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REVIEW ARTICLE

POTENTIAL ANTICLASTOGENIC EFFECT OF HYPERFORIN

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Summary

Hyperforin is a prenylated phloroglucinol derivative which occurs in the plant *Hypericum perforatum* L. It has several medicinally important properties (antidepressant, anti-inflammatory, proapoptotic, antibacterial and antiangiogenic). To enable its medicinal use, it is necessary to investigate its potential genotoxic effect on human cell lines. We also observed an anticlastogenic effect of hyperforin towards the indirect mutagen benzo(a)pyrene. Benzo(a)pyrene is a widespread polycyclic aromatic hydrocarbon. We performed experiments on human tumor cell line HepG2, using the *in vitro* mammalian chromosome aberration test. We investigated two cytogenic parameters: the number of aberrant metaphases and the total number of chromosome aberrations. We found out that hyperforin was not genotoxic on human cell line HepG2. The second part of our results implies that hyperforin had an anticlastogenic effect against the indirect mutagen benzo(a)pyrene in our experimental conditions. In future we will continue our research by using another range of hyperforin concentrations, other cell lines and other chemical mutagens.

Key words: hyperforin; benzo(a)pyrene; genotoxic; anticlastogenic; chromosomal aberrations.

INTRODUCTION

Hyperforin is a major lipophilic constituent of *Hypericum perforatum* L. and forms up to 4% of its dried weight. It is a prenylated phloroglucinol derivative that consists of a phloroglucinol skeleton with lipophilic isoprene chains (Fig. 1) (1). *Hypericum perforatum* L. is widely distributed in Europe, Asia, Northern Africa and North America (2). Hyperforin occurs in leaves of *Hypericum perforatum* L. Hyperforin is very important secondary metabolite, because it has several medical effects such as: antidepressant, anti-inflammatory, as well as antibacterial, antitumoral and antiangiogenic (3, 4, 5, 6, 7, 8, 9). We observed its anticlastogenic effect towards the indirect mutagen benzo(a)pyrene (BaP). BaP is a polycyclic aromatic hydrocarbon which belongs to the most widespread environmental toxicants and carcinogens (10). Benzo(a)pyrene has carcinogenic, mutagenic and teratogenic effects in various species and tissues (11, 12, 13, 14). Besides respiratory exposure through inhalation of polluted air or cigarette smoke, another major route for human BaP exposure is through consumption of BaP-containing food, such as overcooked or barbecued meat (15). The toxicity of BaP is caused by its biotransformation by microsomal cytochrome P450 enzymes (Fig. 2) (10,16).

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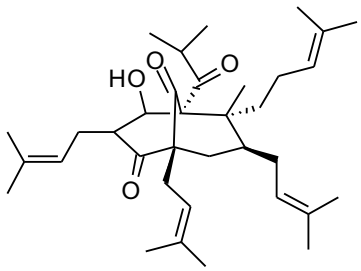

Figure 1. Chemical structure of hyperforin (1)

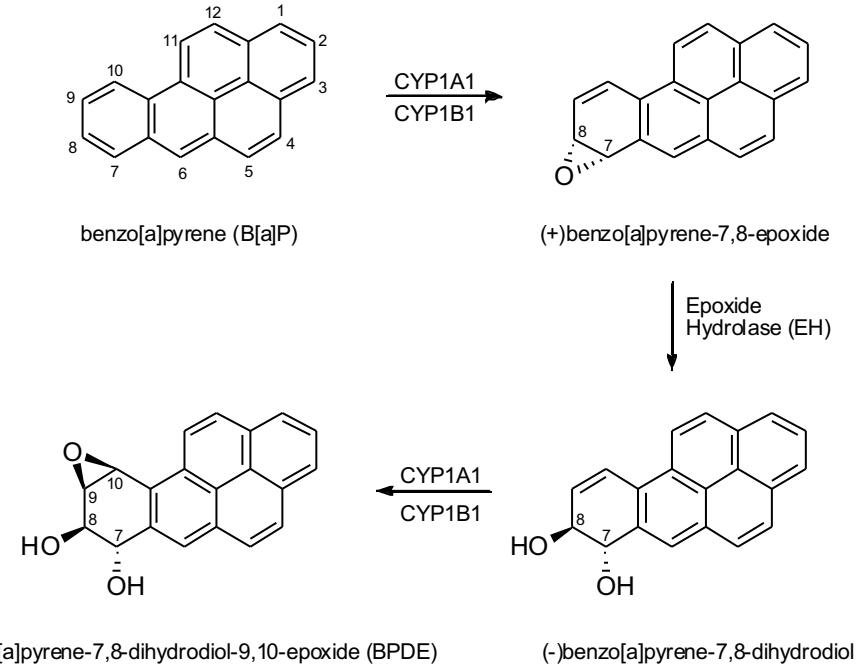

Figure 2. Metabolic activation of benzo(a)pyrene (16)

MATERIALS AND METHODS

Cell culture

The cell line HepG2, derived from human liver tumor, was founded by Dr. B. B. Knowles (Wistar Institute of Anatomy and Biology, Philadelphia, PA, U.S.A.). The cell line was obtained from A. Collins. Cell line HepG2 is capable of metabolic activation of many indirect mutagens by cytochrome P450: CYP1A1, CYP1A2, CYP2C9, CYP3A4, CYP2C19, CYP2A6, CYP2B6, CYP2C8, CYP2D6, CYP2E1 (17). This cell line is characterized by the aneuploid number of chromosomes. The cells were cultured in the Wiliams medium (PAN-Biotech GmbH, Germany) with 10% fetal bovine serum (PAN-Biotech GmbH, Germany) and with antibiotic gentamycin (50 μg/ml) (gentamycin Sandoz, SANDOZ, Slovenia). The cells were cultured in plastic Petri dishes under C02/air (5 % : 95 %) at 37°C similarly as (18).

In vitro mammalian chromosome aberration test

Experiments were made in the light of the OECD recommendations, test No. 473, In vitro mammalian chromosome aberration test (19).

Potential genotoxic effect of hyperforin

HepG2 cell line was treated with 7.5 μM hyperforin (dicyclohexylammonium salt, AppliChem, Germany) in medium, and it was cultivated

in thermostat for 42 hours. 5 μM benzo(a)pyrene (SIGMA) indirect mutagen was used as a positive control (PC) for the pro-mutagen effect.

Potential anticlastogenic effect of hyperforin towards benzo(a)pyrene

The cell line was pre-treated with 0.15 μM hyperforin in medium and then the cells were cultivated in thermostat for 24 hours and cell line HepG2 was treated by 1.25 μM benzo(a)pyrene.

Before use, HF and B(a)P were disolved and diluted to the indicated concentrations in DMSO.

Processing of karyological preparations and their cytogenic analysis

We supplemented colchicin (Lachema, Prague, Czech Republic) into a medium containing the cell line HepG2 for 3 hours before processing the karyological preparations. For each sample, 100 metaphases were analysed. Breaks (chromatid and iso-chromatid) and exchanges (dicentrics, rings, tri-radials, quadri-radials) were analysed. The results were evaluated on the basis of a test difference between two relative values. The percentage of aberrations was compared with negative controls.

RESULTS

Potential genotoxic effect of hyperforin

The cell line HepG2 treated with 7.5 μM hyperforin showed approximately the same amount of aberrant metaphases and chromosomal aberrations in comparison with the *solvent* control. On the contrary, cells treated with 5 μM benzo(a)pyrene varied in the amount of aberrant metaphases and chromosomal aberrations in comparison with the *solvent* control. A significant statistical difference was registered when comparing the cytogenetic parameters with the *solvent* control (Fig. 3). Based on our results, we came to the conclusion that hyperforin did not have genotoxic effect to HepG2 in our experimental conditions.

Potential antimutagenic effect of hyperforin towards indirect mutagen benzo(a)pyrene

HepG2 cells were treated with 1.25 μM benzo(a)pyren after being pre-tretaed with 0.15 μM hyperforin. We found out statistically significant decrease of the monitored cytogenetic parameters (Fig. 4). Based on the experiments, we can conclude that hyperforin had an anticlastogenic effect towards the indirect mutagen benzo(a)pyrene.

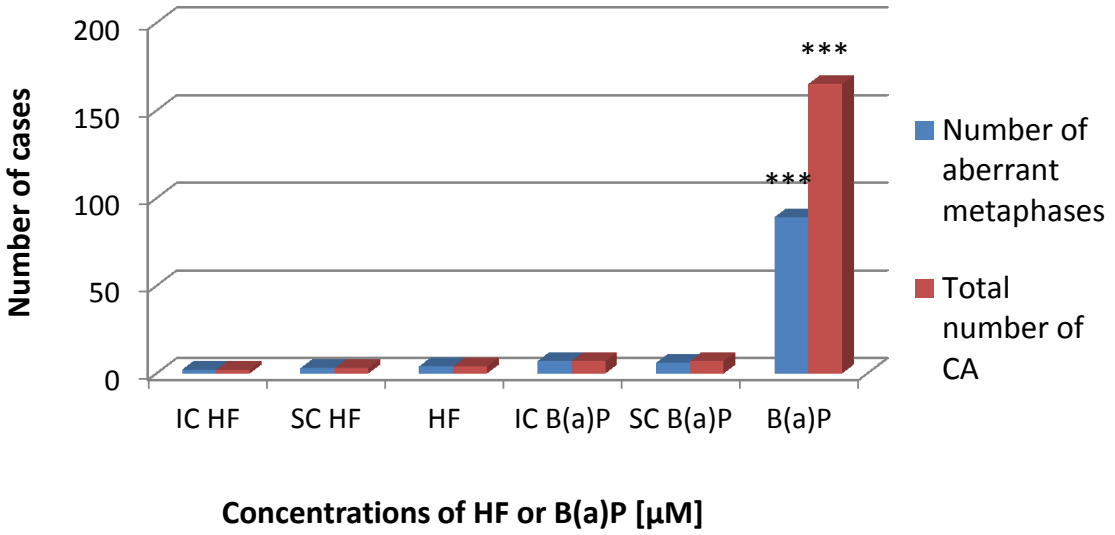

Figure 3. Treatment with 7.5 μM HF (hyperforin) or 5 μM B(a)P (benzo(a)pyrene)

Significant difference in comparison with the *solvent* control - SC is marked with stars: *** p<0,001.
CA - chromosomal aberrations; IC - *intact* control; SC - *solvent* control (DMSO)

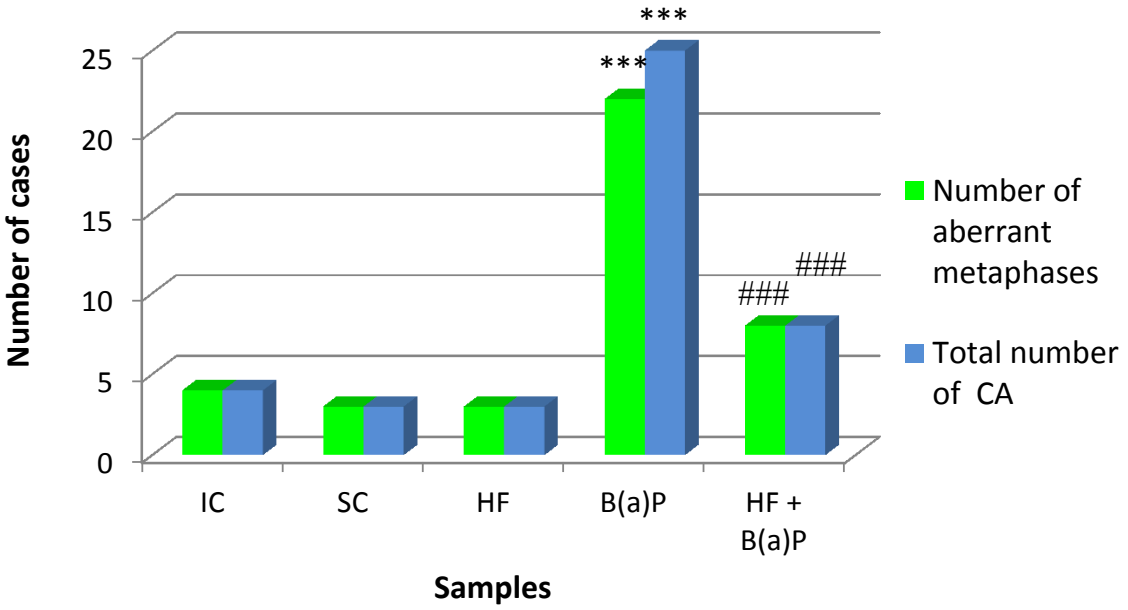

Figure 4. Treatment with 1.25 µM B(a)P (benzo(a)pyrene) after pre-treated with 0.15 µM HF (hyperforin)

Significant difference in comparison with the control is marked with stars: *** p<0,001. Significant decrease between sample treated with B(a)P and sample treated with B(a)P after pre-treated with HF is marked with crosshatch: ### p<0,001.
CA - chromosomal aberrations; IC - *intact* control; SC - *solvent* control (DMSO)

DISCUSSION

In our experiments, we proved that 7.5 µM hyperforin did not have a genotoxic effect on the human cell line HepG2 in our experimental conditions. It is very important to know whether hyperforin is genotoxic or is not genotoxic on human cell lines because it exhibits several important medicinal effects.

Moreover, we also observed an anticlastogenic activity towards the indirect mutagen benzo(a)pyrene. Mutagenicity of benzo(a)pyrene is caused by microsomal cytochrome P-450 enzymes such as: CYP1A1, CYP1A2, CYP1B1, CYP3A4, CYP2C9 which are responsible for a metabolic activation of benzo(a)pyrene on ultimate carcinogen benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide (10, 20, 21). We assume that an anticlastogenic effect of hyperforin towards benzo(a)pyrene was caused by the hyperforin inhibition of the activities of cytochrome P-450 enzymes CYP1A1 and CYP2C9 present in HepG2 cells (14, 17, 21, 22). Our work shows the influence of hyperforin when used simultaneously with medication, when it may have a negative impact on the treatment effect.

CONCLUSION

We observed that hyperforin did not have any genotoxic effect on the tumor human cell line HepG2. Hyperforin exhibited an anticlastogenic effect towards the widespread indirect mutagen benzo(a)pyrene. In future, we will continue to explore hyperforin using other cell lines and other chemical mutagens.

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