REAL-TIME SENSING OF DOXORUBICIN IN AN ISOLATED RAT HEART

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ABSTRACT

Doxorubixin is a highly effective and widely used antracycline antibiotic cytostatic drug used to treat numerous types of tumour diseases, but the cardiotoxic effect significantly limits its application. Doxorubicin has hot great fluorescence properties what can be used to its detection. The detection of the fluorescence of the therapeutics in organisms is limited by the thickness of the tissue the light need to penetrate. An alternative way for increasing the sensitivity of this type of imaging is the elimination of surrounding tissue, leading to *ex vivo* analysis under simulated conditions (i.e. perfusion system for isolated heart). The aim of this study was the combination of perfusion system with the fluorescence *in vivo* imaging system to observe the fluorescence compounds in beating heart.

The study proposes the application of *in vivo* imaging system for fluorescence *ex vivo* analysis of rat heart from the doxorubicin administered rat. The miniaturized Langendorff perfusion system was used. The isolated heart was supply by oxygenated Tyrode solution (37 °C) to ensure the heart beating and nutrition. This arrangement enabled the detection of doxorubicin in the ex vivo heart. The detection concentration was 1 μg of doxorubicin in the heart after the intraperitoneal application of 4 mg doxorubicin.

Key words: doxorubicin, fluorescence imaging, langendorff-perfused heart, cancer, magnetic particles

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INTRODUCTION

Doxorubicin is a highly effective and widely used antracycline antibiotic, important antineoplastic drug intercalating DNA and causing oxidation stress that is used to treat leukaemia and solid tumours (Hynek, Krejcova et al. 2012). However, its application is limited by high cardiotoxicity, therefore it is necessary to monitor the applied dose (Minotti, Menna et al. 2004). The doxorubicin can be sensitively detected due to its fluorescence properties (Changenet-Barret, Gustavsson et al. 2013). Detection of the fluorescence of the therapeutics in organisms is limited by the thickness of the tissue the light need to penetrate Currently, fluorescence imaging techniques are being successfully expanded towards *in vivo* imaging (Bratlie, Dang et al. 2010; Shin, Pierce et al. 2010). However, certain limitations have to be taken into account including relatively high background signal produced by the tissue surrounding and also the scattering and absorption of both excitation and emitted light during its penetration through the tissue (Houston, Sevick-Muraca et al. 2002). An alternative way for increasing the sensitivity of this type of imaging is the diminishing or elimination of surrounding tissue, leading to *ex vivo* analysis under simulated conditions (i.e. perfusion system for isolated heart).

Nowadays the study of cancer treatment is focused on targeted therapy. Nanoparticles, which are widely used in studies of targeted transport of a wide range of substances, are magnetic particles (Mok and Zhang 2013). However, there are certain toxicological risks from the use of magnetic particles in medicine; therefore a number of tests are required. Magnetic particles generally comprise of iron, nickel or cobalt (Gupta, Naregalkar et al. 2007; Thorek and Tsourkas 2008; Tran and Webster 2010; El-Okr, Salem et al. 2011; Nejati and Zabihi 2012; Nakamura, Ueda et al. 2013). Their size is several nanometres - micrometres. Magnetic particles with specific surface modifications can be used for various biomedical purposes, such as drug delivery, hyperthermia, transfection and magnetic resonance imaging (Gupta, Naregalkar et al. 2007; Wu, Ou et al. 2010; Schlorf, Meincke et al. 2011; Nandori and Racz 2012). Drug transport through the magnetic particles may be facilitated by binding to specific nanoparticles, such as lipid (Silva, Santos et al. 2012) and protein carriers (Elzoghby, Samy et al. 2012) which allow selective release of drugs in the required area. Such release may be performed by various mechanisms including photo- (Banerjee and Chen 2009) or thermoiniciated (Li, ten Hagen et al. 2010) or pH triggered release (Xu, Flores et al. 2011).

The aim of this study was to investigate the ability of commercially available fluorescence *in vivo* imaging system to utilize for the perfusion system of rat isolated heart exposed to doxorubicin doxorubicin, and to determine doxorubicin accumulation in the cardiac tissue. For the purpose doxorubicin toxicity reduction, the targeted transport of doxorubicin was studied. The encapsulation of the doxorubicin into the apoferritin and magnetic particle-based targeted was investigated.

MATERIAL AND METHODS

Animal handling

Two male Wistar rats (250 g) were used in this study. One was intraperitonealy administered 2 ml of doxorubicin (2 mg/ml in distilled water). The other animal served as negative control and the same amount of physiological solution was administered i.p. Both, doxorubicin and physiological solutions were preheated to 37°C before application. Forty eight hours after administration the animals were euthanized and both hearts were isolated.

Heart preparation

First, deep inhalation anesthesia by isoflurane was introduced. The chest of rat was then opened with scissors and the heart with sufficiently long piece of aorta quickly cut out. It was placed into a beaker with cold Tyrode solution of following composition: 8 g/l NaCl, 0.2 g/l $CaCl_2$, 1 g/l $NaHCO_3$, 0.05 g/l Na_2HPO_4, 0.1 g/l $MgCl_2$, 1 g/l glucose. The heart was then connected to the miniaturized Langendorff setup inside the *In vivo* imaging instrument and perfused under constant perfusion flow with oxygenated Tyrode solution. The coronary flow was kept by the peristaltic pump at the rate of 9 ml/min.

Fluorescence ex vivo imaging of rat heart

The fluorescence of doxorubicin was registered by Carestream *In vivo* Xtreme Imaging System (Rochester, USA) under following conditions: exposition time: 2 s, binning: 2x2 pixels, f-Stop: 1.1, excitation/emission 480 nm/600 nm. Camera is a cooled back-thinned, back illuminated camera designed for maximum sensitivity. The camera utilizes a two-stage thermo-electric cooler that cools down the CCD below -55 °C absolute. The camera collects the image data on a 2048 x 2048 pixel CCD. Single frame image data is digitized at 16-bits, and presented in software as a 32-bit floating point image. The images were processed by Carestream molecular imaging software (Carestream) and the Carestream Multispectral software was used to eliminate the autofluorescence of the tissue.

RESULT AND DISCUSSION

Commercially available *in vivo* instrument is a highly sensitive machine combining the X-ray and fluorescence imaging modality to give precise spatial visualization of targeted area. The chamber with the imaging area of 20×20 cm provides enough space for miniaturized Langendorff perfusion system. The tubing supplying the isolated heart with the oxygenated Tyrode solution was slid into the imaging chamber through the aperture commonly used to supply the anesthetic gas during animal imaging. Tyrode solution was oxygenated and heated to 37 °C outside the chamber prior to application. In order to prevent its cooling the length of the tubing was minimized. Constant perfusion flow ensured by peristaltic pump was set to 9 ml/min, which is sufficient value for the rat heart. The heated environment of the imaging chamber provided friendly environment for isolated heart. The perfusate was drained by running down the side of the inclined Petri dish placed under the isolated heart.

Finally, the heart isolated from the doxorubicin administered rat was investigated. In Fig. 1 is the photography of heart in the chamber, and the X-ray, fluorescence images and combined X-ray and fluorescence images are. The autofluorescence of the tissue was deducted. According to detected fluorescence intensity of the doxorubicin in the heart the amount of doxorubicin in the heart was determined to 1 μg. The *in vivo* imaging system is useful for the detection of fluorescence compounds in the Langendorff-perfused heart.

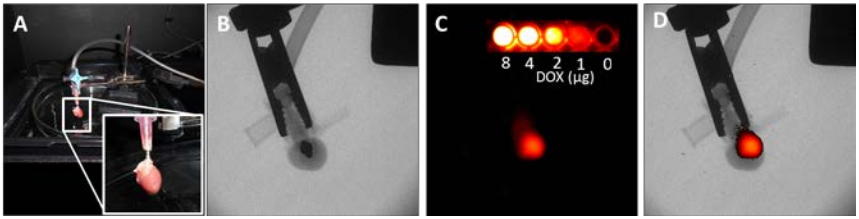

Fig. 1 *Isolated heart of the rat administered doxorubicin (4 mg of DOX 48 hours prior heart isolation): A) photographyof the heart in the chamber; B) X-ray image; C) fluorescence image (excitation 480 nm, emission 600 nm); D) overlay of B and C*

CONCLUSIONS

The *In vivo* imaging system may be considered as a promising tool for both sensing of heart function and the effects of compounds with fluorescent properties on heart tissue

Doxorubicin can be effectively encapsulated into the apoferritin cavity and transported by magnetic field to the site of action. This feature allows the application of apoferritin as a drug nanocarrier with specific low pH initiated release.

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