Evaluation of Hypericin: Effect of Aggregation on Targeting Biodistribution

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ABSTRACT: Hypericin (Hy) has shown great promise as a necrosis-avid agent in cancer imaging and therapy. Given the highly hydrophobic and π-conjugated planarity characteristics, Hy tends to form aggregates. To investigate the effect of aggregation on targeting biodistribution, nonaggregated formulation (Non-Ag), aggregated formulation with overconcentrated Hy in dimethyl sulfoxide (Ag-DMSO) solution, and aggregated formulation in water solution (Ag-water) were selected by fluorescence measurement. They were labeled with ^{131}I and evaluated for the necrosis affinity in rat model of reperfused hepatic infarction by gamma counting and autoradiography. The radioactivity ratio of necrotic liver/normal liver was 17.1, 7.9, and 6.4 for Non-Ag, Ag-DMSO, and Ag-water, respectively. The accumulation of two aggregated formulations (Ag-DMSO and Ag-water) in organs of mononuclear phagocyte system (MPS) was 2.62 ± 0.22 and 3.96 ± 0.30 %ID/g in the lung, and 1.44 ± 0.29 and 1.51 ± 0.23 %ID/g in the spleen, respectively. The biodistribution detected by autoradiography showed the same trend as by gamma counting. In conclusion, the Non-Ag showed better targeting biodistribution and less accumulation in MPS organs than aggregated formulations of Hy. The two aggregated formulations showed significantly lower and higher accumulation in targeting organ and MPS organs, respectively. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:215–222, 2015

Keywords: radiolabeling; aggregation; formulation; fluorescence spectroscopy; reperfused hepatic infarction; distribution; mononuclear phagocyte system; autoradiography; drug targeting; drug effects

INTRODUCTION

Self-aggregation of small organic molecules refers to spontaneous mutual attraction between molecules via noncovalent bonds including van der Waals forces, hydrogen bonds, hydrophobic effect, and π–π stacking to form large clusters. Some molecular drugs that are composed of extended macrocyclic π-conjugated systems and planar structures such as those in porphyrins,[1] phthalocyanines,[2] and hypercins,[3] easily form aggregates in most solvents. The aggregation changes their spectral and energetic characteristics, affects their biological activities, and influences their efficacy in applications.[4–7] Porphyrins and hypercins are usually used as targeting drug delivery vector in the treatment of cancer.[8–10] Therefore, it is very important to acquire information about their aggregation tendency in order to deliver drug more efficiently to targeted tissues and to produce better diagnostic and/or therapeutic efficacy.

Hypericin (Hy; 4,5,7,4′,5′,7′-hexahydroxy-2,2′-dimethylnaphthodianthrone; Fig. 1) is a natural pigment derived from plant *Hypericum perforatum*, usually called St. John's wort. Its medicinal properties can be traced back to ancient times. Nowadays, Hy is widely used for antidepressive, antiviral, antiretroviral, and antineoplastic therapies.[11–14] As a potent photosensitizer, the photocytotoxic action of Hy has been explored in a variety of preclinical and clinical investigations for cancer photodynamic diagnose and therapy.[15,16] Recently, Hy was discovered with a unique affinity to necrotic tissues. As a small molecular necrosis-avid contrast agent, radioiodine-labeled Hy has been studied as a targeting tracer for tissue viability evaluation in imaging diagnosis of myocardial infarction and ischemic necroses.[17,18] Furthermore, a new anticancer approach combining vascular-disrupting agent and radiolabeled Hy has showed promising anticancer efficacy for the treatment of solid tumors.[19,20]

It is known that compounds with planar structure always tend to present poor aqueous solubility, likewise, π-conjugated structure leads to π–π stacking interaction between molecules.[21,22] Because of the π-conjugated planar structure, Hy has the tendency to form aggregates in aqueous environment and in highly concentrated polar organic solution. In a previous study, Hy formed aggregation in common polar organic solvents above the concentration of 10^{-3} mol/L and it was

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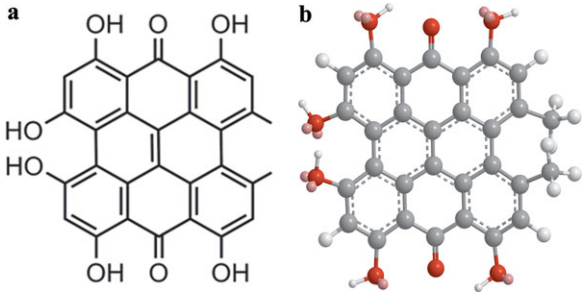

Figure 1. Chemical structure of Hy. (a) 2D structure. (b) 3D structure.

sparingly soluble in nonpolar organic solvents.[23] Under these conditions, Hy built hydrogen bonds to other Hy molecules through the carbonyl and the phenolic hydroxyl groups, which led to forming homoassociates and exhibited weaker fluorescence.[24] These aggregates influence the bioactivity of Hy considerably. Theodossiou et al.[25] observed the aggregation with high concentration of Hy in PAM212 murine keratinocytes and the Hy aggregation exerted poor phototoxicity on cells. Sennoside A is a kind of median dianthrone with similar structure to Hy. However, its structure is spatially arranged in cross with steric hindrance that greatly prevents aggregation. Sennoside A showed an infarct/myocardium ratio of 11.9 and less accumulation in mononuclear phagocyte system (MPS) compared with Hy in one reported article.[26] Therefore, aggregation may be a major reason for high accumulation of Hy in MPS and weaken targeting biodistribution.

Van De Putte et al.[27] studied the impact of aggregation on the biodistribution of Hy in mouse radiation-induced fibrosarcoma (RIF-1) tumor model. The results showed a pronounced uptake in MPS (liver, spleen, and lung) and a slow body clearance with decreased tumor/normal tissue ratios. However, there appeared to be some shortages in their study. The concentration used as nonaggregated Hy in that study was 4 mg/mL, in which aggregation must have actually formed according to the previous study.[23] Such aggregated formulation was most likely with suspended coarse aggregates, which largely differ from the oligomers (especially dimer aggregates) of Hy as concerned elsewhere.[23,28]

Highly concentrated solution is possibly used in clinical relevant preparation of Hy. However, the effect of aggregation at high concentration of polar solvent on biodistribution to target and MPS has not been investigated yet. Therefore, it is necessary to clarify the real difference between such nonaggregated and aggregated formulations in this particular aspect. Accordingly, we compared nonaggregated formulation (Non-Ag) with aggregated formulations of highly concentrated Hy in dimethyl sulfoxide (Ag-DMSO) solution and in water solution (Ag-water) by using ^{131}I-labeled Hy with gamma counting and autoradiography in rat model of reperfused hepatic infarction.

MATERIALS AND METHODS

Hypericin (purity > 98%) was purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Spectrometric grade DMSO was purchased from Tedia Company (Fairfield, Connecticut). Stock solution of Hy in DMSO with a final concentration of 1×10^{-2} M was prepared. Deionized water was used to prepare DMSO–water mixtures. Determination of radiochemical yield and *in vitro* stability were conducted by HPLC with a pump (waters 2695) and waters 2998 UV/Visible detector and HERM LB500 LC radio detector (Berthold Technologies, Milan, Lombardy, Italy) and a Alltima C18 column (250 × 4.6 mm^2, 5 μm; Millipore, Billerica, MA). Flowchart of the whole experimental design is shown in Figure 2.

Fluorescence Measurements

The aggregation of Hy impairs the degree of fluorescence. Thus, fluorescence measurement was used as a method to detect the aggregation of Hy. The fluorescence intensity of Hy, molar concentrations from 1×10^{-7} to 1×10^{-2} M in DMSO and 1×10^{-4} M in DMSO–water mixtures (water content from 10% to 80%), were measured by Cary eclipse fluorescence spectrophotometer (Agilent Technologies, Mulgrave, Victoria, Australia) using an excitation wavelength of 343 nm.

Animal Model of Reperfused Hepatic Infarction

The animal care, use, and experimental protocols were approved by the Animal Affairs Committee of Jiangsu Academy of Traditional Chinese Medicine (Nanjing, China). Adult male SD rats (260–310 g) were anaesthetized with intraperitoneal injection of 10% chloral hydrate at a dose of 3 mL/kg. Under laparotomy, hilum of the right liver lobe was clamped for 3 h, followed by reperfusion and closure of the abdominal cavity.[29] Rats were allowed to recover for at least 12 h before intravenous administration of a tested Hy formulation.

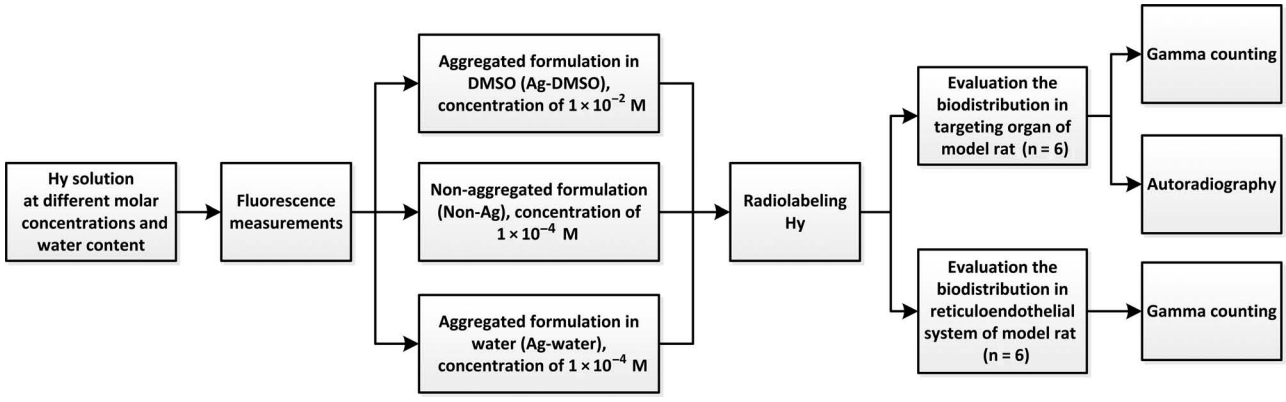

Figure 2. Flowchart of the whole experimental procedures.

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