Hypericin - The Facts About a Controversial Agent

A. Kubin1,2,*, F. Wierrani2,3, U. Burner2, G. Alth1,2, W. Grünberger2,3

1Ludwig Boltzmann Institute for Clinical Oncology and Photodynamic Therapy, Vienna, Austria; 2Vienna Society for Photodynamic Diagnosis and Photodynamic Therapy, Vienna, Austria and 3Department of Gynecology and Obstetrics, Hospital Rudolfstiftung, Vienna, Austria

Abstract: Hypericin is a naturally occurring substance found in the common St. Johns Wort (Hypericum perforatum) species and can also be synthetized from the anthraquinone derivative emodin. As the main component of Hypericum perforatum, it has traditionally been used throughout the history of folk medicine. In the last three decades, hypericin has also become the subject of intensive biochemical research and is proving to be a multifunctional agent in drug and medicinal applications. Recent studies report antidepressive, antineoplastic, antitumor and antiviral (human immunodeficiency and hepatitis C virus) activities of hypericin; intriguing information even if confirmation of data is incomplete and mechanisms of these activities still remain largely unexplained. In other contemporary studies, screening hypericin for inhibitory effects on various pharmaceutically important enzymes such as MAO (monoaminoxidase), PKC (protein kinase C), dopamine-beta-hydroxylase, reverse transcriptase, telomerase and CYP (cytochrome P450), has yielded results supporting therapeutic potential. Research of hypericin and its effect on GABA-activated (gamma amino butyric acid) currents and NMDA (N-methyl-D-aspartat) receptors also indicate the therapeutic potential of this substance whereby new insights in stroke research (apoplexy) are expected. Also in the relatively newly established fields of medical photochemistry and photobiology, intensive research reveals hypericin to be a promising novel therapeutic and diagnostic agent in treatment and detection of cancer (photodynamic activation of free radical production). Hypericin is not new to the research community, but it is achieving a new and promising status as an effective agent in medical diagnostic and therapeutic applications. New, although controversial data, over the recent years dictate further research, re-evaluation and discussion of this substance. Our up-to-date summary of hypericin, its activities and potentials, is aimed to contribute to this process.

Key Words: Fluorescence diagnosis, photodynamic therapy (PDT), photophysical diagnosis (PPD), antiviral, antiretroviral, photosensitizer.

INTRODUCTION

Hypericin is a plant derived substance, which historically has been used for medicinal application. Today, it is still of remarkable interest and is a main topic of discussion concerning its potential scope of clinical applications. Anti-depressive, antiviral, antiretroviral, antineoplastic, antitumor, photodynamic and photodiagnostic activities of hypericin are currently under investigation. However, when the focus is narrowed down to those activities which are generally accepted, specific medicinal and clinical applications become more restricted. Recent reviews ascribe the importance of hypericin to the treatment of cancer [1] and discuss the relevance of its chemical and physical properties to pharmaceutical application [2, 3].

The aim of this paper is to summarize the extensive amount of laboratory and clinical data in order to illuminate this controversially discussed active agent. Publications (recently reviewed: [4, 5]) about extracts of Hypericum perforatum (St. John’s Wort) are not considered in this study. We restrict our discussion to data which specifically concern hypericin with the exception of pharmacological investigations which include the uptake and elimination studies of orally administered extracts of St. John’s Wort.

CHEMISTRY OF HYPERICIN

The first detailed report of the isolation of hypericin from Hypericum perforatum was published in 1939 by Brockmann et al. [6]. This precedes the reference in the MERCK Index (11th and 12th Edition) in which a paper from 1942 is cited. The first correct chemical formula of hypericin was reported in 1942 by Brockmann et al. [7]: C₁₆H₁₂O₄ and 8 years later the correct structure was published by the same author [8].

In the literature, hypericin is occasionally referred to as a naphthodianthrone derivative and in chemical abstracts as 1, 3, 4, 6, 8, 13-Hexahydroxy-10, 11-dimethylphenanthro[1, 10, 9, 8-opqra]perylenne-7, 14-dione. Fig. (1) shows the structure of hypericin and the region of possible deprotonation at the bay-position. Principally, deprotonation is possible at the phenolic hydroxyl groups at the peri- and bay-positions [2] with different acidities. Hypericin is regarded as a vinylogous carboxylic acid. Its stabilized form following deprotonation of the bay-region with a pKa value of 1.6 shows an estimated pKa value of 1.8 in aqueous solutions [2, 9]. Deprotonation of a peri-hydroxyl group at pKa value of 9.4 with an estimated pKa value in aqueous solutions of 9.2 leads to destabilization due to alkaline conditions [2].

The chemical synthesis of hypericin follows the pattern of biogenesis. Emodin anthrone is the precursor of hypericin synthesis and is obtained either from reduction of emodin isolated from the bark of the breaking buckthorn (Cortex...
frangulae) [10] or by synthesizing emodin as first described by Brockmann [11]. A new high yield synthetic route to prepare emodin anthrone with commercially available orthocresotinic acid as precursor was developed by Falk [12]. A more practical overview on the synthesis of emodin and hypericin is compiled by Falk [2].

Oxidation of emodin to emodin bianthrone leads after further oxidation directly to hypericin (first described by Brockmann et al. [11, 13] then modified and improved by Banks et al. [14] and Cameron et al. [15]). Alternatively, it was found that oxidation of emodin bianthrone in concentrated aqueous ammonia results in protohypericin, which can be photochemically converted into hypericin [2, 14].

Hypericin is hydrophobic and insoluble in water, oil, methylene chloride and most other nonpolar solvents. It is soluble in alkaline aqueous solutions, organic bases such as pyridine, and polar organic substances including acetone, ethanol, methanol, ethyl acetate, ethyl methyl ketone and other solvents yielding red solutions with fluorescence emission maximums of about 600 nm (Fig. 2).

Hypericin is soluble in biological media, which is made possible by complex formation of hypericin with biological macromolecules [3] (e.g. DNA, human serum albumin and other plasma proteins, membrane fragments and cellular compounds.) These properties are used in fluorescence diagnostic tools in vivo and in clinical trials.

As a gold standard for purity of hypericin, the molar extinction coefficient is used for evaluation of quality. Recent investigations of hypericin showed that molar extinction coefficients at about 590 nm vary from 27.000 to 52.000 [16] depending on various factors including solvent, raw material, production process, aggregation, storage conditions, moisture etc. Upon addition of water to hypericin solutions, a dramatic change in absorption properties is observed [17]. This is due to the tendency of molecules in concentrated hypericin solutions to aggregate and slowly form insoluble pellets in a process that appears to be irreversible. These aggregates are nonfluorescent, and show visible absorption spectra in the same region as free hypericin, but have much lower extinction coefficients [18]. This may be a result of the formation of dimers found at

![Fig. (1). Structure of phenolate ion of hypericin upon deprotonation of the bay-region.](image1)

![Fig. (2).](image2) Fluorescence spectrum of hypericin (5 µmol/L) in methanol: excitation 287 nm, emission maximum 590 nm.
concentrations above $10^3$ mol/L suggesting that greater concentrations induce formation of high molecular weight aggregates. For this reason most of the product specifications of commercially available hypericin do not specify this critical value.

**ACTIVITY OF HYPERICIN ON ENZYMES**

Several pharmacological activities of hypericin including antidepressant, antiproliferative, and antiviral effects are ascribed to inhibition or induction of enzymes. One important family of enzymes which hypericin interacts with is protein kinase C (PKC).

The PKC family currently consists of several structurally distinct isoforms that have been separated into classic calcium dependent PKCs (α, β1, β2, γ) and PKCs, which possess activity in the absence of calcium. Measurements of enzyme activity of hypericin are affected by several factors including definition of assays and methods, enzyme isoforms, influence of temperature, proteins and above all presence of light and oxygen because of strong photocatalytic activity of hypericin.

Takahashi et al. [19] measured inhibition of PKC from rat brain and protein kinase A (PKA) from bovine heart by hypericin. Hypericin was found to inhibit PKC from rat brain with an IC50 value of 3.37 µmol/L whereas inhibitory activity on PKA was > 80 µmol/L (IC50). Antiproliferative activity of hypericin on mammalian cells (BALB 3T3/Her-ras [19]) was consistent with the toxicity of hypericin to human cells (MOLT-4 and HUT-78 [20]). Additionally, Takahashi et al. [19] observed corresponding effects of hypericin on PKC and mammalian cell lines and suggested that the cytotoxic activity of hypericin is due to inhibition of PKC. Table 1 shows inhibitory activity of hypericin against several enzymes as published. Due to the use of different units to describe enzyme activity, we calculated IC50 in µmol/L in order to be able to compare results.

The inhibition of PKC [21] and PTK (Protein tyrosine kinase) [22] by hypericin has also been demonstrated to be strongly light-dependent implying that enzyme activity values have to be verified under dark conditions if standard experiments were carried out under light conditions. Utsumi et al. [23] observed an IC50 of about 0.1 µmol/L for hypericin against PKC at a light intensity of 0.029 mW/cm² (for 3 min). Reducing light intensity to 0.005 mW/cm² required 1 µmol/L hypericin for IC50. The inhibitory effect of hypericin was increased by increasing the intensity of light. Only weak activity of hypericin against PKC was observed under dark conditions.

Bruns et al. [24] demonstrated experimentally light dependent inhibition of PKC by calphostin (a perylenequinone with structures and properties similar to hypericin). Photonsensitized inhibition of protein tyrosine kinase activities (PTK) of the epidermal growth factor receptor and the insulin receptor, and of Ser/Thr protein kinases (PK CK-2, PKC and mitogen-activated kinase: MAP-K) has been observed with nanomolar concentrations of hypericin [21]. Hypericin is largely inactive against cytosolic protein tyrosine kinases: Lyn, Fgr, TPK-IIIB and CSK. Furthermore, hypericin shows inhibitory activity against casein kinase – 1 and casein kinase - 2 in the presence of light and no activity in dark reactions [21, 22].

No effect of dark or ambient lightning conditions on the inhibition of PtdIns-3-kinase could be observed by Frew et al. [25]. Hypericin was found to be a general inhibitor of signaling kinases but with a greater than 5-fold selectivity for phosphatidylinositol-3-kinase (PtdIns-3-kinase) [25]. Enzyme activity of hypericin, however, could be activated by light by decreasing IC50 for inhibition of epidermal growth factor receptor (PTK) to 0.044 µmol/L. No activity of hypericin as an inhibitor of the growth factor signaling enzyme phosphatidylinositol specific phospholipase C (PtdInsPLC) could be found at concentrations of about 100 µmol/L [25].

Flow extracts of *Hypericum perforatum* efficiently inhibit binding of [1H]Nalazem (antagonist of benzodiazepine) to rat brain benzdiazepine binding sites of the GABAA-receptor in vitro. This effect is not caused by pure hypericin (IC50 > 1 µmol/L) but by amentoflavone (IC50 = 0.015 µmol/L), which occurs in high concentrations in *Hypericum* flowers [26].

Dopamine-β-hydroxylase (D-β-H) is inhibited by hypericin with an IC50 of 3.8 µmol/L [27]. Enzymatic activity and potential influence of hypericin was measured by HPLC quantification of dopamine as substrate and noradrenaline as product. One test system including polarographic determination of oxygen uptake with tyramine as a substrate analoge showed an IC50 of 21 µmol/L of hypericin against D-β-H [28].

Results of inhibitory activity of hypericin on reverse transcriptase (RT) are inconsistent. Hypericin interacts non-specifically with proteins such as albumin. In the presence of bovine serum albumin (BSA), inhibitory effects of hypericin on HIV-1 reverse transcriptase (HIV-1 RT) are much lower than in the absence of BSA [29]: hypericin IC50 against HIV-1 RT = 0.77 µmol/L, but in presence of 20 µg/ml BSA hypericin IC50 against HIV-1 RT = 10 µmol/L. This effect suggests that the enzyme inhibitory activity is dependent on the amount of exogenous protein present.

Mereulo et al. [20] observed suppression of RT activity (Friend leukemia virus, radiation leukemia virus) although hypericin did not inhibit reactions catalyzed by commercially purified RT from avian or murine viruses (possible influence of light (day light) and protein concentration in enzyme assay may not have been considered). Similar to the findings of Mereulo, Tang et al. [30] and Lavie et al. [31] could not inhibit purified RT directly.

Mitochondrial succinoxidase is inhibited by hypericin 6-fold greater under light irradiation than under dark conditions. The most effective wavelength is visible light of about 600 nm corresponding to the absorption maximum of hypericin of about 592 nm [32]. Thomas et al. [32] observed a positive relationship between succinoxidase inhibition and generation of singlet oxygen ($^{1}O_2$), suggesting that hypericin-induced photosensitized inhibition of succinoxidase occurred predominantly by a type II mechanism with ($^{1}O_2$) as the primary oxidant.
Table 1. Inhibitory Effect of Hypericin on Several Enzymes as Published

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Origin</th>
<th>IC 50 µmol/L</th>
<th>Source</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein kinases Ser/Thr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein kinase C (PKC)</td>
<td>Rat brain</td>
<td>3.37</td>
<td>[19]</td>
<td></td>
</tr>
<tr>
<td>Protein kinase C (PKC)</td>
<td></td>
<td>0.027</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Protein kinase C (PKC)</td>
<td>Rat brain</td>
<td>0.1</td>
<td>[23]</td>
<td>+ light</td>
</tr>
<tr>
<td>Protein kinase C (PKC)</td>
<td>Rat brain</td>
<td>NA</td>
<td>[23]</td>
<td>dark</td>
</tr>
<tr>
<td>Protein kinase A (PKA)</td>
<td>Rat brain</td>
<td>&gt; 3.4</td>
<td>[25]</td>
<td></td>
</tr>
<tr>
<td>Protein kinase A (PKA)</td>
<td>Bovine heart</td>
<td>&gt; 80</td>
<td>[19]</td>
<td></td>
</tr>
<tr>
<td>Protein kinase A (PKA)</td>
<td>Bovine</td>
<td>10</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Cdc2 kinase</td>
<td>Xenopus oocytes</td>
<td>&gt; 20</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Casein kinase – 1 (CK-1)</td>
<td>Porcine spleen</td>
<td>3.0</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Casein kinase – 1 (CK-1)</td>
<td>NA</td>
<td></td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>Casein kinase – 2 (CK-2)</td>
<td>Recombinant</td>
<td>0.006</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Casein kinase – 2 (CK-2)</td>
<td>NA</td>
<td></td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>Mitogen-activated protein kinase (MAP-K)</td>
<td>Xenopus oocytes</td>
<td>0.004</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Protein tyrosine kinases (PTKs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermal growth factor – Receptor (EGF-R)</td>
<td>Mouse</td>
<td>0.4 – 8.7</td>
<td>[22]</td>
<td>**</td>
</tr>
<tr>
<td>Epidermal growth factor – Receptor (EGF-R)</td>
<td>Mouse</td>
<td>0.044</td>
<td>[22]</td>
<td>+ light</td>
</tr>
<tr>
<td>Epidermal growth factor – Receptor (EGF-R)</td>
<td>Bovine</td>
<td>0.035</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Insulin-Receptor (Ins-R)</td>
<td>Bovine</td>
<td>0.029</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Cytosolic PTKs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lyn</td>
<td>Rat spleen</td>
<td>4.0</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>Fgr</td>
<td>Rat spleen</td>
<td>&gt;20</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>PTK-IIB</td>
<td>Rat spleen</td>
<td>&gt;20</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>e-Scr kinase (CSK)</td>
<td>Recombinant</td>
<td>&gt;20</td>
<td>[21]</td>
<td>+ light</td>
</tr>
<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myosin light chain kinase (MLCK)</td>
<td>Chicken gizzard</td>
<td>&gt; 40</td>
<td>[19]</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylinositol-3-kinase (PtdIns-3- kinase)</td>
<td>Bovine brain</td>
<td>0.18</td>
<td>[25]</td>
<td>ambient light</td>
</tr>
<tr>
<td>Phosphatidylinositol-3-kinase (PtdIns-3- kinase)</td>
<td>Bovine brain</td>
<td>0.18</td>
<td>[25]</td>
<td>dark</td>
</tr>
<tr>
<td>Dopamin-ß-Hydroxylase</td>
<td></td>
<td>3.8</td>
<td>[27]</td>
<td></td>
</tr>
<tr>
<td>Dopamin-ß-Hydroxylase</td>
<td></td>
<td>21</td>
<td>[28]</td>
<td></td>
</tr>
<tr>
<td>DNA polymerase α</td>
<td>PBMC</td>
<td>14.7</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>HIV-1 reverse transcriptase</td>
<td>Recombinant</td>
<td>0.77</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>Reverse transcriptase (RT)</td>
<td>Recombinant</td>
<td>??</td>
<td>[29]</td>
<td>[20,30,31]</td>
</tr>
<tr>
<td>Monoaminooxidase A (MAO A)</td>
<td>Rat brain</td>
<td>68</td>
<td>[36]</td>
<td></td>
</tr>
<tr>
<td>Monoaminooxidase B (MAO B)</td>
<td>Rat brain</td>
<td>420</td>
<td>[36]</td>
<td></td>
</tr>
<tr>
<td>MAO A, MAO B</td>
<td>Rat brain</td>
<td>&gt;&gt;10</td>
<td>[33]</td>
<td></td>
</tr>
</tbody>
</table>
Inhibition of MAO is one of the key mechanisms used in conventional therapy for depression. Unlike the crude extract of *Hypericum perforatum*, hypericin lacks significant inhibitory activity of MAO<sub>A</sub> or MAO<sub>B</sub> *in vivo* [33-35]. The IC<sub>50</sub> values published by Suzuki *et al.* [36] of 68 µmol/L for MAO<sub>A</sub> and 420 µmol/L for MAO<sub>B</sub> are not relevant for effective pharmaceutical applications. Hypericin clearly shows affinity for the glutamate receptor N-methyl-D-aspartate (NMDA) [33]: see Table (2).

The “dumbbell” disintegration reaction is a screen for testing inhibitors of integrase (i.e. HIV-virus encoded enzyme which is important for integration). Hypericin was found to be active with an IC<sub>50</sub> of 10-15 µmol/L [37]. Hypericin also displays inhibitory activity against subviral preintegration complexes (PICs) of about 100 µmol/L.

The effect of hypericin on various CNS receptors was studied by radioligand binding techniques in order to determine a profile of pharmacological activity *in vivo* [39]. Binding to opioid receptor subtypes µ, κ, and δ was inhibited by hypericin in the micromolar range whereas binding to serotonine receptors 5-HT was inhibited by hypericin approximately three times less potently [39]. Strong inhibition activity by hypericin against corticotropin releasing factor (CRF) receptors with urocortin as the standard ligand was measured at an IC<sub>50</sub> of about 0.3 µmol/L.

Light dependent inhibitory effects of hypericin against different neurotransmitter receptors were investigated by Gobbi *et al.* [40]. Unfortunately, dark reaction experimental conditions were not standardized. Neuropeptide Y (NPY), Y<sub>1</sub> and Y<sub>2</sub> receptors showed much less affinity to their labeled ligands PYY in presence of hypericin and light than under dark conditions (similar to the results SIGMA receptor tests).

*In vivo* inhibition of the antioxidant enzymes glutathione reductase, selenium-dependent glutathione peroxidase, glutathione S-transferase, and superoxide dismutase was investigated by Johnson *et al.* [38]. Glutathione reductase was the most sensitive with an IC<sub>50</sub> of about 2 µmol/L both in the dark reaction and with light irradiation suggesting that inhibition of glutathione reductase activity by hypericin is not light dependent. Additionally, hypericin shows less inhibitory activity against the other antioxidants tested (µmol/L ranges); under dark conditions both glutathione peroxidase and glutathione S-transferase showed even lower IC<sub>50</sub> values of hypericin.

Recently hypericin has been suspected to adversely affect the metabolism of various co-administered drugs via inhibition of several species of cytochrome P450 (CYP). In an investigation with several St. John’s Wort constituents and recombinant heterologously expressed human CYP enzymes, hypericin demonstrated 50 % competitive inhibition at concentrations below 10 µmol/L for CYP2D6, CYP3A4, and CYP2C9 activities [41]. Budzinski *et al.* [42] measured inhibitory effects of hypericin against CYP3A4 of IC<sub>50</sub> of 330 µmol/L using 7-benzylxyoresorufin as the substrate.

Steroid X and xenobiotic receptor SXR (an orphan nuclear receptor) induces hepatic CYP3A gene expression in response to diverse endogenous steroids, xenobiotics and drugs. Unlike St. John’s Wort extract and isolated hyperforin, hypericin does not activate SXR and thus does not induce expression of the SXR target gene, CYP3A [43].
In spite of inhibitory activity of hypericin against CYP3A4, activation of CYP3A gene expression would lead to antagonistic action of hypericin against drugs which are metabolized as CYP3A4 substrates [44]. These effects could lead to a reduction of plasma drug levels since CYP3A4 metabolizes roughly 50% of drugs used today. Clinical investigations are currently being conducted with St. John’s Wort (e.g. plasma level reduction of indinavir after St. John’s Wort intake [45]), whereas studies testing pure hypericin are still lacking.
Drug interactions are known to cause either inhibition of cytochrome P450 enzymes or induction of gene expression for synthesis of cytochromes. The former leads to an increase of the concentration or efficacy of the administered drugs and the latter to a decrease.

Apparently, hypericin is involved in both mechanisms making an accurate forecast of pharmacodynamic interactions and risk of adverse reactions difficult. Numerous studies headed by Ruschitzka et al. [46] report that St John’s Wort extracts endanger the success of organ transplantation due to decreasing immunosuppressant cyclosporin. Due to low effects on gene expression for CYP3A biosynthesis [43] of hypericin, drug interactions could possibly be excluded by pure hypericin. Further studies of the effects of pure hypericin are necessary to clarify this issue.

CELL DEATH INDUCED BY HYPERICIN: APOPTOSIS OR NECROSIS

In vivo responses to PDT are influenced by a number of factors: the photosensitizer used and its concentration in the tissue, irradiation protocol including light source, wavelength and energy intake, type of tumor, its level of oxygenation and local pH-value and the subcellular localization of hypericin [1, 47].

Today, it is believed that the mode of cell death by PDT is often apoptosis at low sensitizer concentrations and low light fluency (J/cm²; the radiant energy per unit area), whereas necrosis occurs in assays using high sensitizer concentrations and higher light fluency [47, 48]. Further initiation of apoptosis by photosensitization depends on subcellular localization of the sensitizer [49] (i.e., an apoptotic response can be obtained when lysosomes or mitochondria are targeted for light induced damage).

Apoptosis has been observed in HeLa cell cultures in response to 80 – 250 nmol/L hypericin activated by light (4 J/cm²) whereas increasing the hypericin concentration to 1 µmol/L led to necrosis after irradiation [47].

Ali et al. [48, 49] observed in various cell lines a largely apoptotic cellular response to hypericin concentrations of 0.313-1.25 µmol/L upon photoactivation. Hypericin concentrations of 2.5 - 5 µmol/ml in cell culture media led to necrosis after light irradiation. Variations of light doses provoke a similar shift from apoptosis to necrosis. It is evident that in a photodynamic process apoptosis is the basal cell response followed by necrosis after light and sensitizer dosages are increased.

Rapid collapse of mitochondrial transmembrane potential is induced by hypericin [54]. Lysosomal and mitochondrial apoptotic pathways are well defined: Cathepsin D is a lysosomal aspartic protease, which is released in increased amounts in radiation-induced apoptosis. Cathepsin D activity is also increased in cytosol of irradiated cells exposed to hypericin [49]. Cathepsin D activates caspase-3, which stimulates additional apoptotic mechanisms. The release of enzymes from lysosomes into cytosol interacts with mitochondrial membrane causing the release of cytochrome c into the cytosol and inducing apoptosis of cells by activating caspase-8, caspase-9 and caspase-3 sequentially [52, 55]. Cytosolic cytochrome c acts as a cofactor in the formation of a complex with Apaf-1 (apoptotic protease activating factor 1), procaspase-9 and apoptosome dATP/ATP leading to the activation of caspase-9. Caspase-9 leads to the activation of

Table 4. Qualitative Activity of Photoactivated Hypericin in Apoptosis Pathway

<table>
<thead>
<tr>
<th>Apoptotic response photoactivated by hypericin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrinking in cell size</td>
<td>Yes</td>
</tr>
<tr>
<td>Chromatin condensation</td>
<td></td>
</tr>
<tr>
<td>Fas/FasL involvement</td>
<td>Yes</td>
</tr>
<tr>
<td>Caspase activation</td>
<td>Yes</td>
</tr>
<tr>
<td>Cytochrome c release</td>
<td>Yes</td>
</tr>
<tr>
<td>Cathepsin D release</td>
<td>Yes</td>
</tr>
<tr>
<td>Increase of p53 expression</td>
<td>Yes</td>
</tr>
<tr>
<td>PS externalization</td>
<td></td>
</tr>
<tr>
<td>Bcl-2 involvement</td>
<td>Yes</td>
</tr>
<tr>
<td>DNA laddering/fragmentation</td>
<td>Yes</td>
</tr>
<tr>
<td>Appearance of a sub-diploid peak</td>
<td>Yes</td>
</tr>
<tr>
<td>Human tumor cells</td>
<td>Yes</td>
</tr>
<tr>
<td>Dark reaction</td>
<td>No</td>
</tr>
</tbody>
</table>
executioner caspases (-3, -6, -7), which catalyze cleavage of vital proteins [1].

The mitochondrial pathway involves pro-apoptotic members of the Bcl-2 family, which are partly under the control of the tumor suppressor gene p53. A hypericin dose-dependent expression of p53 gene could be observed by Ali et al. [49].

Tumor cell death induced by photoactivated hypericin is also mediated by caspase proteases [50]. In presence of caspase inhibitors apoptotic signals do not occur. Apoptotic signals may be triggered not only by hypericin acting as a photosensitizer but also possibly by its ability to inhibit protein kinase C (PKC) [56]. Controversial findings by Jarvis et al. [57] reported that DNA fragmentation by hypericin (0.1 – 100 µmol/L) in HL-60 promyelocytic leukemia cells could not be observed.

Ali et al. [53] reported that the formation of H₂O₂ and other reactive oxygen species (ROS) via photoactivation of hypericin is associated with cytochrome c release and activation of caspase-3 with a subsequent drop in mitochondrial membrane potential. Today it is well established that many inducers of apoptosis converge on the activation of caspase-3 proteases, which then trigger the cascade of apoptosis. Caspase-3 activity is determined by western blotting cleavage products of the caspase-3 specific substrate poly(ADP-ribose)polymerase (PARP).

Scavenging of H₂O₂ production causes cells to fail to undergo apoptosis [53]. H₂O₂ scavengers such as the reductant glutathione (GSH), N-acetylcysteine (NAC), catalase and other antioxidants might inhibit apoptotic cell death triggered by photoactivated hypericin.

In conclusion, apoptotic signals are switched on by hypericin and light via mitochondrial and lysosomal pathways. These pathways can be triggered by reactive oxygen species as H₂O₂ which in turn, can be prevented by radical scavengers such as GSH, NAC, catalase and superoxide dismutase (SOD). It has been suggested that nitric oxide (NO), Fas/FasL apoptotic pathway and the role of Bcl-2 family might also be involved in apoptosis triggered by hypericin [48].

PHARMACOLOGY

Pharmacokinetic and pharmacodynamic studies in humans using pure hypericin (0.05 mg per kg body weight (group 1: n = 12)) orally once a day showed an elimination half-life of 33.8 ± 18.8 h, similar to subjects receiving 0.1 mg hypericin per kg body weight: 36.1 ± 22.6 h (group 2: n = 7) [58]. Bioavailability was approximately 20 %. Steady state was reached at two weeks. No discernible drug accumulation from daily dosing could be verified. No metabolism of hypericin could be observed corresponding to the mean area under the curve (AUC) determinations of 1.5 (group 1) and 3.1 (group 2) µg/ml × hr [58]. The time to reach maximal drug concentration was 4.4 ± 2.5 h. AUC and mean values of the maximal drug concentration (Cₘₐₓ: 28.1 ± 11.8 and 79.2 ± 30.4 ng/ml for the doses of 0.05 and 0.1 mg/kg), the minimal drug concentration (Cₘᵢₜ: 15.4 ± 5.3 and 41.7 ± 15.9 ng/ml for the doses of 0.05 and 0.1 mg/kg) and steady state levels in serum showed a dose dependent relationship.

Similar results were obtained in a single dose and steady state study in which plant-derived extract preparations of hypericin were orally administered to thirteen volunteers [59]. Estimated amounts of 250, 750 and 1500 µg hypericin resulted in median peak levels in plasma (Cₘₐₓ) of 1.3, 7.2, and 16.6 µg/L. AUC determinations, elimination half-life time (41.3 h) and steady state kinetics were roughly comparable to the results in the above mentioned phase I dose escalation study. Interestingly, the Cₘₐₓ and the AUCs for the lowest dose were disproportionally lower than those for the higher doses, which was also confirmed by other authors [60]. Daily intake of total hypericin (Hypericum perforatum extracts of 1 – 10 mg) over several weeks of conventional herbal drug therapy resulted in maximal plasma concentrations of up to 100 µg/L (equivalent to 0.2 µmol/L) [60]. The terminal elimination half-life of hypericin increased significantly with higher doses. And comparable to other studies, the time to reach maximal drug concentration in plasma was 4 - 5 h. Kinetic parameters after intravenous administration of hypericin (115 µg) corresponded to those estimated after an oral dosage. Systemic availability (14 %) was low.

Comparable pharmacokinetic results concerning AUC determinations and elimination half-life times were demonstrated in another study with Hypericum extract LI 160 on twelve healthy volunteers. Single doses of dried St. John’s Wort extract (300, 900 or 1800 mg containing 250, 750 or 1500 µg hypericin) were administered orally and led to maximal plasma levels of 1.5, 4.1, and 14.2 µg/L hypericin. During long-term dosing (3 x 300 mg/day), a hypericin steady state was reached after 4 days and mean maximal plasma level during the steady state treatment was 8.5 µg/L [61].

A phase I study of hypericin as an antiretroviral drug in HIV-infected adults was designed with multiple high doses of pure hypericin applied intravenously (iv.) [62]:

- group 1: 0.25 mg/kg body weight, iv., twice weekly 8 – 24 weeks (n = 11)
- group 2: 0.50 mg/kg body weight, iv., twice weekly 8 – 24 weeks (n = 11)
- group 3: 0.25 mg/kg body weight, iv., three times weekly 8 – 24 weeks (n = 11)
- group 4: 0.50 mg/kg body weight, orally, per day 8 – 24 weeks (n = 11)

Almost all patients experienced phototoxicity of grade 2 or higher (ACTG toxicity scale; grade 2: erythema, numbness, pain, temperature sensitivity causing tolerable discomfort and requiring non-narcotic analgesia; grade 3: intolerable erythema, numbness, pain, temperature sensitivity, etc.).

Probands of group 4 received hypericin orally.

Hypericin is resorbed in the intestinal compartment without being metabolized. Based on its chemical structure and molecular size (> 500 Da), it is presumed to be excreted in the bile [59, 60]. It is not detectable in urine, even after incubation of urine with glucuronidase and sulfatase [59].
Prolonged uptake lag time for hypericin may be due to absorption at distal enteral sites, which could lead to a broad variation of absorption triggered by pharmacokinetic variability such as food interaction after oral intake. Lag times are also consistent with hypericin uptake experiments with Caco-2 cells (model of intestinal mucosa, Caco-2 model) [63], which demonstrated a significant cellular accumulation (4-8 %). Absorption by passive transcellular diffusion is characterized by saturation of Caco-2 cells after 3 hours [64]. Furthermore, hypericin shows a high non-specific affinity to proteins [3], detergents and lipids [65].

Kinetic data available from mice show a distribution half-life for hypericin of 2.0 h and an elimination half-life of 38.5 h [66]. These data are surprisingly similar to human studies. Murine lungs show a five-fold higher uptake than the spleen followed by liver, blood, kidneys, heart, gut, various xenografted tumors, stomach, skin, muscle and brain [67].

Hypericin was also administered as an intravenous bolus dose of 2 mg/kg (n = 3) or 5 mg/kg (n = 1) [68] in non-human primates to study plasma pharmacokinetics and cerebrospinal fluid penetration. Bi-exponential elimination of hypericin from plasma was demonstrated with an average terminal half-life of 26 ± 14 h. Hypericin was not detected in the cerebrospinal fluid (CSF) of any animal (lower limit of detection 0.1 µmol/L) and CSF penetration was less than 1 %.

Conventional therapy with St. John’s Wort extract for depression does not normally cause phototoxic side effects [69]. Single doses of 2 – 11 mg total hypericin do not result in increased sensitivity to UVA irradiation [60]. Both the minimal erythema dose and minimal tanning dose decreased about 20 % during multiple dosing of 5.62 mg total hypericin per day. Multiple dose administrations of hypericin (approximately 0.2 mg per kg body weight) effective against viral-, bacterial-, or enzymatic-caused diseases lead to unacceptable photosensitivity, pain and temperature sensitive side effects. Gulick et al. [62] defined 0.25 mg/kg as the maximum tolerated dose for bi-weekly intravenous administration.

**ANTIVIRAL ACTIVITY**

Viral diseases join cancer as one of the most serious medical and social problems of mankind. Although the virucidal and antiviral activities of hypericin and its mode of action have been widely studied in the past two decades, the results of *in vivo* and *in vitro* activity remain controversial. Likewise, the physico-chemical mechanisms of the biological activity of hypericin at the cellular level are still unclear. To offer an overview we have summarized the qualitative results of several studies in Table 5.

Reports rapidly established 2 important facts which will be discussed in more detail: (I) hypericin inactivates a wide variety of lipid containing (i.e. enveloped) viruses and is inactive against viruses lacking membranes, and (II) the reported virucidal or antiviral potency strongly depends on the *in vivo* and *in vitro* experimental protocol.

For example, in several early studies no consideration was given to the role of light [20, 29-31, 70-72]. It is not clear whether any of the experiments described in these reports used defined doses of light, ambient light, or if they were conducted in the dark. All demonstrated some degree of virus inactivation, but not however, without apparent discrepancies between several of the results. It is conceivable that these discrepancies can be explained by the failure to control for light exposure in the various hypericin-virus reactions [73-76].

Light is known to be required for some bioactivities of the photodynamic dye hypericin. In the presence of visible light, hypericin produces singlet oxygen and other ROS which can damage membranes [77-79]. Ambient light conditions as well as most of the fluorescent lamps found in virology laboratories and in biosafety cabinets could conceivably generate sufficient energy of the appropriate wavelength to photosensitize hypericin and consequently generate ROS.

Several studies have, indeed, been published with the aim to investigate the influence of light on the virucidal activity of hypericin. Unfortunately clarification of the issue has not been satisfactorily achieved. Carpenter et al. [74], Lenard et al. [80], and Hudson et al. [76] reported that the virucidal activity of hypericin is entirely dependent on the presence of light, and light is an absolute requirement for antiviral activity. Hudson discussed the dependence on light in more detail. He published that under conditions in which hypericin caused substantial inactivation of HIV-1 (human immunodeficiency virus type 1), there was a strict requirement for visible light. Only when the concentration of hypericin approached cytotoxic levels was there also a light-indepenedent antiviral effect [76]. These findings contrast somewhat with reports that the virucidal effect on viruses with membranes as well the intracellular antiviral effect is enhanced by but not dependent on the presence of light. In the absence of light activities are diminished but remain significant [73, 75]. In summary, however, it can be stated that light increases the virucidal potency of hypericin by at least 100-fold [73-86].

As discussed above, hypericin activated by light generates ROS. Consequently, the role of oxygen in the activity of hypericin was investigated. Early studies by the group of Petrich et al. observed that oxygen is not required for the virucidal activity of hypericin [82, 87]. Later, they re-examined the importance of oxygen and published that there was a significant reduction of light-induced antiviral activity of hypericin under hypoxic conditions (>100 fold) although hypericin retained measurable virucidal activity [88]. These findings are in accordance with data published by the group of Meruelo who demonstrated that in the presence of radical quenchers such as sodium azide, β-carotene, or crocetin, the virucidal effect of hypericin was significantly reduced [89, 90].

Several speculations on these possible reactions have been published. Redepenning et al. [91] reported electrochemical results that account for a strong oxidizing potential of hypericin in the excited state. Redepenning also hypothesized possible mechanisms involving superoxide radical anion and hypericinium ion thereby implicating type I mechanisms. Recently the group of Petrich et al. [92] suggested another oxygen-independent alternative origin of the photoinduced virucidal activity of hypericin which may be related to its ability to acidify its environment by a
photogenerated pH-drop [92-98]. This proposed mechanism of an excited-state proton-transfer has also been supported by the group of Miskovsky et al. [99-101].

Apart from the photoinduced more global bioactivities of hypericin, a series of specific interactions of hypericin with virions and/or their replication cycle has been proposed. The compound hypericin appears to inactivate free virions and interfere with steps in the replication cycle. Meruelo et al. for example, proposed a mechanism by which hypericin prevents viral budding and shedding from cells by interfering with the proper assembly and maturation of viral cores of budding particles [20, 31]. Tang et al. have found that it is efficient in inactivating viruses endowed with lipid coats, while it is ineffective against non-enveloped viruses suggesting that inactivation might depend on the presence of a viral lipid membrane [30]. This would agree with conclusions derived from studies with cells [77] as well as studies about the effect of hypericin on viral fusion. Lenard et al. have shown that the fusion activity of several viruses is inactivated by hypericin in an absolutely light dependent process [80]. In this context it is interesting to refer to fluorescent microscopic observations where it has been shown that hypericin can be incorporated in the phospholipid bilayers of the cell plasma membranes [31, 102]. Loss of the fusion function might be one main reason that enveloped but not non-enveloped viruses are inactivated [103].

Photochemically induced alterations (cross-linkings) of viral membrane proteins have also been shown to be promoted by hypericin [80, 84, 104]. Degar et al. published an increased density of retrovirions as well as altered protein patterns after hypericin treatment of cells [85]. The group of Meruelo also proposed that inactivation of HIV-1 is characterized by photochemical alteration of the HIV major capsid protein p24 and the p24-containing gag precursor and is accompanied by an inability to release RT-activity (reverse transcriptase-activity) from the treated virions [84, 85]. They published that incubation of retroviral-infected cells with hypericin drastically reduced detectable RT-activity of mature virions (in accordance with [74, 80]), but it had no effect on total viral mRNA. They concluded that suppression of RT-activity was not a direct result of hypericin activity on the enzyme [20, 30, 31]. In contrast, Schinazi et al. found that hypericin could inactivate HIV-1 replication in cultured cells and could inhibit HIV-1 RT-activity in vitro at submicromolar concentrations ([29], Table 1).

Studies indicate that serum is important for the activity of hypericin. It has been shown that the efficacy of virucidal activity can be significantly decreased by the presence of serum, apparently due to serum components binding to the compound. Inhibitory concentrations are published to be > 0.1 % v/v, [76, 83, 105]. However, in contrast to these results, Fehr et al. reported that 1-10 % v/v, serum provided comparable results [82]. These effects of serum are significant to prospective applications in antiviral therapy in vivo where hypericin may encounter interfering and/or promoting molecules similar to those found in serum [106].

In vitro experiments have shown that hypericin possesses either (I) virucidal activity by inhibiting viral infectivity in a hypericin-preincubation and light-dependent inactivation reaction and/or (II) antiviral activity by inhibiting viral replication in cell cultures.

In vitro virucidal activity has been shown against a variety of enveloped viruses including HIV-1 [31, 73, 75, 76, 80, 84, 89, 90], HSV-1 (herpes simplex virus type 1) [30, 72, 83, 107], HSV-2 (herpes simplex virus type 2) [72], BVDV (bovine viral diarrhea virus) [90], influenza A (influenza virus type A) [30, 80, 83], Para-3 (parainfluenza virus type 3) [72], RadLV (radiation leukemia virus) [31, 85, 89], Mo-MaLV (Moloney murine leukemia virus) [30], VV (Vaccinia virus) [72], FLV (Friend leukemia virus) [23, 81], VSV (vesicular stomatitis virus) [72, 80], MCMV (murine cytomegalovirus) [73, 75], Sendai virus [80], SV (Sindbis virus) [73, 75, 105], EIAV (equine infectious anemia virus) [74, 82, 86, 88, 108], DHBV (duck hepatitis B virus) [104], BIV (bovine immunodeficiency virus) [109], and HCMV (human cytomegalovirus) [71].

Inhibition of virus replication in cell cultures by an antiviral reaction has been published for HIV-1 [29, 31], HSV-1 [72], HCMV [71], MCMV [75], RadLV [20, 31], VSV [72], and EIAV [70].

However, the ability of hypericin to act as antiviral drug in vitro and to inhibit virus replication without preincubation of cell-free virus with hypericin has been a controversial topic. Tang et al. [30] for example, have not found any antiviral activity against HSV-1 (in contrast to [72]) and have shown that hypericin has no antiviral potency against several viruses for which virucidal activity has been described [30].

In contrast, non-enveloped viruses such as adenovirus-2 (adenovirus type 2) [30], poliovirus-1 (poliovirus type 1) [30] and HRV-2 (human rhinovirus type 2) [72] are resistant to hypericin virucidal and antiviral activity under in vitro conditions in which membrane-containing viruses are readily inactivated [30].

In vivo tests in mice have shown that hypericin was effective against RadLV [20], LP-BM5 (murine leukemia virus) [31], FLV [20, 23, 30, 31, 81] and HSV-1 [30]. Even though, the potency of hypericin as an antiviral drug under these in vivo conditions has also been a topic of controversy. Only the group of Meruelo and Lavie in the late eighties found that hypericin markedly suppressed the spread of murine retroviral infections in vivo [20, 31]. All the other investigative groups reported that hypericin is only active as a virucidal drug in mice when preincubated with the virus and illuminated. Tang et al. [30] for example found that mice susceptible to and infected with FLV or HSV-1 are not sensitive to hypericin suggesting that in vivo hypericin is only active if preincubated with virus and ineffective if not [30]. In contrast to Meruelo and Lavie [20, 31] who reported an antiviral effect with a single dose of hypericin for mice exposed to the compound some hours prior to or after viral inoculation, Tang et al. could not demonstrate any in vivo therapeutic effect for hypericin when administered in a single dose [30]. Also, contrary to the early enthusiastic reports, Stevenson et al. [81] could find no protection of mice from FLV-induced splenomegaly even with 100 μg hypericin per mouse under several conditions of administration (mixed with inoculum, 1 day p.i. (past infection), 30 days p.i. (post infection),
light or dark). The inoculation dose of FLV was readily inactivated, however, by prior illumination in the presence of hypericin in a direct virucidal reaction [23, 81].

Summing up these reports on the in vivo efficacy of hypericin on enveloped viruses in mice, illumination of hypericin with the virus seems to be an absolute requirement for hypericin antiviral (virucidal) effects, thereby unequivocally limiting its potential usefulness as an antiretroviral drug [30, 81]. Unfortunately, these preliminary data from studies in animal models have also been confirmed by in vivo studies in humans.

Although several early studies on hypericin as an antiretroviral drug against HIV have reported immunological and clinical benefits for HIV-infected persons [110-115], recent phase I dose escalation studies could not confirm any potential usefulness as an antiretroviral drug [58, 62]. The general practitioner Dr. Steinbeck-Klose presented a long-term (40 months) treatment of 18 HIV-infected persons in 1993 [114]. A subsequent report from her in a German journal described a case study of one female patient, who consumed Hypericum extract orally at dosages of 0.028 mg/kg/day for 30 months and 0.056 mg/kg/day for another 10 months (dosages based on hypericin content of plant extract). Her study reported no detectable HIV RNA after this 40-months treatment [111] and surprisingly no side effects [111, 114]. In contrast, subsequent phase I dose escalation studies with hypericin have observed remarkable phototoxic reactions with comparable [58] or higher dosages of hypericin [62].

Recently, BVDV was found to be completely inactivated by hypericin in vivo in the presence of light [90]. Since BVDV is a pestivirus that has structural similarities to the hepatitis C virus (HCV), a phase I dose escalation study was conducted to determine the safety and antiviral activity of hypericin in vivo in 19 patients with chronic HCV infection [58]. Neither a significant change was observed in the plasma level of HCV RNA, nor an improvement in the elevated serum liver enzyme levels in any of the probands treated with an 8-week course of hypericin (either 0.05 or 0.10 mg/kg/day orally). In addition, 11 patients developed considerable phototoxic reactions [58]. Similar results have also been published in the most recent phase I dose escalation study of hypericin as an antiretroviral agent in HIV-infected adults [62]. 30 HIV-infected patients were administered hypericin intravenously with doses of either 0.25 mg/kg or 0.50 mg/kg for up to 24 weeks two or three times weekly or orally 0.50 mg/kg daily. Sixteen of 30 patients discontinued treatment early because of phototoxic effects. Nine of the 13 remaining patients continued the 8-week course, and only 2 patients completed 24 weeks of therapy. Severe cutaneous phototoxicity was observed in 11 of 23 evaluable patients; dose escalation could not be completed. Viral markers such as HIV p24 antigen level, HIV titer and HIV RNA copies did not change significantly although CD4 cell counts decreased during the study in the patients tested. In accordance with the study [58], hypericin caused significant phototoxicity and had no antiretroviral activity in the limited number of patients in this in vivo phase I study [62]. Summing up the human in vivo studies on HCV and HIV-1, hypericin did not show any evidence of detectable significant anti(retro)viral activity in patients with chronic infections.

Since illumination of hypericin with the virus seems to be absolutely required for hypericin antiviral (virucidal) effects, any possible therapeutic potential of hypericin as an antiretroviral drug in humans is likely to be limited by its need to be activated by light. Thus the absence of light in many regions of the body may preclude the use of hypericin as a therapeutic compound for the treatment of viral infections in vivo. To overcome this limiting factor, the group of Petrich et al. discussed the implication of exploiting chemiluminescence as a 'molecular flashlight' for effecting photodynamic therapy against virus cells and tumor cells [108]. They reported a strategy to place luciferin and luciferase in the proximity of hypericin as a chemiluminescent light source so that photodynamic therapy could be extended to all regions of the body. The chemiluminescent light-generating system was however, not as effective in activating hypericin as was illumination from a continuous source (1000 times more effective), suggesting that optimal activation depends on the local concentration of energy donors [108]. Furthermore, since the luciferin / luciferase system consumes oxygen and may lead to localized hypoxic conditions, depletion of oxygen may be a limiting factor in this system [88]. Recently, this group published the synthesis of a covalently linked derivative of hypericin, a tethered pseudohypericin-luciferin-compound [92, 116], which should ensure the proximity of the light source to the antiviral agent. They have shown that the relevant photophysical parameters of this tethered molecule in bulk solvent and in micelles are very similar to the parent molecule and it maintains antiviral activity [116]. However, both pseudo-hypericin and the tethered molecule were about 1000 times less effective than illuminated hypericin in reducing virus infectivity in vitro [116].

Another potential application of hypericin as a virucidal agent in human medicine, namely the ex vivo treatment of blood components, was discussed by the group of Meruelo [89, 90]. They reported that hypericin is a potent virucidal agent against HIV-1 and BVDV, which acts as a model for HCV. They showed complete inactivation of 10^6 tissue culture-infective doses of HIV-1 in whole blood and in diluted packed red cells upon illumination of 20 µg/ml and 50 µg/ml hypericin [89]. BVDV was even more sensitive to inactivation by hypericin than HIV [90]. The same group also investigated the effects of photosensitization on hemopoietic cell lines carrying quiescent integrated HIV-1 provirus (a model used for evaluating virus inactivation in latently infected cells; an absolute requirement for transfusible blood products). Phorbol ester-induced virus production by these cells was effectively prevented by photosensitization with hypericin at conditions similar to those found to be effective in inactivating cell-free HIV-1 and BVDV [90]. Although photodynamically induced virus inactivation appears promising in preventing transmissions of enveloped virus infections in transfusible blood products, the major conflicting consideration in photodynamic virus inactivation in blood is virucidal efficacy, which is improved by higher doses of photosensitization versus toxicity to red blood cells by irradiation [90].
Table 5a – b. Anti(retro)viral and Virucidal Activities of Hypericin

Table 5a

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cultures</th>
<th>System</th>
<th>Light</th>
<th>Origin</th>
<th>Antiviral*</th>
<th>Virucidal*</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus-2</td>
<td>HeLa cells</td>
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<td>[30]</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>EREP cells</td>
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<td>[109]</td>
<td>+</td>
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<tr>
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<td>RD420 cells</td>
<td>in vitro  + light</td>
<td>[90]</td>
<td>+</td>
<td>human red cells</td>
<td></td>
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</tr>
<tr>
<td>DHBV</td>
<td>D2 cells</td>
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<td>[104]</td>
<td>+/-</td>
<td>light dependence</td>
<td></td>
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<tr>
<td>EIAV</td>
<td>ED cells</td>
<td>in vitro</td>
<td>[70]</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>ED cells</td>
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<td>[74]</td>
<td>+</td>
<td>light dependence</td>
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<td></td>
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<tr>
<td>EIAV</td>
<td>ED cells</td>
<td>in vitro  + light</td>
<td>[108]</td>
<td>+/-</td>
<td>chemiluminescent light source luciferin / luciferase</td>
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<tr>
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<td>ED cells</td>
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<td>[82]</td>
<td>+</td>
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<td>ED cells</td>
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<td>[88]</td>
<td>+</td>
<td>oxygen and light dependence</td>
<td></td>
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<tr>
<td>EIAV</td>
<td>ED cells</td>
<td>in vitro  + light</td>
<td>[86]</td>
<td>+ light/dark</td>
<td>light dependence</td>
<td></td>
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<tr>
<td>FLV</td>
<td>BALB/c mouse</td>
<td>in vivo mouse</td>
<td>[20]</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>BALB/c mouse</td>
<td>in vivo mouse</td>
<td>[31]</td>
<td>+</td>
<td></td>
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<tr>
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<td>-</td>
<td>- 4°C/+ 37°C</td>
<td>temperature dependence</td>
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<td>in vivo mouse</td>
<td>[81]</td>
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<td>+</td>
<td>light dependence</td>
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<tr>
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<td>+</td>
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<td>HCMV</td>
<td>Hs-68 and MRC-5 cells</td>
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<td>[71]</td>
<td>+</td>
<td>+</td>
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<td>[58]</td>
<td>-</td>
<td>in vivo human phase I</td>
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<td>CEM cells</td>
<td>in vitro  + light</td>
<td>[75]</td>
<td>+ light/dark</td>
<td>light dependence</td>
<td></td>
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<td>[31]</td>
<td>+</td>
<td></td>
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<td>[29]</td>
<td>+</td>
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<tr>
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<td>CEM cells</td>
<td>in vitro  + light</td>
<td>[73]</td>
<td>+ light/dark</td>
<td>light dependence</td>
<td></td>
<td></td>
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<tr>
<td>HIV-1</td>
<td>MT-4 and ACH-2 cells</td>
<td>in vitro  + light</td>
<td>[80]</td>
<td>+</td>
<td></td>
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<tr>
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<td>in vitro  + light</td>
<td>[76]</td>
<td>+</td>
<td>cytotoxicity and serum influence</td>
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<td>[89]</td>
<td>+</td>
<td>human blood and human red cells</td>
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<td>HIV-1</td>
<td>CEM cells</td>
<td>in vitro  + light</td>
<td>[90]</td>
<td>+</td>
<td>human blood and human red cells, HSA (human serum albumin) complex, dependence on excited oxygen-species quenchers</td>
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<td>HRV-2</td>
<td>HeLa cells</td>
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<td>[72]</td>
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### Table 5b

<table>
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<th>Virus</th>
<th>Cultures</th>
<th>System</th>
<th>Light</th>
<th>Origin</th>
<th>Antiviral*</th>
<th>Virucidal†</th>
<th>Note</th>
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<tr>
<td>HSV-1</td>
<td>BSC-1 cells</td>
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<td>HSV-1</td>
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<td>[72]</td>
<td>+</td>
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<td>[107]</td>
<td>+ light/dark</td>
<td>light dependence</td>
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<td>[83]</td>
<td>+</td>
<td>dependence on serum</td>
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<tr>
<td>Influenza A</td>
<td>MDCK cells</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<td>[80]</td>
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<td>hemolysis-test for viral fusion</td>
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<td>+ light</td>
<td>[83]</td>
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<td>dependence on serum</td>
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<td>[73]</td>
<td>+ light/dark</td>
<td>light dependence</td>
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<td>in vitro</td>
<td>[20]</td>
<td>+</td>
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<tr>
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<td>[31]</td>
<td>+</td>
<td></td>
<td>+</td>
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<td>[80]</td>
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<tr>
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<td>MEF cells</td>
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<td>+ light</td>
<td>[75]</td>
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</tr>
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<td>SV</td>
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<td>in vitro</td>
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<td>[73]</td>
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<td>light dependence</td>
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<tr>
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<td>+ light</td>
<td>[105]</td>
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<td>[72]</td>
<td>+</td>
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</tr>
</tbody>
</table>

1 anti(retro)viral activity by inhibiting viral replication
2 virucidal activity by inhibiting viral infectivity
* as concluded by corresponding authors

In addition to direct photoinduced damage to blood components (red cells in particular), the lack of specificity of photodynamic agents has been a constant problem in attempts to use them for disinfecting blood fractions [117]. Presumably for that reason plans to illuminate the blood of HIV patients after administration of a photodynamic dye have not been followed up on (i.e. in preliminary observations, activation of psoralen after extra-corporeal illumination of blood has been reported [118]).

While hypericin may be a suitable agent for ex vivo photodynamic inactivation of enveloped viruses in blood and blood products (where phototoxicity is not an issue and the enhancement of hypericin antiviral activity by light can be used), it is still unclear how this compound could be therapeutically useful as an anti(retro)viral drug in the systemic treatment of humans.
ANTITUMOR ACTIVITY

Numerous investigations have shown that hypericin is toxic to cells from normal mammalian tissues and tumors upon irradiation with visible or UV light. It appears, however, that studies of antiproliferative, antineoplastic, antibiotic and tumoricidal effects on most in vitro cell cultures refer solely to hypericin in the photoactivated state. Photocytotoxic activity must be preceded by preincubation of hypericin for several hours at concentrations of approximately 0.1 – 1 µmol/L and higher in a light and concentration dependent manner. Kamuthabwa et al. [119] recently reported on photodestruction of AY-27 rat bladder carcinoma cells by hypericin. Incubation of the cells with 0.8 – 1.6 µmol/l hypericin and treatment with light resulted in a cytotoxic and antiproliferative effect dependent on the fluence of light. No cytotoxic effects were observed when the cells were shielded from light or when irradiated in the absence of hypericin. Delaey et al. [120] investigated the cytotoxicity and antiproliferative photoeffect of hypericin on human cervix carcinoma, skin carcinoma, and breast adenocarcinoma cell lines incubated with hypericin. In dark conditions, no cytotoxic or antiproliferative effect was observed in spite of subcellular uptake of hypericin into the endoplasmatic reticulum, Golgi apparatus and other membranous regions of the cells. Former investigations found 100 % survival rates of both normal epithelial cell lines and neoplastic cell lines at hypericin concentrations of 0.2 µmol/L in the absence of light. Ninety percent survival rates could be observed in the same model at 2 µmol/L hypericin [121]. Dark toxicity of hypericin also did not occur after treatment of tumor cells (human colon, bladder and nasopharyngeal carcinoma cells) and normal fibroblasts with hypericin at 20 µmol/ml as reported by Ali et al. [48].

It is not surprizing that the absence of dark toxicity of hypericin on cell cultures has been compared by numerous authors to its weak effect on enzymes, receptors and viruses under similar dark conditions. However, in some investigations hypericin has indeed shown toxic activities in the dark on mammalian and human tumor cell lines. In 1989, Takahashi et al. [19] reported cytotoxic properties of hypericin corresponding to its inhibitory activities on PKC (see also table 1). Antiproliferative activity of hypericin on mammalian cells was examined revealing a growth inhibition of BALB 3T3/H-ras cells (H-ras transfected mouse BALB 3T3 cells) at an IC50 value of 12.5 µmol/L. This result is similar to the hypericin toxicity on MOLT-4 and HUT-78 human cells at cell culture concentrations of about 20 µmol/L (Meruelo et al. [20]). In contrast, RadLV- and FLV-infected lymphoblastoid mouse cell lines were uneffected by hypericin doses below 50 µmol/L even after a prolonged exposure time of 9 days in the dark.

At hypericin concentrations of 2 - 40 µmol/L, Vander Werf [122] observed only weak dark toxicity on pulmonary human squamous cell carcinoma (UCLA-P3) and human fibrosarcoma cells (TE671), whereas colon adenocarcinoma cells (HT29), oral squamous cell carcinoma (CAL33), and breast adenocarcinoma cells (-231) showed no suppressed effects.

Bank et al. [123] countered with their report on cytoidal effects of hypericin in a light and concentration dependent manner and on cytostatic activities of hypericin on several cell types in the dark. Cytostatic (tumorcidal) efficacy was demonstrated by in vitro growth inhibition of highly metastatic murine breast adenocarcinoma and squamous cell carcinoma (SQ2 SCC) tumor cells. It was also shown that hypericin reduced the size of these tumors in mice and prolonged animal survival in the complete absence of light. Apoptosis could not be detected but DNA synthesis was strongly suppressed. In vivo inhibition of tumor growth in the dark by hypericin led to increased longevity by a few percentage points but did not cause total remission of highly invasive breast adenocarcinoma in mice.

In addition to these findings, growth inhibition combined with induction of apoptosis has been observed in pituitary adenoma cell lines [124] and in glioma cell lines [125]. In vitro glioma cell invasion was inhibited but cell attachment and proliferation were not [126]. Hypericin also enhanced the radiosensitivity of glioma cells [127].

In other studies, no toxicity was observed in three human melanoma cell lines (one pigmented cell line (G361) and two amelanotic cell lines (M18 and M6)) with culture medium concentrations of hypericin of 0 to 1 µmol/L [128]. The authors concluded that toxicity is strongly light dependent without any response in the dark. Thomas et al. [102] could not show any dark toxicity of hypericin against EMT6 mouse mammary carcinoma cells even at the highest hypericin concentrations tested (50 µmol/L). No dark activities of hypericin against adenocarcinoma cells [129], human fibroblast cells [130], epidermoid carcinoma cells (as squamous cell carcinoma) [131] could be shown.

HYPERICIN IN PHOTODYNAMIC THERAPY (PDT) AND PHOTOPHYSICAL DIAGNOSIS (PPD)

Photodynamic therapy (PDT) and photophysical diagnosis (PPD) are innovative and attractive methods for the treatment and detection of small and superficial tumors [132]. Most prominent in the postwar history of PDT are the porphyrin substances and three main groups of investigators: Schwartz et al. [133], Lipson et al. [134] and Dougherty [135, 136]. During the past three decades, a wide variety of substances, including porphyrins, chlorins, purpurins phthalocyanines, etio-purpurins, lutetium tetraphyrin and many others have been proposed as potential candidates for PDT. Of all the above investigated sensitizers, porphyrins and their derivatives and the remarkable porphyrin precursor 5-aminolevulinic acid (5-ALA) have obtained regulatory approval for clinical PDT.

PDT requires a photosensitizer, a light source and oxygen. The sensitizer should be administrable locally or systemically and should accumulate preferentially in tumor tissue. After an incubation time of several hours, the region of interest is irradiated by laser light or a broadband light source. Upon light absorption, the excited sensitizer generates singlet oxygen (type II mechanism) and reactive oxygen species such as superoxide radical anions, hydroxyl radicals and peroxides (type I mechanism). The radicals are cytotoxic and react with cell constituents and are the initial point of apoptosis. The standard for photodynamic activity is the “quantum yield” $(\Phi_x)$, which specifies the amount (Mol) of generated radicals or energy transitions per Mol absorbed.
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photos. Quantum yield depends on reaction media, temperature, pH-value and various other physical and chemical influences. Hypericin bound to liposomes shows a singlet oxygen quantum yield of: $\Phi_1 = 0.4$ [137]. Jardon and coworkers estimated a singlet oxygen quantum yield of hypericin in ethanol of $\Phi_1 = 0.35$ and in water of less than 0.02 [138]. However, energy transfer of excited hypericin results not only in generation of radicals but also in heat and photon emission (fluorescence). Fluorescence of sensitizers accumulated in tumors is one of the most promising aspects of PDT and PDD. Particularly photodiagnostics in bladder cancer is becoming a reliably successful and safe method for detection of small tumors of the urothelium [139-142].

Recent investigations have shown that photodynamic therapy with hypericin successfully inhibits tumor growth in various mouse tumor models. This occurs via apoptosis and necrosis resulting in vascular damage [130, 143, 144]. Hypothetically, hyperthermia could potentiate the antitumor effect of hypericin-mediated photodynamic therapy [145]. However, clinical applications of hypericin to investigate its photodynamic and phototherapeutic potentials are rare.

In small-scale clinical investigations, 100 – 500 $\mu$g hypericin / cm$^2$ tumor have been injected intratesionally into basal cell carcinomas and squamous cell carcinomas [146, 147]. Photodynamic therapy was performed with visible light at a total dose of 200 J/cm$^2$. Clinical remissions (total and partial) were observed 6 – 8 weeks after therapy. Hypericin administered topically as a gel did not show any photodynamic effect [147].

One case report of a malignant mesothelioma of the tunica vaginalis testis dexter (74 year old patient) reported a combination therapy of interstitial HDP (hematoporphyrin derivative) and superficially applied hypericin [148]. Light irradiation had no effect on the HPD-photosensitized area but there was tumor destruction in the field with both administered sensitizers.

Promising results have been published on hypericin and tumor diagnostic applications. In one study, 40 patients with bladder cancer underwent instillation of 40 ml hypericin solution (80 $\mu$mol/L) into the bladder over a period of 2 hours. Fluorescence detection of the cancer cells was carried out using a conventional endoscopic light system with an excitation wavelength of 380 – 450 nm and a wavelength pass-filter for fluorescent light greater than 520 nm [140]. Localization of small tumor lesions was potentiated by the red/orange fluorescence of hypericin which was enriched in the tumor tissue. The sensitivity for detecting carcinoma in situ was 93 % and the specificity was 98.5 %. This confirmed that hypericin accumulates specifically in superficial urothelial lesions thereby fulfilling one of the most important prerequisites, which make hypericin interesting for photodynamic therapy and fluorescence diagnostic tools.

In a larger investigation of 87 patients, the sensitivity and specificity for detecting carcinoma in situ was 94 % and 95 % [149]. Due to formation of nonfluorescent aggregates of hypericin in water, investigators used 1 % plasma protein solution to ensure monomolecular distribution of hypericin [140]. Other investigators used Polyvinylpyrrolidone (PVP) to dissolve hypericin for instillation in the bladder [139, 150, 151]. Pytel et al. [139] recommended the use of hypericin for fluorescent cytological purposes because of its high specificity and photostability. Olivo et al. [142] suggested a new concept of combining confocal endomicroscopy and 3-dimensional fluorescence imaging of bladder epithelium following instillation of hypericin to obtain a high diagnostic accuracy of almost 100 % sensitivity and specificity.

In another study, fluorescence diagnosis of human gastric tumors was performed on 21 patients using extract of Hypericum perforatum, which was administered orally at a total hypericin dosage of 5 – 7 mg [152, 153]. A helium cadmium laser (442 nm) was used for excitation. Fluorescence of hypericin enriched tissue ranged from 510 – 725 nm with maximum of 603 nm. Specificity was 85 %, which encourages further investigations of laser-induced fluorescence with hypericin in detection of early stage malignant lesions.

CONCLUSION

Hypericin has several attributes, which makes it particularly attractive for investigating its clinical use: it possesses minimal dark toxicity, is not metabolized and is a photodynamic active molecule with marked fluorescence emission in orange/red regions. Hypericin accumulates in superficially located small tumors, which can be detected by fluorescence diagnostic tools. Due to high sensitivity and specificity of hypericin at low concentrations in human hollow organs such as bladder and stomach, hypericin is a tumor selective agent. Further investigation may support higher concentrations of hypericin for administration in hollow organs for photodynamic tumor therapy. Ideal properties for photodiagnostic and photodynamic applications of hypericin are light absorption by many wavelengths, photostability, rapid clearance from normal tissue and/or slow uptake in normal cells resulting in high tumor selectivity. Studies of bio-distribution using confocal laser microscopy show penetration and accumulation of hypericin in cytoplasm and endoplasmatic reticulum of human tumor cell lines [150, 151]. Subcellular distribution of hypericin in several tumor cell lines has been determined by a number of studies [32, 49, 102, 154]. Hypericin locates preferentially in the cytoplasm and after long-term incubation also in the nucleus [102, 154]. Ali et al. [49] supposed localization of hypericin in mitochondria and Golgi apparatus.

A dramatic difference in the enzyme inhibitory activity of hypericin can be shown using different assays and the presence or absence of light and proteins. It was not possible to evaluate all the cited methods determining enzyme activity, but screening of published data revealed two contrary species of inhibitory effects of hypericin. Inhibitory activity was shown to be activated by copious amounts of light and was also found under specific dark conditions in which only a few substances (PKC, PtdIns-3-kinase, CYP2C9, and CYP 3A4) affected. However, the inhibition of these enzymes by hypericin has also been demonstrated to be strongly light-dependent indicating that if standard experiments were carried out under day light conditions, then enzyme activity values must be verified under dark conditions.

Inhibition of dopamine-$\beta$-hydroxylase (D-$\beta$-H) could possibly show beneficial effects on patients suffering from mental depression. Upon measurement of enzymatic activity
and potential influence of hypericin via HPLC quantification of dopamine as the substrate and noradrenaline as the product, hypericin showed an IC50 of 3.8 µmol/L [27]. Using a test system including polarographic determination of oxygen uptake with tyramine as a substrate analog, the IC50 of hypericin was 21 µmol/L against D-β-H [28]. Influence of daylight during enzyme reaction was not considered. To verify these results, oxygen consumption and light dependence of hypericin-inhibited enzyme reactions have to be observed apart from the photodynamic activities on the test system.

Hypericin lacks significant inhibition of MAOΑ or MAOβ [33, 35, 36, 155]. Inhibitory activity at concentrations of IC50 = 100 µmol/L against MAOΑ [36] could not be reached in organism after oral or intravenous administration without severe phototoxic side effects. Plasma concentrations during conventional oral therapy with St. John’s Wort extract did not exceed 0.2 µmol/L maximum [60]. Furthermore, a plateau of 0.015 µmol/L mean plasma concentration of hypericin was measured during multiple dose Hypericum treatment. Hence, the weak and non-selective inhibitory effect of hypericin against MAO (> 100 µmol/L) is not the vital mechanism of the antidepressive effects of St. John’s Wort. The high IC50 for hypericin to inhibit MAO is irrelevant for pharmaceutical applications.

With respect to the IC50 values obtained for hypericin to some CYPs, it is important to note that results depend on substrate used, origin of CYP, quality of hypericin, influence of oxygen and light and performance of assays. In vitro IC50 value determination can be used to assist in estimation of possible drug-drug interactions but do not replace clinical testing. Implications of CYP inhibition are evident; products shown to inhibit CYP could be used as drug-sparing agents when taken concomitantly with conventional medicines in order to decrease the dosage, adverse side effects and costs of expensive drug regimes. Based on the published inhibitory activity of hypericin on CYP [41, 42, 156], the following examples of conventional drugs could be affected: CYP2C9: warfarin, diclofenac, ibuprofen, tamoxifen; CYP2D6: tricyclic antidepressants, codeine, propafen; CYP3A4: (approx. 50 % of all commercially available drugs) cyclosporin, erythromycin, verapamil, lovastatin, nifedipine, lidocaine, and several others.

Intake of total hypericin per day during conventional herbal drug therapy (over several weeks) by Hypericum perforatum extracts is 1 – 10 mg resulting in plasma concentrations up to a maximum of 100 µg/L [60]. 100 µg/L are equivalent to 0.2 µmol/L. Pharmacokinetic studies in humans using pure hypericin or preparations of St. John’s Wort extract led to similar results: oral administration showed an elimination half-life of 34 – 42 h, the bioavailability was about 20 % and the time to reach the maximal drug concentration was 4 – 5 h. These concentrations are the reference point for evaluation of possible drug and enzyme interactions by hypericin.

Therefore, it does not seem reasonable that hypericin could be present at physiologically relevant concentrations in plasma during conventional therapy with St. John’s Wort extract and simultaneously cause antagonistic or inhibitory side effects.

Furthermore, a number of clinically significant interactions (decrease in concentration or efficacy of prescribed medicines) were observed when Hypericum extracts were co-medicated with i.e. phenprocoumon, cyclosporin, HIV protease inhibitors, theophylline, digoxin and oral contraceptives. Hypericin may play a role in interacting with CYP liver enzymes but needs to be confirmed both in vivo and in vitro under dark conditions [157].

Regarding hypericin in association with anxiety, depressive illness and ethanol consumption, hypericin affinities to neurotransmitter receptors might be of interest. However, inhibition of GABA, serotonin, NPY and SIGMA receptors have been observed in vitro at relatively high hypericin concentrations and were also light dependent [40]. Hypericin shows a stronger affinity to opioid and CRF receptors [39].

Reviewing the often published and non-acceptable data concerning hypericin antiviral and antiretroviral activity, inconsistent results are most likely the product of differing and inaccurate designs of tests and assays. Hudson et al. who initially discussed the very important role of reaction parameters in antiviral assays stated that virucidal effects are not influenced significantly by temperature provided that light exposures are controlled [105]. In contrast, Tang et al. [30] reported a temperature–dependence for the reaction in vitro, which would not be expected on the basis of the photodynamic behavior of hypericin in other biological systems. However, because the experiments were not controlled for light conditions, effects ascribed to temperature might in fact be due to the presence or absence of light exposure.

Reactions have also been reported to be strongly affected by the order of incubation of the components: virus, hypericin, serum, and light. If virus and hypericin are preincubated, even in the dark, then subsequent addition of serum cannot prevent the virucidal effect. On the other hand, preincubation of hypericin with serum completely prevents the virucidal activity toward subsequent addition of virus [105].

Consequently, detailed understanding of the interaction of hypericin with cellular components such as membranes, proteins and nucleic acids is of fundamental biological importance. Hypericin is known to complex with macromolecules such as DNA and HSA in particular. Specific interactions of hypericin with DNA and its model compounds were studied by resonance Raman and surface enhanced Raman spectroscopy [158-161]. Interestingly, although hypericin has been investigated extensively in the early 1990s for its antiviral and antiretroviral activity, a mechanism of activity based on direct interaction of the compound with DNA or RNA was not proposed until 1995 [158]. The interaction with DNA is structure dependent and has been determined as very weak [158-161].

Hypericin also binds to HSA, a major transport protein in the blood plasma [3, 89, 162, 163] as well as LDL and HDL [89]. Hypericin was found to retain its virucidal activity in association with HSA [164] but varies with the molar ratio between hypericin and HSA [90]. Interestingly, the interaction between hypericin and HSA has been shown to impede excited state proton transfer and thus also a pH-drop.
Hypericin is bound to the IIA subdomain of HSA, BSA and RSA. This interaction is mainly hydrophobic. Hypericin forms a hydrogen bond between N-H group of Trp and C=O group of hypericin in IIA subdomain which impede excited state proton transfer in hypericin [162, and summarized in 3]. The virucidal activity of lipoprotein-bound hypericin has been shown to diminish beyond concentrations of 2 to 5 µg/ml [89]. Furthermore, hypericin is negatively charged and forms organic and inorganic monobasic salts (ion pairs) in physiological pH. Various ion pairs have been shown to differ in their virucidal activity [89]. Hypericin-lysine, for example, was shown to fail to inactivate HIV-1 in vitro [89].

To date, the equivocal results published demonstrate that the physico-chemical mechanisms of the virucidal and antiviral activity of hypericin at the cellular level still remain unclear. Because the molecular targets of hypericin are not yet well defined, we can do little more than speculate at this time. Further studies will be needed in order to elucidate the mechanisms of action.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Austrian Ministry of Science, Research and Culture; a research grant from the Austrian Ministry of Health and Consumers Care; and by the Vienna Society for Photodynamic Therapy and Photophysical Diagnosis.

ABBREVIATIONS

ACH-2 Cells = T-lymphoblastoid, chronically infected T-Cell line with HIV provirus
Adenovirus-2 = Adenovirus type 2
Apaf-1 = Apoptotic protease activating factor 1
BIV = Bovine immunodeficiency virus
BSA = Bovine serum albumin
BVDV = Bovine viral diarrhea virus
CEM Cells = CD4+ human T-cell line
CRF = Corticotropin releasing factor
CYP = Cytochrome P450
DHBV = Duck hepatitis B virus
D-ß-H = Dopamine-ß-hydroxylase
ED Cells = Equine dural cells
EIAV = Equine infectious anemia virus
EREp Cells = Embryonic rabbit epithelial cell line
FLV = Friend leukemia virus
GABA = Gamma amino butyric acid
HCMV = Human cytomegalovirus
HCV = Hepatitis C virus
HDL = High density lipoprotein
HeLa Cells = Human epithelial cervical carcinoma cells
HIV-1 = Human immunodeficiency virus type 1
HIV-1 RT = HIV-1 reverse transcriptase
HRV-2 = Human rhinovirus type 2
Hs-68 = Human foreskin fibroblasts
HSA = Human serum albumin
HSV-1 = Herpes simplex virus type 1
HSV-2 = Herpes simplex virus type 2
Influenza A = Influenza virus type A
LDL = Low density lipoprotein
LP-BM5 = Murine leukemia virus
MAO = Monoaminoxidase
MAP-K = Mitogen-activated kinase
MCMV = Murine cytomegalovirus
MEF = Mouse embryo fibroblasts
Mo-MuLV = Moloney murine leukemia virus
MRC-5 = Human lung fibroblasts
NMDA = N-methyl-D-aspartat
NPY = Neuropeptide Y
p.i. = Past infection
Para-3 = Parainfluenza virus type 3
PARP = Poly(ADP-ribose)/polymerase
PBMC = Human peripheral blood mononuclear cells
PtdIns-3-kinase = Phosphatidylinositol-3-kinase
PICs = Preintegration complexes
PKA = Protein kinase A
PKC = Protein kinase C
Poliovirus-1 = Poliovirus type 1
PtdInsPLC = Phosphatidylinositol phospholipase C
PTK = Protein tyrosine kinases
RadLV = Radiation leukemia virus
RL-12 Cells = Murine T-cell line
ROS = Reactive oxygen species
RT = Reverse transcriptase
SV = Sindbis virus
U1 Cells = Monocytoid, chronically infected cell line with HIV provirus
VSV = Vesicular stomatitis virus
VV = Vaccinia virus

REFERENCES

References 165-167 are related articles recently published in Current Pharmaceutical Design.

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