factors, NF-κB and AP-1, has been highlighted in mutagenesis and carcinogenesis [25,26] and tetrandrine is known to inhibit the activation of NF-κB in the alveolar macrophage [22]. Nevertheless, the identification of the ultimate mechanism of the antimutagenic effect of the BBI alkaloids awaits further study.

As described above, the significant antimutagenic potency against AO was observed for the two protoberberines studied herein. The potency of jatrorrhizine alone is comparable to that found for the overall BBI fraction and the potency of berberine is even by more than 2 orders of magnitude higher. To our knowledge, the ability of protoberberines to reduce the mutagenicity of environmental chemicals has not yet been reported so that the mechanism of their antigenotoxic action was not discussed. However, the cytotoxic activity of berberine and its analogues has been attributed to DNA topoisomerase I (TOPO-I) and II (TOPO-II) poisoning [11–13]; recently, a mechanistic model [12] for TOPO-I poisoning by protoberberines was developed according to which berberine binds to DNA and TOPO-I at the interface of the binary complex DNA – TOPO-I in such a manner that rings C and D (Fig. 1) intercalate into the DNA helix, while rings A and B protrude out of the helix interior into the minor groove, where they are accessible to interact with specific functional groups on the enzyme. Thus, both drug-DNA and drug-enzyme interactions contribute to the overall affinity (potency) of the drug. In this light it is reasonable to suggest that the antimutagenicity of berberine against the genotoxic effect of AO on plastid DNA is exerted by a similar, if not identical, mechanism. A comparison of the an-

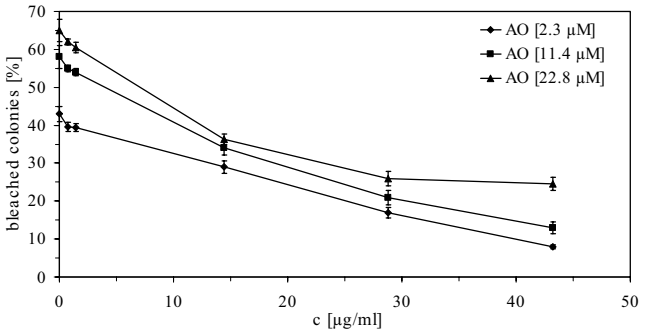

Figure 5
A plot of percentage of bleached colonies of *E. gracilis* vs. concentration of jatrorrhizine at 3 concentrations of acridine orange (AO). Symbols and brackets have the same meaning as in Fig. 2.

timutagenic potency of the two protoberberines tested here supports this notion. The only difference between berberine and jatrorrhizine (Fig. 1) is restricted to the substitution on the A ring (2,3-methylenedioxy in berberine vs. 2-methoxy-3-hydroxy substitution in jatrorrhizine), i.e. to the portion of the molecule which is involved in interactions with TOPO-I. Thus, insertion of the protoberberine molecule between the base pairs of DNA prevents the interaction of the AO mutagen at the same binding site as AO is known as a frameshift mutagen due to strong intercalative binding. A similar detrimental effect of the free hydroxy group on the TOPO-II poisoning activity has also been observed for berberrubine [13]. In this context it is important to note that camptothecin, a natural product currently in clinical use for the treatment of a variety of cancers [12,25], has a pharmacological profile very similar to that of protoberberines; its antineoplastic and antimutagenic activity was demonstrated against many organic mutagens and also the DNA-TOPO-II complex was identified as its molecular target [26]. It is therefore reasonable to consider that the drug-induced poisoning of TOPO enzymes may be of general importance for anticancer and antimutagenic drugs.

Table 1: Antimutagenic potency (AP) [%] of berberine in reducing the acridine orange (AO)-induced bleaching of *Euglena gracilis* cells.

mutagen	concentration of berberine [μg/ml]				
AO [μM]	0.03	0.06	0.15	0.30	0.45
2.3	89 ± 2.2	88 ± 5.3	90 ± 6.2	86 ± 1.7	78 ± 6.5
11.4	80 ± 3.0	86 ± 4.2	84 ± 1.6	83 ± 1.1	82 ± 3.6
22.8	54 ± 3.5	54 ± 1.8	49 ± 4.1	38 ± 2.3	36 ± 1.7

Conclusions

In summary, the data presented here showed that the BBI alkaloid fraction and especially the protoberberines, jatrorrhizine and berberine, inhibited the AO-induced mutagenicity in the *E. gracilis* assay. Berberine demonstrated significant antimutagenic activity against the chloroplast damaging effects of AO with a high potency. The protective effects of the latter compound against the chemical carcinogen suggest its potential chemopreventive ability, which is under investigation using other bacterial tests as well as animal tumour models.

Competing interests

None declared.

Acknowledgements

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