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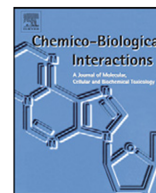

Whole body and tissue imaging of the butyrylcholinesterase knockout mouse injected with near infrared dye labeled butyrylcholinesterase

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A B S T R A C T

Butyrylcholinesterase (BChE) has proven to be an effective bioscavenger against nerve agents and organophosphates. Phase I safety trials of human BChE are currently being conducted and large-scale production of recombinant BChE is underway. Information on the real-time distribution of BChE from the injection site has not been well characterized. This study utilized the BChE nullizygote (BChE−/−) mouse and tetrameric equine BChE labeled with LI-COR® fluorescent IRDye 800CW to track, quantify and determine the retention time of BChE in vivo following intramuscular injection. In vivo images were acquired with Xenogen's IVIS® 200 imager and the LI-COR Odyssey® Imaging System fitted with the MousePOD™. Plasma and tissues were tested for BChE activity. The 2 mg of BChE spread from the injection site to heart, liver, intestine, kidneys, lungs, salivary glands, and muscle, but did not enter the brain or the skin. Fluorescence intensity in organs and BChE activity in plasma peaked on day 1. BChE activity in plasma was undetectable by day 16, at a time when there was still significant fluorescent signal and BChE activity in the liver (0.32 units/g), injected quadriceps (0.13 units/g) and in most of the organs analyzed. It is concluded that the tetrameric BChE glycoprotein of 340 kDa diffuses from the muscle injection site to blood and peripheral organs and has a longer residence time in the organs than in blood.

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1. Introduction

Butyrylcholinesterase (BChE; accession # gi:116353) is an effective bioscavenger of nerve agents and organophosphates (OP) [1–5]. This protective property has made BChE a candidate for use in the treatment of humans following OP or nerve agent exposure. Production of recombinant human BChE from transgenic goat milk will provide large quantities of enzyme for use in bioscavenger trials [3,6]. The in vivo pharmacokinetics of horse BChE and of native and recombinant human BChE have been studied in rodents [1,5,7–11] and in non-human primates [2,5,12,13] by measuring circulating plasma enzyme activity and in some cases activity in tissue extracts. The real-time distribution of BChE from the site of injection to organs has not been reported in the literature. We outline a method by which the distribution of BChE following an intramuscular (IM) injection can be visualized and tracked in whole animals and tissues over the entire residence time with the use of a near infrared fluorescent dye bound to horse BChE protein. The absorbance spectra of body fluids and tissues are at a low in the near infrared region, therefore naturally occurring auto-fluorescence is reduced [14]. The challenge of distinguishing the BChE activity of exogenously delivered enzyme from native enzyme was solved by the use of the BChE nullizygote (BChE−/−) mouse. This animal has

Abbreviations: BChE, butyrylcholinesterase; −/−, nullizygote; IM, intramuscular.

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no detectable BChE in the plasma or in any tissue [15]. To test the clearance and non-specific binding characteristics of the dye a carboxylate form of the dye was injected intramuscularly and tracked until no fluorescence above background was detected. The free dye was cleared by 32 h post-dosing. Following injection with the dye conjugated BChE the maximum plasma activity was reached at 30 h post-intramuscular injection. At 12 days post-dosing the plasma had an activity of 1 unit/ml, a level similar to the 1.9 units/ml present in wild-type mice. It was found that fluorescent signal and BChE activity remained in the liver (0.32 units/g), at the muscle injection site (0.13 units/g), as well as in the intestine, kidneys, lungs, salivary glands, spleen, diaphragm, stomach and in the uninjected muscle 16 days post-injection at a time when there was no detectable BChE activity in the plasma.

2. Experimental methods

2.1. Animal model

Animal work was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. Formal approval to conduct the experiments was obtained from the animal subjects review board at the University of Nebraska Medical Center.

The BChE knockout mouse was developed in our laboratory [15]. All tissues and plasma in the BChE−/− mice are devoid of BChE activity. BChE−/− mice have no distinguishable phenotype. Their genetic background is strain 129Sv. Both male and female adult mice were used in this trial. The absence of BChE ensured that any BChE enzyme that was detected had been delivered exogenously.

2.2. Labeling of BChE with fluorescent dye

The lyophilized horse BChE from Sigma (C-1057) contains salts so that 2.0 mg of BChE is present in 20.4 mg of Sigma product. Two milligrams of horse BChE contain 1440 units of BChE activity where units are defined as micromoles butyrylthiocholine hydrolyzed per minute at pH 7.0, 25 °C. Horse BChE (2.0 mg) was labeled with IRDye 800CW (Protein Labeling Kit–High MW #928-38040, LI-COR®, Lincoln, NE) following the product insert instructions. This dye contains an *N*-hydroxy-succinimide ester reactive group that couples to primary amines, forming stable conjugates with lysines. The conjugate was dialyzed extensively against phosphate buffered saline to remove excess nonreacted dye. The labeled BChE bound 5 dye molecules per mole of protein and lost 60% of its initial activity. The labeled BChE was examined on a nondenaturing gel where it was found to consist primarily of tetramers of molecular weight 340,000.

2.3. Preparation of the BChE KO mouse for injection and imaging

At least 24 h prior to injection all test animals were placed on a diet of Ensure Fiber (Abbott Laboratories, Abbott Park, IL). The liquid diet eliminated the background fluorescence in the stomach and intestines that results from the chlorophyll in standard pelleted diets. The mice were shaved and Nair® was used to remove the hair from the ventral and dorsal surfaces of the mice. Hair is removed because up to 45% of the fluorescent signal is blocked by hair. Each animal was imaged prior to injection to establish a background level of fluorescence.

2.4. Injection protocol

BChE−/− mice were injected intramuscularly (IM) into the left quadriceps posterior thigh muscle with either 1 nmol IRDye 800CW carboxylate ($n = 2$), 2 mg of horse IRDye 800CW labeled horse BChE conjugate ($n = 7$) or unlabeled horse BChE 2 mg ($n = 3$).

2.5. Plasma collection for determination of BChE half-life

Plasma was collected from the saphenous vein into heparinized microvette tubes (Sarstedt, Nümbrecht, Germany) at 15, 30, 60, 120, 180, 240, and 360 min post-dosing and every 24 h for 16 days. The volume of blood collected at each time point was approximately 50 μl.

2.6. Tissue collection for determination of BChE activity and imaging of frozen sections

BChE−/− mice treated with horse IRDye 800CW labeled horse BChE conjugate IM ($n = 2$ per day) were euthanized by CO_2 asphyxiation on days 1, 3 or 16 post-dosing. The animals were thoroughly perfused intracardially with 75 ml of 0.1 M PBS, the tissues were removed and imaged on the Odyssey Imaging System. Immediately after imaging one half of each tissue was flash frozen and cut into 7 μM sections, while the other half was homogenized for determination of residual BChE activity and visualization on a nondenaturing gradient gel.

2.7. Scanning procedure

Xenogen's IVIS® System 200 imager was used to scan in vivo fluorescence in the live animals. This is a real-time in vivo imaging system using white light excitation. The ICG background filter and the ICG emission filter were selected for use with the IRDye 800CW. Animals were anesthetized with 2% isoflurane to maintain sedation during the imaging procedure.

Whole body images from the Odyssey Imaging System (LI-COR®) equipped with the MousePOD™, an accessory for administering anesthesia and maintaining animal temperature, were compared with the images from the Xenogen IVIS® System 200. The Odyssey Imaging System uses two monochromatic diode lasers for excitation and collects the emissions with microscope optics below the surface of the target. The Odyssey Imaging System was also used to image the perfused tissues, frozen sections and the tissue homogenates on polyacrylamide native gels. The fluorescent intensity of the tissues was quantified using the Odyssey software package.

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