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Mechanisms of Hyperforin as an anti-angiogenic angioprevention agent

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ABSTRACT

Hyperforin, the major lipophilic compound contained in extracts of *Hypericum perforatum*, is responsible for the antidepressant activity associated with the extract. Recently, several other biological properties of Hyperforin have been unveiled including inhibition of tumour invasion and angiogenesis. The mechanism of the anti-angiogenic activity of Hyperforin remains to be fully elucidated. We show that treatment with non-cytotoxic concentrations of Hyperforin restrains, in a dose-dependent manner, the capacity of endothelial cells to migrate towards relevant chemotactic stimuli. Hyperforin inhibits the organisation of HUVE endothelial cells in capillary-like structures *in vitro*, and potently represses angiogenesis *in vivo* in the Matrigel sponge assay in response to diverse angiogenic agents. Immunofluorescent staining shows that in cytokine-activated endothelial HUVE cells Hyperforin prevents translocation to the nucleus of NF-κB, a transcription factor regulating numerous genes involved in cell growth, survival, angiogenesis and invasion. Under Hyperforin treatment *in vivo*, the growth of Kaposi's sarcoma – a highly angiogenic tumour – is strongly inhibited, with the resultant tumours remarkably reduced in size and in vascularisation as compared with controls. Hyperforin has also been reported to have anti-inflammatory properties. Here we show that Hyperforin inhibits neutrophil and monocyte chemotaxis *in vitro* and angiogenesis *in vivo* induced by angiogenic chemokines (CXCL8 or CCL2). These results highlight the potential for Hyperforin as an anti-inflammatory angioprevention agent, acting as a strong inhibitor of inflammation- or tumour-triggered angiogenesis, and provide new therapeutic approaches to halting pathology-associated angiogenesis.

1. Introduction

Extracts of St. John's wort (*Hypericum perforatum*, Guttiferae) have been in use since the time of Hippocrates for treating wounds, and was known to Paracelsus for the treatment of psychiatric disorders long before depression was recognised as a well-described pathology.[1,2] Today, controlled trials confirm the efficacy of this plant extract over placebo in the treatment of mild to moderately severe depression, through inhibition of neurotransmitter reuptake, such as dopamine,

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noradrenaline and gamma-aminobutyric acid.[3] Of the different classes of *H. perforatum* secondary metabolites, the prenylated acylphloroglucinol Hyperforin (Hyp) – isolated first by Bystrov in 1975[4] – has emerged as a key player in anti-depressant activity.[5]

In addition to its anti-depressive properties, Hyp has other beneficial effects, including anti-inflammatory activity through inhibition of the proliferation and induction of apoptosis of peripheral blood mononuclear cells (PBMC)[6]; blocking of 5-LOX and COX-1, i.e. two crucial enzymes in the biosynthesis of pro-inflammatory eicosanoids[7]; and significant relief of mild to moderate atopic dermatitis in topical treatment.[8] Hyp thus appears to act as an *in vivo* anti-inflammatory agent[9], though underlying mechanisms are still under debate. It has been demonstrated that Hyp inhibits the generation of reactive oxygen species (ROS) as well as the release of leukocyte elastase (degranulation) and Ca^{2+} mobilisation in human isolated polymorphonuclear (PMN) leucocytes.[10] Quiney et al. have shown that Hyp promotes apoptosis and capacity to secrete MMP-9 on neoplastic B lymphocytes,[11] while for T lymphocytes, Schempp et al. have demonstrated that Hyp suppresses the proliferation of PBMC in a dose-dependent manner.[12]

Further, Hyp is able to lower the proliferation rate of different mammalian cancer cell lines, such as squamous cell carcinoma, malignant melanoma, chronic myeloid leukaemia and lymphoma, by altering the balance between tumour cell proliferation and cell death rates.[13] Similar activities were found for a soluble derivative of Hyp, aristoforin.[14] Moreover, Hyp has been shown to effectively inhibit cancer growth and metastatic spreading without inducing adverse toxic effects.[15]

Recent studies show that Hyperforin and several synthetic derivatives also have anti-angiogenic properties[16–20]; however, the mechanisms of action of this anti-angiogenic action remain to be fully elucidated. Based on the anti-angiogenic and anti-inflammatory properties of Hyp, we hypothesised that Hyp could have direct actions on endothelial cells as well as the potential to inhibit inflammation-associated angiogenesis and innate immune cell activities.

In order to create new blood vessels, endothelial cells must migrate, differentiate into tubular structures and colonise the target tissue. Previous studies have found that Hyp is able to inhibit matrix metalloproteinase 9 (MMP-9),[11,19,20] a principally leucocyte-derived metalloprotinase involved in cellular invasion. We investigated the ability of Hyp as the stable and crystalline dicyclohexylammonium salt (Hyp-DCHA; Fig. 1),[21] a convenient storage form, to inhibit endothelial cell growth, migration and morphogenesis *in vitro* in the absence of apoptosis and to restrain angiogenic tumour growth *in vivo* in the Kaposi's sarcoma xenograft model.

It is now well recognised that inflammation-triggered angiogenesis plays a crucial role in cancer proliferation, growth and spread. Innate immune cells – tightly interplaying with endothelial cells – act as promoters of the angiogenic process, and their recruitment correlates with increased malignant phenotype of the tumours. Contrasting inflammation-triggered angiogenesis could thus be a crucial additional front of attack against the carcinogenesis process of solid tumours.[22,23] Here we show that Hyp inhibits migration of monocytes and PMNs *in vitro* and inflammation-triggered angiogenesis *in vivo*. Finally, we show that Hyp inhibits nuclear translocation of NF-κB, a central hub in both angiogenesis and inflammation. Our findings reveal that Hyperforin acts as a broad-spectrum anti-angiogenic compound and, given its anti-angiogenic anti-inflammatory properties and low toxicity, it may be an effective chemopreventive agent.

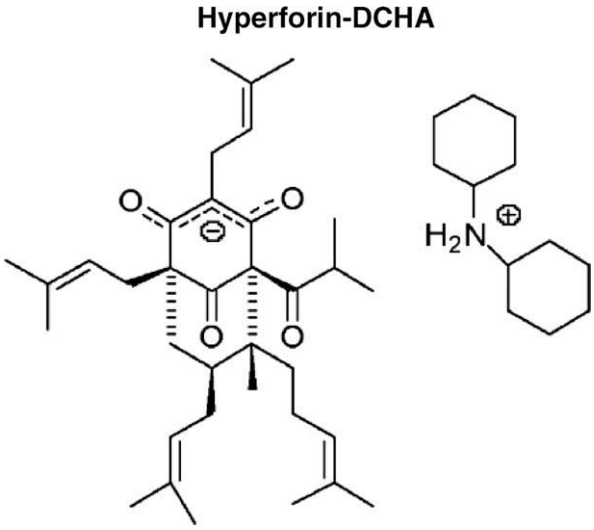

Fig. 1 – The chemical structure of Hyperforin-DCHA (dicyclohexylammonium) salt.

2. Materials and methods

2.1. Chemicals and cell cultures

The stable dicyclohexylammonium salt of Hyperforin (Hyp-DCHA, Fig. 1) was prepared according to the method described in patent PCT WO 99/41220 1999 Aug 19,[4] and solubilised 10 mM in DMSO, the latter also included in appropriate controls without Hyp-DCHA. Human recombinant VEGF-A, EGF, acid and basic FGFs, and TNF-alpha were obtained from Peprotech, heparin and hydrocortisone from Sigma, and Matrigel from the Engelbreth-Holm-Swarm sarcoma as previously described.[24] Monocytes and PMNs (essentially neutrophils) were freshly isolated (>96% pure) from human peripheral blood of healthy donors using standard Ficoll and Percoll gradients, and grown in 10% heat-inactivated foetal bovine serum (FBS), supplemented with 1% L-glutamine in RPMI medium (Sigma). Human umbilical vascular endothelial cells (HUVEC) were obtained from Interlab Cell Line Collection, IST Genoa, freshly isolated from umbilical veins and grown on 0.1% gelatine-coated tissue culture plates in M199 endothelial growth medium (Sigma), supplemented with 10% heat-inactivated FBS supplemented with 1% L-glutamine, FGF (1 μg acid-FGF plus 1 μg basic-FGF/100 ml), EGF (1 μg/100 ml), heparin (10 mg/100 ml) and hydrocortisone (0.1 mg/100 ml). In all *in vitro* experiments, cells were used between the 8th and 10th *in vitro* passages. Human Kaposi's sarcoma cells (KS-Imm) were derived from a spontaneously immortalised cell line from the iatrogenic form of KS,[25] and were routinely grown in 10% heat-inactivated FBS, supplemented with 1% L-glutamine in DMEM medium (Sigma).