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The Anticancer Activity of Hypericin in Photodynamic Therapy

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Abstract

Photodynamic therapy (PDT) is a cancer treatment that requires the interaction of a photosensitizer (PS), light and oxygen. A PS is characterised as a non-toxic drug or dye which is excited using light from a laser source at a specific wavelength. The excited PS will react with oxygen present in biological tissue to produce reactive oxygen species (ROS) that destroys cancerous cells by inducing cell death. The PS uptake by cancerous tissue in combination with localised light delivery makes PDT an effective oncology treatment that prevents damage to surrounding normal healthy tissue. To date, most photosensitizers (PSs) for PDT have been chemically synthesized and modified to satisfy the demands for an ideal PS. However, a naturally occurring red plant pigment (Hypericin) has drawn increased interest recently as a new generation PDT drug. It is known to have high quantum yields, tumor selectivity and low production costs. Other beneficial properties of hypericin include low photobleaching, low cytotoxicity in the absence of light and no mutagenicity. Several *in vitro* and *in vivo* studies have established its anticancer potential upon irradiation with laser light.

Keywords: Hypericin; Photodynamic therapy; Photosensitizer; Cancer; Anticancer agent

Introduction

In literature hypericin is historically referred to plant (St. John's Wort or Hypericum perforatum) derived substance which has been used for different medical applications (e.g. antidepressant, antiviral, antiretroviral, antitumor etc). Review articles have discussed the physical properties, chemical properties and pharmaceutical applications of hypericin [1-3].

Hypericin is hydrophobic in character making it insoluble in water and non polar solvents. It is soluble in alkaline aqueous solutions, organic bases, polar organic substances and biological media. The molar extinction coefficient of hypericin can be easily measured and used to determine the purity or quality of the hypericin extraction that is prepared or commercial purchased [2]. At a wavelength of 590 nm the molar extinction coefficient can vary from 27, 000 to 52, 000 depending on the solvent, raw material, production process, aggregation and storage conditions. The absorption properties of hypericin are likely to change when water is added to the hypericin solution. In the presence of water hypericin in high concentrations aggregate to form insoluble pellets [2]. These hypericin aggregates are non fluorescent and show visible absorption spectra in the same region as free hypericin molecules. The insoluble hypericin pellets have much lower extinction coefficients than free hypericin molecules [2].

Pharmacology

Pharmacology and biodistribution studies have shown that conventional therapy with St. John's Wort extract for depression does not cause phototoxic side effects [2]. Hypericin is reabsorbed in the intestinal compartment without being metabolized and based on its chemical structure or molecular size (> 500 Da), it is presumed to be exerted in the bile. It is not detectable in the urine and in the cerebrospinal fluid. A kinetic study with mice showed a distribution half-life for hypericin of 2 h and elimination half-life of 38.5 h and similar data was obtained in a study conducted using humans [2]. The uptake of hypericin by murine lungs shown to be five-fold higher than the spleen followed by the liver, blood, kidneys, heart, gut, various xenografted tumors, stomach, skin, muscle and the brain [2]. An administration dose of approximately 0.2 mg per kg body weight is effective against viral, bacterial or enzymatic caused diseases. The use of hypericin at this dose during multiple administrations leads to photosensitivity, pain and temperature sensitive side effects. Literature has defined 0.25 mg/kg as the maximum tolerated dose for bi-weekly intravenously administration [2].

Hypericin in PDT

It is the above mentioned pharmacology features and other characteristics of hypericin, that makes it a potentially attractive photosensitizing agent for PDT. It is known to possess minimal dark toxicity and it cannot be metabolized. This photodynamic active molecule has a marked fluorescence emission in the orange/red region which can be used as a fluorescence diagnostic tool for the detection of cancer. Hypericin is considered to be a tumor selective agent therefore low concentrations of hypericin can be used in human hollow organs (e.g. bladder or stomach) for photodynamic tumor therapy [2,4]. It is also photostable and absorbs light at many different wavelengths. There is rapid clearance from normal healthy tissue and slow uptake in normal healthy cells resulting in high tumor selectivity [2]. Previous *in vitro* investigations have successfully displayed the anti-cancer activity of hypericin mediated PDT in different cancer cell lines (Table 1).Cellular localisation studies in cancer cells revealed that hypericin in a time – dependent manner (2 - 4 h) accumulated in the membranes, of the endoplasmic reticulum and Golgi complex [5].The use of hypericin

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Cell line	Hypericin concentration	Light Source	Wavelength (nm)	Light dose (J/cm^2)	Results	Reference
Human skin carcinoma (A431)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 & 0.16 μM	[6]
Human cervix carcinoma (ME-180)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 & 0.16 μM	[6]
Human cervix carcinoma (HeLa)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 & 0.16 μM	[6]
Human prostate carcinoma (DU 145)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 &0.16 μM	[6]
Human prostate adenocarcinoma (PC-3)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 & 0.16 μM	[6]
Human breast carcinoma (MCF-7)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 & 0.16 μM	[6]
Human prostate carcinoma	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 &0.16 μM	[6]
Human fibrosarcoma (Hs913T)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 & 0.16 μM	[6]
Mouse fibroblast cells (Swiss 3T3)	100 μM	Fluorescence lamps	530 620	4	IC_{50} 0.10 & 0.16 μM	[6]
Mouse Mammary carcinoma cells, (EMT6)	0.5 μM 1.0 μM	Fluorescent bulbs		1.5	Nontoxic 0.5 μM + 1.5 J/cm^2; Moderately cytotoxic 1.0 μM + 1.5 J/cm^2	[7]
Human lymphoma (U937)	0.2 μM	Argon dye laser	599	2.5 4 -5 8 10 18	No effect (2.5 J/cm^2) Critical effects (4-5 J/cm^2) Lethal doses (8-10 J/cm^2)	[8]
Murine C26 colon carcinoma cells	1 - 4 μM	fibre optic noncoherent light delivery system	550 590	0.3 0.6 1.2	LD_{50} 1 μM 1.60 J/cm^2 55O nm	[9]
Human Squamous oesophageal (Kyse-140)	10 nM - 1 μM	100W halogen lamp	400 – 800	30	IC_{50} 27nM-28nM	[10]
Human adenocarcinoma Oesophageal (OE-33)	10 nM - 1 μM	100W halogen lamp	400 – 800	30	IC_{50} 27nM -28nM	[10]
Human Glioblastoma (U373)	1-100 μM	Argon laser	488	1 - 20	30% killing 100 μM 20 J/cm^2	[11]
Human bladder (T24 & RT)	20 μg/ml 60 μg/ml	Argon-pumped dye laser	630	1 – 2 4 8	LC_{50} 3.6 J/cm^2 60 μg/ml	[12]
Osteosarcoma cell line (U2OS)	0.06-2 μg/ml	300W halogen lamp		2.9 4.8 9.6 14.4	100% killing 1 μg/ml + 9.6 J/cm^2; 0.5 μg/ml + 14.4 J/cm^2; IC_{50} 0.4 μg/ml + 9.6 J/cm^2	[13]
Human breast adenocarcinoma (MCF-7)	0.021 μM	11 L18 W/30 lamps	530 - 620	4.4	80% survival after 24hrs & 60% survival after 48 hrs	[14]
Human Breast adenocarcinoma (MCF-7)	0.0084 – 0.021 μM	Lamp	530 – 620	4.4	Greater reduction in cell viability after 48 h	[14]
Human epidermoid carcinoma (A431)	1 μM	Red light diode array	610	1.5	LD_{50} 0.31 μM	[15]

Table 1: The photodynamic effect of hypericin on different cancer cell lines.

as anticancer drug means it has the capacity to simulate cell death (e.g. necrosis, apoptosis or autophagy) in cancer cells when it is activated with laser. Hypericin – induced apoptosis was first seen in PDT treated human malignant glioma cells by using the DNA fragmentation assay thereafter hypericin - induced cell death mechanisms were investigated for other cancer cells lines [5]. The inhibition of PKC (protein kinase C) by photoactivated hypericin was suggested to be the triggering event in this type of killing (apoptosis). A study shown that HeLa cells treated with 80 – 250 nmol/l of hypericin and a light dose of 4 J/cm^2 induced apoptosis, and an increased hypericin concentration (1 μmol/l) with the same light dose of 4 J/cm^2 induced necrosis. This is an indication that variation in hypericin doses after laser irradiation could provoke a shift from apoptosis to necrosis [2,5].

Recently, *in vivo* and clinical studies have been published demonstrating the antitumor effects of hypericin in PDT [16-21]. A clinical investigation for basal and squamous cell carcinoma used hypericin at a concentration of 100 - 500 μg per cm^2 of the tumor in

combination with a total light dose of 200 J/cm^2 [2]. Some clinical trials have successfully confirmed the potential of hypericin-PDT for the treatment of recurrent mesothelioma, basal and squamous cell carcinoma [2,5]. However, clinical applications of hypericin to investigate its photodynamic potential for other cancers are still lacking. Therefore, small – scale *in vitro* and *in vivo* photodynamic studies using hypericin still needs to be performed and this would eventually lead to investigations in clinical settings for a wider range of cancers.

Conclusion

PDT is a promising targeted therapy, created to overcome growing problems of convectional surgery and existing oncology treatments, such as drug toxicity, drug resistance and specificity [22]. Its anticancer efficacy predominately depends on the type of PS and laser activation parameters (treatment light dose) used during PDT. Hypericin does not only have the characteristics of ideal PS but has also proven to be a potent photosensitizing agent in various investigations. It also has shown to posses the ability to be used as a diagnostic tool or fluorescent selective marker for the diagnosis of different cancers. Researchers still need to explore the anticancer effects of hypericin during PDT on various other existing cancers, to find the suitable laser parameters in combination with the complementary hypericin concentrations that can be used for the treatment of primary and secondary tumors.

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