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Photochemical and phototoxic activity of berberine on murine fibroblast NIH-3T3 and Ehrlich ascites carcinoma cells

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Abstract

The present study demonstrates photoinduced generation of superoxide anion radical and singlet oxygen upon UVA irradiation of berberine chloride, and its cytotoxic/phototoxic effects on murine fibroblast non-cancer NIH-3T3 and Ehrlich ascites carcinoma (EAC) cells. The EPR spectra monitored upon photoexcitation of aerated solutions of berberine evidenced the efficient activation of molecular oxygen via Type I and II mechanisms, as the generation of superoxide anion radical and singlet oxygen was observed. The EAC cell line was more sensitive to the effect of non-photoactivated and photoactivated berberine than the NIH-3T3 cell line. UVA irradiation increased the sensitivity of EAC cells to berberine, while the sensitivity of NIH-3T3 cells to photoactivated berberine was not changed. Berberine significantly induced direct DNA strand breaks in tested cells, oxidative lesions were not detected, and the effect of irradiation of cells after berberine treatment did not affect the increase of DNA damage in EAC and NIH-3T3 cells. The DNA damage generated by a combination of berberine with UVA irradiation induced a significant blockage of EAC cells in the S and G_2/M phases and the stopping/decrease of cell proliferation after 24 h of influence. On the other hand, after 36 h or 48 h of berberine treatment, the DNA damage induced necrotic or apoptotic death of EAC cells. Whether these divergences are caused by differences in the properties of two non-isogenic cell lines or by different berberine uptake and cell localization will be analyzed in our further investigations.

Keywords: Berberine; EPR; Spin trap; Phototoxicity; NIH-3T3 and EAC cells; Cell death; DNA damage

1. Introduction

Berberine is now an extensively studied isoquinoline alkaloid that generates a wide variety of biochemical and pharmacological effects [1–3]. Berberine has demonstrated significant antimicrobial activity against a variety of organisms including bacteria, fungi, viruses, chlamydia and protozoans [4–8]. It can also be used as an antidiarrhea, antihypertension, antiarrhythmias and an antiinflammatory agent [9–17]. Berberine has been demonstrated to possess antitumor activity in vitro and in vivo [14,18–24]. Through investigation of the biochemical effects of berberine it has been discovered that berberine inhibits a number of enzymes, e.g. NADH oxidase, reverse transcriptase and diaminooxidase [25], topoisomerase [26]; activator protein 1 and cyclooxygenase-2 [18,27]. It interacts in vitro with DNA, poly(A) fragments of mRNA and tRNA by the mechanism of intercalation [22,25] and it induces apoptosis through a mitochondria/caspase pathway in human hepatoma cells [28].

Berberine has long been used in Chinese medicine; now it has been employed to treat skin diseases, including psoriasis and eye infections. The topical application of berberine to the skin or eyes raises the possibility that an adverse

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phototoxic reaction may result from an interaction between the alkaloid and light [29].

The interaction of the compound (natural or synthetically prepared) with light can lead to the production of singlet oxygen and radical species. Such compounds are called photosensitizers. A photosensitizer is described as a compound that, in the presence of oxygen and upon light stimulation, generates superoxide radicals (Type I reaction), which in turn may form peroxide and hydroxyl radicals, or (non-radical) singlet oxygen molecules ($^1O_2$) (Type II reaction) [30].

In the presence of UV light some photosensitizers that induce phototoxicity, may exhibit genotoxic potential and photocarcinogenesis [31,32]. On the other hand, many bioactive phytochemicals have been shown in recent years to be photosensitizers, i.e. their toxic activities against viruses, microorganisms, insects or cells are dependent on or are augmented by light of certain wavelengths. These activities are often selective, and this has led to the concept of therapeutic prospects in the control of infectious diseases, pests and cancer. Reaction mechanisms commonly involve singlet oxygen and radicals, which are thought to cause photodamage to membranes or macromolecules [33].

Berberine has been reported by various authors to be a photosensitive agent which is able to produce singlet oxygen and radical species in the presence of UVA irradiation [10,29,34,35].

In our previous studies [21–23] we found cytotoxic/antiproliferative effects of berberine on cell lines L1210, B16, EAC, HeLa, U937, as well as NIH-3T3 and V79 cells, which showed concentration- and time-dependence. Berberine demonstrated necrotic or apoptotic cell death depending on its concentration and the type of cell line. High concentrations of it induced intercalation of berberine with DNA, inhibition of DNA synthesis and caused cell death of EAC cells. Lower concentrations induced clear apoptotic cell death, which was followed by inhibition of protein synthesis.

We also monitored the ability of protoberberinium salts (berberine, palmatine and jatrorrhizine iodides) to produce superoxide anion radicals and singlet oxygen upon photoexcitation in dimethyl sulfoxide solvent [36]. The application of EPR spectroscopy confirmed the generation of $O_2^{\cdot-}$ and $^1O_2$ during continuous irradiation of berberine, but the photochemical activities of palmatine and jatrorrhizine were substantially lower. The fluorescence spectra of protoberberinium iodides measured in DMSO or ethanol revealed the role of iodide counter-ion as an efficient fluorescence quencher.

Based upon these results, in the present study we investigated by EPR spectroscopy the photoexcitation of berberine chloride, resulting in the formation of ROS. Further, we studied the in vitro cytotoxic effect of berberine in the dark and in the presence of UVA light, using NIH-3T3 and EAC cells. The effect of berberine alone or in combination with UVA irradiation on the cell cycle, on the induction of necrosis/apoptosis, and on DNA damage was monitored.

2. Materials and methods

2.1. Chemicals

Berberine (2,3-methylenedioxy-9,10-dimethoxyprotoberberine chloride, Scheme 1), was obtained from the Sigma Company (Slovakia). The compound was stored at 4 °C under dark conditions. The stock solution of berberine for EPR experiments and incubation with cells was prepared in DMSO and further diluted in the cell culture medium. The final DMSO concentration in the medium was 0.1% (in either control or treated samples), which did not affect cell viability. DMSO was purchased from Merck.

Spin trapping agents 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) and α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) from Aldrich were stored at −18 °C. DMPO was distilled before application and saved under argon. Stable free radical 4-hydroxy-2,2,6,6-tetramethylpiperidine *N*-oxyl (TEMPOL) and singlet oxygen agent 4-hydroxy-2,2,6,6-piperidine (TMP) were purchased from Aldrich. Superoxide dismutase (SOD), propidium iodide (PI), normal melting point agarose (NMP agarose), Triton-X 100, trypan blue, ethidium bromide (EtBr), RNase, and Hoechst 33342 were from Sigma–Aldrich.

Low melting point agarose (LMP) was purchased from Invitrogen (Great Britain) and phosphate-buffered saline (Dulbecco A) (PBSa) was from OXOID (Great Britain).

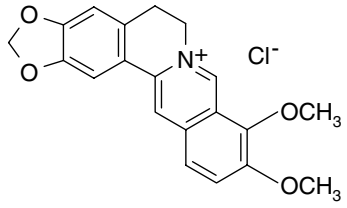
Ethylenediaminetetraacetic acid (EDTA), ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), and sodium hydroxide (NaOH) were from Lachema (Brno).

RNase, proteinase K and 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) were obtained from the BIOCOM Company (Slovakia).

Formamidopyrimidine–DNA–glycosylase (FPG) and Endonuclease III (Endo III) were obtained from A.R. Collins (University of Oslo, Oslo, Norway). The crude extracts of FPG and Endo III were diluted in 40 mM HEPES–KOH, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/ml bovine serum albumin, pH 8.0 (1:3000 and 1:1000, respectively) just before use.

2.2. Cell lines

Normal NIH-3T3 cells were obtained from the American Type Culture Collection, Rockville, MD (USA). Before a uniform monolayer of NIH-3T3 cells was formed, cells were freed from the surface of the culture dish by a



Scheme 1. Chemical structure of berberine chloride.