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Evaluation of Berberine as a DNA-Specific Fluorescent Dye and DNA-Intercalating Antitumor Alkaloid

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Berberine is a *pentacyclic isoquinoline alkaloid,* contained in *Hydrastis canadensis (Rannunculaceae), Chelidonium majus (Papaveraceae)* and in some medical plants from *Coptis* and *Berberidaceae families.* A lot of *in vitro* and *in vivo berberine's mechanisms* as *a nuclear* and *chromosomal DNA-specific fluorochrome*, as well as like *antitumor alkaloid,* have been reviewed.

Key words: berberine (berberine sulfate), DNA-specific fluorochrome, nuclear and chromosomal fluorescence, antitumor alkaloid.

1. The alkaloid berberine

Berberine (Fig. 1) is an isoquinoline alkaloid, contained in large amounts in roots, rhizomes and bark of some plants, such as *Hydrastis canadensis* (*Rannunculaceae*); *Chelidonium majus* (*Papaveraceae*); *Coptidis japonica, Coptidis chinensis* and *Coptidis rhizoma* – from *Coptis* family, as well as in the plants from *Berberidaceae.*

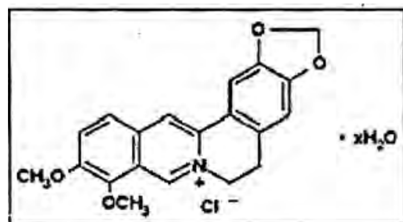

Fig. 1. $C_{20}H_{17}O_4N$. x xH_2O

DNA-specific and antitumor activities of berberine could be explained with its ability to intercalate with DNA [16, 23]. This process is attributed to the planar structure of berberine – a property, which facilitates intercalation and subsequent π-stacking with DNA-base pairs, mainly with the participation of berberine's methoxy groups [10]

2. Berberine and its salts as fluorochromes

2. 1. Berberine as a DNA-specific fluorochrome and antitumor alkaloid

Some biochemical data [23] concerning interaction between berberine sulfate and nucleic acids, have shown that it binds specifically with DNA. The fluorescent cytochemistry of nucleic acids gave a ground to recommend the berberine sulfate application as a DNA-specific fluorochrome in the cytological and cytogenetical practice [5 – 9, 25].

The differences established in the DNA-fluorescence of active (euchromatin) and inactive (heterochromatin) nuclear zones by post-DNA-partial denaturation and fluorochromation with berberine sulfate have been established for detection of cell maturity (proliferation, differentiation) and/or malignisation [5 – 9], as well as for chromosome staining and microfluorometric analysis [9, 25].

The ability of the highly condensed nuclear chromatin in the small thymocyte nuclei and the more loosely organized chromatin in the hepatocytes to interact with the DNA-specific fluorochrome has been also determined by microfluorometry and the results have been compared with those, obtained with other fluorochomes, interacting only with double-stranded nucleic acids [2, 3]. In comparison with other DNA-fluorochrome techniques, based on the application of quinacrine, acroflavine, chloroquine etc., the berberine sulfate one secures an intensive and long-lasting fluorescence with a high stability to actinic effects: the interphase nuclei and chromosomes keep well and for a long time their structure and the intensity of fluorescence [5, 6, 25]. An important advantage of the berberine sulfate fluorescent technique is the specific binding of the fluorochrome not only with double strand-DNA, but also with a single strand-DNA – in cases of pathological changes of the chromatine, as well as after DNA-denaturation treatments [6, 25].

The significantly decreased (weaker) nuclear fluorescence in some types of immature and/or neoplastic cells (lymphoblasts, myeloblasts, paraleucoblasts, cancer cells) and certain chromosomal regions, would be due to the greater amounts of active euchromatine (slow reassociating or unstable to DNA-denaturation), not binding to a sufficient degree the fluorochrome applied. On the other hand, it could be assumed that the different amounts of histones and non-histone proteins in the different types of chromatine are responsible for the changes in DNA (DNP) as far as its denaturation and fluorochromation properties are concerned. The results show that the differences established in the nuclear and chromosomal chromatin fluorescence by the technique of partial DNA-denaturation and fluorochromation with berberine sulfate is due to the chromatin functional differences. Hence, the ability of nuclear and chromosomal chromatin to interact with various types of fluorochromes might be useful parameter in subsequent studies of the nuclear and chromosomal chromatin organization [2 – 9, 25].

The biochemical, cytofluorometrical and molecular bases of DNA-berberine interactions at the level of cell nuclei and chromosomes – in the course of their fluorochromation, could be the same as more recently described mechanisms of berberine-induced cytostatic and/or apoptotic action against tumor cells in vivo and in vitro [15].

A lot of experimental results [1, 13, 18] have shown that berberine induced cytostatic activity against tumor cells in vitro, as well as their apoptosis, may be explained with intracellular DNA-intercalating action.

A current experimental model [12, 15] suggested that the DNA-binding- and intercalative components of berberine are important for the inhibition of DNA topoisomerase. In this way, a dual – topoisomerase I and II, "poisoning activity" of berberine and hence a possibility for apoptotic cell death, has been recently reported

[12, 16]. In the treated with berberine HL-60 human promyelocytic leukemia cell line, a decline in telomerase activity has been also observed [22].

The results from another study [14] suggested that berberine exhibits the ability to induce nuclear morphological changes and internucleosomal DNA-fragmentation, characteristic of apoptosis in HL-60 human promyelocytic leukemia cells. The experimental results suggested that the intercellular DNA-interacting action of berberine, as well as other important cellular processes may be involved in the berberine-induced apoptosis in tumor and leukemia cell lines. The anticancer (cytotoxic) activity of berberine against human HeLa tumor cell line and murine L1210 leukemia cells, has been also obtained [11]. In both cases a concentration-dependent decrease of cell number in S phase and an increase in G_2/M phases have been detected. Besides that, the sensitivity of leukemia L1210 cells to berberine was higher than that of HeLa cells [11]. In L1210 cells, treated with 10-50 μg/ml berberine, G_0/G_1 cell cycle arrest has been observed.

Cell cycle analysis of the treated with berberine in vitro esophageal cancer cell (ECC) lines (YES-1 – YES-6) [4, 8] has shown the accumulation of the alkaloid in the cells in the G_0/G_1 phases at a dose-dependent manner and a relative decrease of cell number in the S phase [8].

In determining the morphological changes of fibroblasts from the mouse Balb/c 3T3 cell line – after berberine treatment (in concentrations 100 μg/ml and 200 μg/ml, respectively), it has been established that the high berberine concentration (200 μg/ml) correlated with the high number of apoptotic cells [24]. On the other hand, cells, treated with this high berberine concentration, accumulated berberine in the nuclei. When the cells have been treated with berberine in low concentration (100 μg/ml), accumulation of cells in G_2/M phases – with the highest concentrations of berberine in the cytoplasm, has been observed in the time of *apoptosis induction.*

2. 2. Berberine sulfate fluorochromation for polysaccharides

Berberine sulfate staining for heparin in mast cells has been performed in unstimulated and antigen-stimulated mice [17, 19, 21]. The results of these experiments have demonstrated a significant contribution of the mast cells to the immune complex-induced inflammation [21]. The fluorescence of mast cells in the rat hypothalamus – after berberine sulfate staining, indicated also the presence of heparin in their cytoplasm [20].

In agar colonies of human bone marrow cells, stained with metachromatic dyes, only macrophages with large granulated cytoplasm were positively stained with berberine sulfate (but they are negative for histamine, chloracetate-transferase and mast cell triptase) [13]. However, such changes were not observed in the cytoplasm of mast cells precursors.

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