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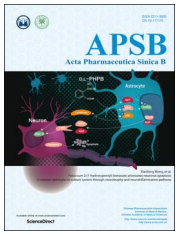

ORIGINAL ARTICLE

^{131}I-Evans blue: evaluation of necrosis targeting property and preliminary assessment of the mechanism in animal models

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KEY WORDS

^{131}I-Evans blue;
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Abstract Necrosis is a form of cell death, which is related to various serious diseases such as cardiovascular disease, cancer, and neurodegeneration. Necrosis-avid agents (NAAs) selectively accumulated in the necrotic tissues can be used for imaging and/or therapy of related diseases. The aim of this study was to preliminarily investigate necrosis avidity of ^{131}I-evans blue (^{131}I-EB) and its mechanism. The biodistribution of ^{131}I-EB at 24 h after intravenous administration showed that the radioactivity ratio of necrotic to viable tissue was 3.41 in the liver and 11.82 in the muscle as determined by γ counting in model rats. Autoradiography and histological staining displayed preferential uptake of ^{131}I-EB in necrotic

Abbreviations: CE-T1WI, contrast-enhanced T1WI; CT-DNA, calf-thymus DNA; DMSO, dimethylsulfoxide; DWI, diffusion-weighted imaging; EB, evans blue; H&E, haematoxylin-eosin; Hyp, hypericin; ^{131}I-EB, ^{131}I-evans blue; %ID/g, percentage of the injected dose per gram of tissue; MRI, magnetic resonance imaging; MPS, mononuclear phagocyte system; NAAs, necrosis-avid agents; PI, propidium iodide; RCP, radiochemical purity; RFA, radiofrequency ablation; RPLI, reperfused liver infarction; SD rats, Sprague–Dawley rats; T1WI, T1-weighted imaging; T2WI, T2-weighted imaging; TLC, thin layer chromatography

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tissues. *In vitro* nuclear extracts from necrotic cells exhibited 82.3% of the uptake in nuclei at 15 min, as well as 79.2% of the uptake at 2 h after ^{131}I-EB incubation. The DNA binding study demonstrated that evans blue (EB) has strong binding affinity with calf-thymus DNA (CT-DNA) ($K_{sv}=5.08\times10^5$ L/(mol/L)). Furthermore, the accumulation of ^{131}I-EB in necrotic muscle was efficiently blocked by an excess amount of unlabeled EB. In conclusion, ^{131}I-EB can not only detect necrosis by binding the DNA released from necrotic cells, but also image necrotic tissues generated from the disease clinically.

1. Introduction

Necrosis characterized by irreversible loss of plasma membrane integrity is a distinguished feature of many pathological conditions such as cancer, cardiovascular diseases, neurodegenerative disorders, autoimmune diseases and others[1]. And necrosis is also one of the major risk factors for accelerated deterioration of diseases[2,3]. The ongoing efforts have been put into understanding the role of cell necrosis in a range of diseases and therapeutics[4,5]. But to date, few appropriate cell necrosis imaging probes have been reported for clinical use in human.

Necrosis-avid agents (NAAs) which can selectively accumulate in the necrotic tissues are expected for both imaging and therapeutic applications[6,7]. Radiolabeled NAAs have been used as markers for noninvasive 'hot-spot imaging' to localize necrotic tissues such as myocardial infarction[8]. They provide a clear-cut distinction between viable and necrotic myocardium, which is crucial for myocardial viability determination and subsequent therapeutic decisions in clinical cardiology[9]. On the other hand, radioiodinated NAAs, which are preferentially taken up in tumor necrosis induced by antineoplastic drugs[10–12], emit radiation to kill and/or restrain adjacent residual cancer cells[13].

Porphyrin-based NAAs are still studied in preclinical experiments by different research centers[14,15]. Some porphyrin derivatives with superb necrosis targetability have been applied for visualization of necrosis including brain infarction and lesions of radiofrequency ablation (RFA). However, their clinical utility was limited by phototoxicity, unsatisfactory clinical tolerance and other potential side effects[16]. Hypericin (Hyp) has been recognized as one of the NAAs that exhibit a peculiar affinity for necrotic tissue. Nevertheless, Hyp is nearly insoluble in water and most nonpolar solvents, and shows unwanted retention in organs of the mononuclear phagocyte system (MPS)[17]. It will take a long time for Hyp to be used in clinical. Therefore, there is a pressing need for seeking safe and effective necrosis targeting probes with theragnostic purposes.

Evans blue (EB) is an artificial dye that may emit a bright red fluorescence as implied by its structure (Supplementary Information Fig. S1a)[18,19]. Interestingly, agricultural researchers apply EB staining to characterize maize endosperm cell death and identify key differences related to premature endosperm degeneration[20]. As a rapid and reproducible method, EB can stain damaged or dead cells in the plants[21,22]. In general, cell death in animals or in plants share many similar morphological and biochemical characteristics[23]. Moreover, EB has been used clinically as an intravenously injectable dye for determining the total blood volume in human, even in pregnant women and newborn babies, without adverse reactions[24,25]. Hence, we hypothesized that EB as a safety compound may exhibit necrosis targeting properties and could be applied for necrotic tissue imaging in animals.

The disruption of cytomembrane of necrotic cell results in the exposure of genomic DNA, which becomes the target of NAAs[26]. The previous study revealed that the planar-structured compounds have the potential to intercalate with double stranded DNA by forming stable complexes[27]. Therefore, we speculate that EB may target on the necrotic tissues by binding with DNA exposed on the necrotic cells.

In this study, necrosis targetability of ^{131}I-EB was evaluated by tissue γ counter, autoradiography, fluorescence microscopy and histochemical staining in rat reperfused liver infarction (RPLI). Nuclear extraction, fluorescence microscopy, DNA binding and blocking experiments were designed to examine EB necrosis targeting mechanism on cellular/tissue level.

2. Materials and methods

2.1. Materials and reagents

Sprague–Dawley rats (SD, male, 260–280 g) were provided by the Experimental Animal Center of Academy of Military Medical Sciences. All experimental protocols were approved by the Ethical Committee of Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School. The care and treatment of all animals were maintained in accordance with NIH publication No.85-23 (revised in 1996) on "Principles of laboratory animal care". EB was commercially available from Sigma Chemical Co. (St Louis, MO, USA) with purity greater than 98%. Sodium iodide's ($Na^{131}I$) radionuclidic purity was >99% and specific activity was 555 MBq/mL, which was supplied by HTA Co., Ltd. (Beijing, China).

2.2. Labelling of EB by ^{131}I

The iodogen coating method was conducted successfully in this study. Radioiodination was carried out by adding $Na^{131}I$ solutions and EB (1 mg/mL) (volume ratio, 1:4) into iodogen (1,2,4,6-tetrachloro-3α, 6α-diphenylglycoluril; Sigma, St. Louis, MO, USA) tube (containing 100 μg of iodogen). The compound was shaken and incubated for 6.5 h at 70 °C, and terminated by removal of reaction solution. Radiochemical purity (RCP) of ^{131}I-EB was determined by thin layer chromatography (TLC).

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