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ORIGINAL ARTICLE

Application of quantum dot-based biosensor to the conjunctiva in Wistar albino rats

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Abstract

This study was conducted to get an idea about the distribution of the lymphatic fluid in conjunctiva throughout the body parts. For this purpose, Qdot655 (QD), fluorescence nanoparticles, spread onto the conjunctiva were used on Wistar albino rats. Drainage of QD particles from conjunctiva was followed up via fluorescence images at different hours on body parts such as eye, ears, forearms, hind legs and tails. The first fluorescence signals within the 30th minutes following administration of QD were observed in the nasal region and the anterior extremities. Whereas within 60th minutes following QD spread, fluorescent signals were obtained from the ears, forearms, hind legs and tail of the female and male rats.

KEYWORDS

biosensor, conjunctiva, eyelid lymphatics, in vivo imaging, quantum dot, rat

1 | INTRODUCTION

Anatomically, tunica conjunctiva palpebrarum was relatively richer in terms of vessels than tunica conjunctiva bulbi (Dursun, 2014; König & Liebich, 2014).

The tunica conjunctiva palpebrarum of Wistar rats have proliferative epithelium. This means that the epithelial stem cells in the conjunctiva are located essentially in the palpebral epithelium in Wistar rats. This explanation gave rise to a new perspective on the ocular epithelial development. It was also important in conjunctival injury repair (Chen, Ishikawa, Yamaki, & Sakuragi, 2003). The deep lymphatic system drained the conjunctiva was still undefined. The lymphatic system is an important component of the circulation, and it played a critical role in the regulation of the body fluid and in macromolecular homoeostasis, lipid absorption, immune function and metastasis (Schmid-Schonbein, 1990; Steenbergen, Lash, & Bohlen, 1994). There was no clear view of lymphatic transport in different organs yet. It had been observed that the lymphatic drainage increased after subconjunctival drug administration in both tumour and inflammation cases (Robinson et al., 2006; Sugar, Riazi, & Schaffner, 1957).

Aqueous humour used conjunctival lymph vessels to drain into lymph nodes (Camelo, Kezic, Shanley, Rigby, & McMenamin, 2006; Camelo et al., 2008; Nur, Saçmacı, Ertürk, & Orhan, 2018). The immune system in the lymph node is very important for the defence of the eye (Raviola, 1983). Lymphatic vessels are drained into the parotid lymph node. As it has been reported in studies conducted with fluorescent agents, the lymph liquid, which reaches a parotid lymph node first, was then drained into the retropharyngeal lymph nodes (Freeman & Troutt, 1985). In addition, the lymphatic vessels of the upper and lower eyelids opened to the parotid lymph node (Shoukath et al., 2017).

Stereomicroscopic research on the cornea, limbus and the lymph vessels in the conjunctiva of cattle revealed that there were no valves or invaginations in the lymphatic vessels, while there were diverticula's like humps in the vessels (Gruntzig, Nolte, Schad, & Pfankuchen, 1987). However, according to Guo et al. (2012), there were cover-like formations in the conjunctiva of monkeys. As the contraction of the lymphatic vessels was in the shape of rings, they functioned like pumps and in this way, they were regarded as the factor carrying forward the lymphatic liquid (Lohrberg & Wilting, 2016). The lymphatics of the conjunctiva were also associated with ventral nasal concavities. The inferior nasal concavity (Concha nasalis ventralis) contained numerous lymphatic vessels. The dense lymphatic vascular network found here was also associated with the tear duct (nasolacrimal duct). Nasal conchae were also connected with the lymphatic drainage of the brain (Aspelund et al., 2015; Johnston, Zakharov, Papaiconomou, Salmasi, & Armstrong, 2004).

Finally, the current study was designed to investigate the dispersion time and location of the absorption of Qdot in the conjunctiva of Wistar albino rats by using QD-based biosensor probe. Currently, no data was found in the literature survey as to whether the lymphatic drainage in the conjunctiva joined to the general circulation system via the lymphoid system or via the lymphovenous anastomosis. This study reports that biosensor probe drops on the rat eye can reach far extremities such as the ears, forearms, hind legs and tail.

2 | MATERIALS AND METHODS

2.1 | Collecting animal samples

Five-week-old Wistar albino rats with an average weight of 250–300 g were used in this study. Five female and five male Wistar albino rats were obtained from the animal research centre called DEKAM at Erciyes University (ERU). The rats were kept at room temperature in cages under continuous 12-hr light/dark cycles and fed ad libitum with the standard food and water before and during the experiments.

This experimental study was approved on 15.03.2017 with the approvement code: 17/026 by the Committee for the Ethics of Animal Research (ERÜHADYEK) at the University of Erciyes-Kayseri-Turkey and conducted in compliance with the ethics guidelines (Nur et al., 2018).

2.2 | Anaesthesia procedure

General anaesthesia was applied by the intra-peritoneal route to correct position and handle animals. For this purpose, 0.3 ml of aesthetic combination (0.6 ml xylazine, 0.24 ml ketamine) was used for the male rats, and 0.2 ml of aesthetic combination (0.6 ml xylazine, 0.14 ml ketamine) was used for the female rats.

2.3 | Subpalpebral space injection of quantum dot (QD) and in vivo hyperspectral fluorescence imaging

The head and neck region were roughly trimmed by a hair trimmer. The non-invasive surgical procedure was applied to the rats. QD probe is an excellent far-red–fluorescent label with outstanding brightness and photostability for imaging with an emission maximum of ~655 nm. A single dose of 1 µl of QD (Qdot® ITKTM Carboxyl Quantum Dots, Invitrogen) was dropped with the Hamilton syringe (Hamilton Company) under the upper left eyelid. In order to fallow the dispersion of QDs, in vivo images of the rats were collected at the 1st, 20th, 30th and 60th minutes following the single drop per rat. The in vivo fluorescence images were recorded via the Syngene GBOX-XRQ instrument.

3 | RESULTS

The fluorescence signals were observed over the eye, nose, forelimb, hind limb, ear and the tail region at the 1st, 20th, 30th and 60th minutes after following a single dose of 1 µl QD spreading on the conjunctiva. Time-wise changing on the strength of the observed fluorescence within body parts were presented in Table 1. A tracking way of QD particles from frontal body parts to posterior parts was clearly shown in Table 1. There was no fluorescence signal in some other body parts, except eye within 20 min. The strength of signal enriches after 30 min and mostly appears on the nose and thoracic limb regions. Within 30 min, fluorescence signal strength within the front limb for a male rat is stronger

TABLE 1 The strength of the observed fluorescence on the body parts of male and female rats was presented at the 1st, 20th, 30th and 60th minutes

	1st minute		20th minute		30th minute		60th minute	
Body parts	F	M	F	M	F	M	F	M
Eye	+++	+++	+++	+++	+++	+++	+++	+++
Nose	–	–	–	–	++	+++	–	–
Ear	–	–	–	–	–	+	+++	++
Thoracic limb	–	–	–	–	++	+++	+++	++
Pelvic limb	–	–	–	–	+	–	+++	++
Tail	–	–	–	–	–	–	+++	+++

Note: Fluorescence signal: (–): absent; (+): weak; (++): mild; (+++): strong. F: Female (*n*:5); M: Male (*n*:5).

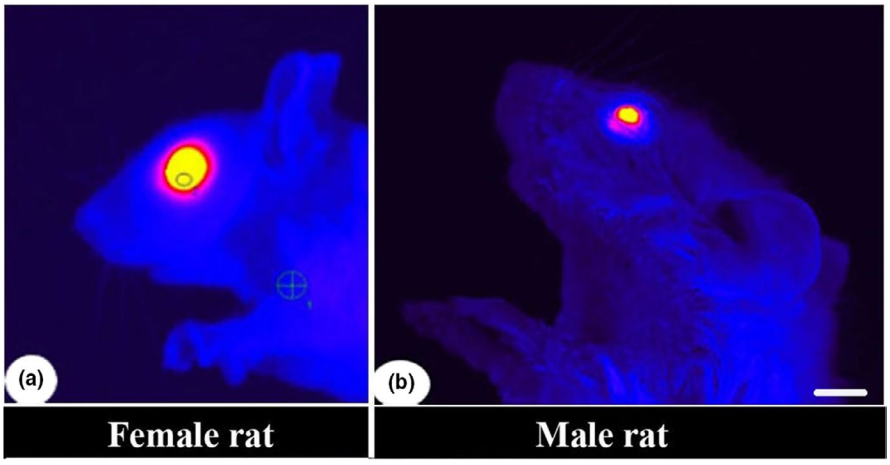

FIGURE 1 Comparing fluorescence Qdot655 distribution on left eyes of the male and female rats at the 1st minute: no difference on the appearance of the fluorescence imaging on the left conjunctiva bulbi of the female and male rats right after Qdot655 1 μl dropped. Scale, 10 mm. 100 × 52.20 mm (300 dpi)

than female rats. Whereas after the 30th minute, preauricular signals for female rats were stronger than male rats when the time reached to the 60th minute.

When time advances, fluorescence signals around the eye region have not lost its strength. However, the signals increase in the other parts of the body, reaching up to the tail, regardless of gender.

The dispersion of Qdots and in vivo images of the rats were collected at the 1st, 20th, 30th and 60th minutes were shown in Figures 1, 2, 3 and 4, respectively.

It was found out that there were no signal differences between both genders in the images obtained right after 1 μl QD was spread in the conjunctiva within the 1st minute (Figure 1). No images could be obtained during the first 20th minute in the other body parts of the rats, except for the eye (Figures 1 and 2). QD within 20 min was only widening continuous dispersion around the eye region (Figure 2). However, the fluorescent images primarily on the nose and anterior limbs were obtained only after the 30th minute (Figure 3).

When QD was given, it was thought that the chemical material would quickly pass to the lacrimal duct within 20th minutes at the medial angle of the eye first and then also passes quickly to the nasolacrimal duct. It was expected that fluorescent images should be observed immediately in those regions. But surprisingly, there are not any images for the nasal area as expected, so fast (Figures 1 and 2). The passage of QDs from eyes-to-nose region has taken about at least 30 min (Figure 3).

The spread of fluorescence QD particles to conjunctiva had reached the ears, the hind limbs and the tail area within 60 min (Figure 4). Somehow, QD within the conjunctiva passes to lymphatic drainage and then joins to the general circulation system via the lymphoid system or via the lymphovenous anastomosis.

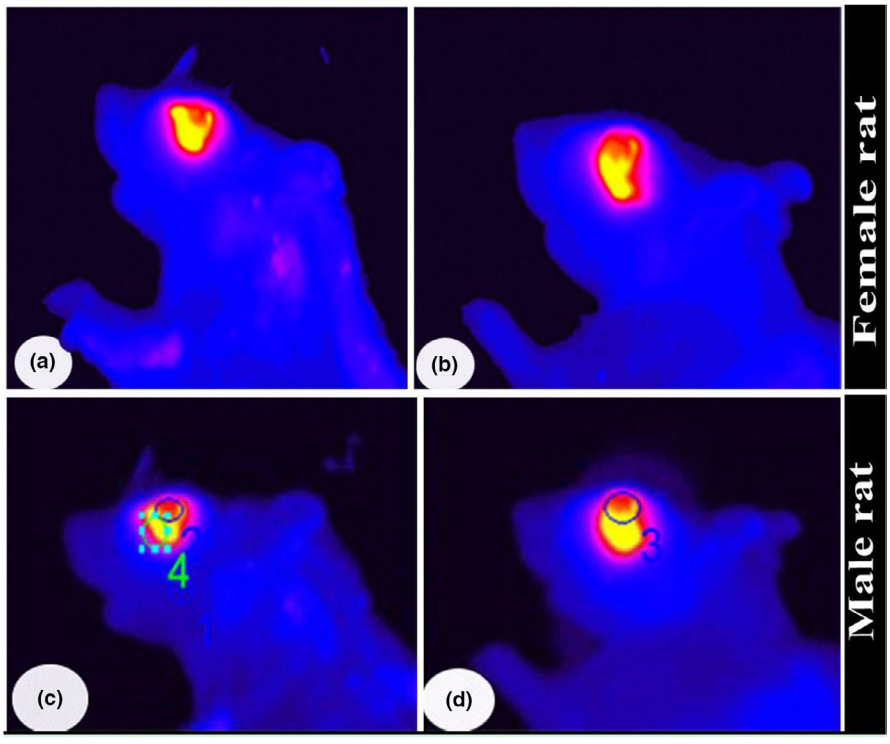

FIGURE 2 Qdot655 within 20 min is advancing dispersion around the left eye region for both genders. No signal around the ear, nose or any other body parts at this time. (a, b) More dispersion of the fluorescence signals around the left eye of the female rat. (c, d) Less dispersion of the fluorescence signals around the left eye of male rats. Scale, 10 mm. 100 × 82.97 mm (300 dpi)

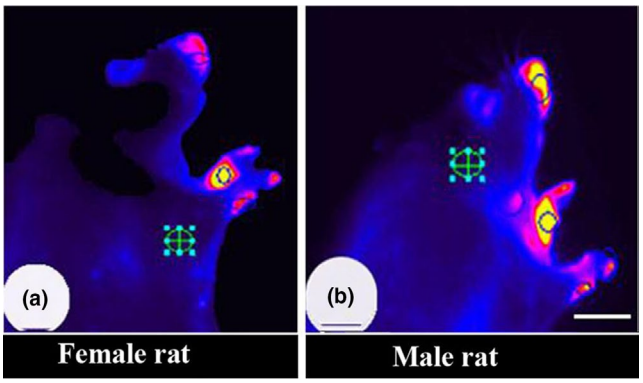

FIGURE 3 The appearance of fluorescence signals around the nose, ear and thoracic limb region of female and male rats at the 30th minutes following after Qdot655 drop. The fluorescence signals were taken from nasal, the underside of the carpal joint and at the tip of the carpal joint with the carpal joint level in the female (a) and, male (b) rats. Scale, 10 mm. 100 × 58.93 mm (300 dpi)

4 | DISCUSSION

The current investigation was prepared to give more anatomical information about the duration of the joining of the lymphatic liquid in the conjunctiva of the male and female rats to the general circulatory system.

The general approach was to drop the fluorescence agent (QD) onto conjunctiva and follow up at different hours on the body parts, and see how fluorescence substance dissolves and distributes throughout via the lymphatic system from the eye to other parts.

Today, studies on both internal and external drainage of the eye are very rare. The drainage of the aqueous humour drains occurred via two pathways: the conventional outflow through the trabecular meshwork and Schlemm's canal, unconventional outflow through the ciliary body. The two pathways of aqueous humour drainage were well studied as reported by (Gobel, Rufer, & Erb, 2011; Swaminathan, Oh, Kang, & Rhee, 2014; Tamm, Braunger, & Fuchshofer, 2015). While there was a third pathway for the drainage of the aqueous humour as reported by (Yücel et al., 2009). Many studies have confirmed that aqueous humour was drained into the submandibular lymph node (Tam, Gupta, Zhang, & Yücel, 2013; Yücel et al., 2009). Leptomeningeal lymph vessels also join to this lymph node. Therefore, it means that the lymph nodes may assume significant roles in the treatment of many illnesses such as glaucoma, MS and Parkinson's disease (Schloetzer Schrehardt, 2007).

No data was found in the literature survey as to whether the lymphatic drainage in the conjunctiva joined the general circulation system via the lymphoid system or via the lymphovenous anastomosis. Although, it was initially thought that we could immediately obtain images (Figure 1) from the nose as the conjunctiva joined to the nasolacrimal duct via the lacrimal canal at the medial angle of the study did not yield such images within the 20 min (Figures 1 and 2). Slow distribution can be explained that either fluorescence particles (QD) were not reached those areas, perhaps due to limited drain or not measured due to tissue thickness, the deepness of the drainage, etc. or could be other limiting reasons. However, 30 min after fluorescence QD dispersion onto conjunctiva, QD particles reached, nasal and carpal regions (Figure 3). When the time reached 60 min after fluorescence spread, fluorescence QDs drainage extended to the ears, forearms, hind legs and tail as seen in Figure 4.

The reason for getting fluorescent images in the preauricular region, as stated in the literature (Echegoyen, Hirabayashi, Lin, & Tao, 2012), originated from the ventral and rostral extraorbital lacrimal glands because the channels of these glands and the channels of the intraorbital lacrimal gland opening to the conjunctiva both combine at the dorsolateral region of the eye. For this reason, the fluorescence image in our study was strong around the eyes within 20 min (Figure 2). The fluorescent particles reaching the preauricular region in rats had been observed within the 30th minutes (Figure 3) and finally 60th minutes (Figure 4). After 60 min following QD dispersion on the conjunctiva, fluorescence strength on eye remains unchanged, contrary florescence diffused locally. This may indicate that drainage on conjunctiva exists, but somehow weak. However, it must have an interconnection with a general circulation system to reach far body parts.

According to Freeman and Troutt (1985), the fluorescent matter in the conjunctiva of calves joined first to parotid lymph node and then to the retropharyngeal lymph node. In our rat study, apparently, no images obtained at retropharyngeal lymph node or in the parotid lymph node regions, perhaps either image could not be caught on right time interval or other obstacles (skin thickness, shift on the emission wavelength, etc.) prevented observation.

In light the of the data above, the study supports the views that the conjunctiva is rich in lymphatic vessels. Lymphatic system of the eye takes an active role in the drainage of intraocular pressure. The parotid and submandibular lymph nodes can possibly contribute drainage of the lymphatic liquid in the conjunctiva.

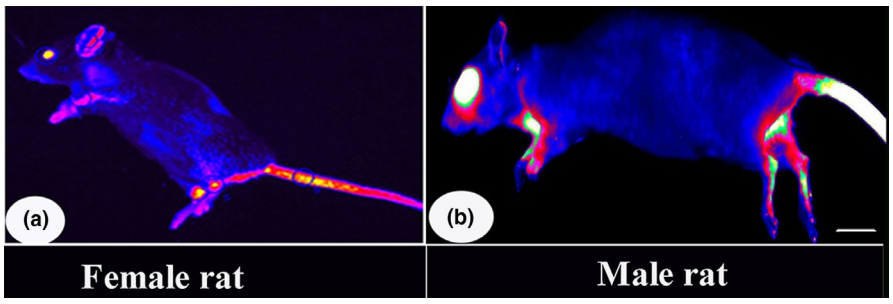

FIGURE 4 Expansion of fluorescence signal to the other body parts at the 60th minute following after the Qdot655 application. Distribution of fluorescent signal was clearly shown on the ears, forearms, hind legs and tail of the female (a) and male (b) rats. Scale, 10 mm. 160 × 5.27 mm (300 dpi)

Somehow, Qdot655 within the conjunctiva passes to lymphatic drainage and then possible joins to the general circulation system via the lymphoid system or via the lymphovenous anastomosis.

This study indicated that conjunctiva interconnected with almost all parts of the body (from the eye to tail etc.). So in the future, the eye (conjunctiva) could be possibly used the administration of certain drugs and tracers, etc. to the other parts of the body for perhaps therapy or scanning.

5 | CONCLUSION

The current investigation considered as the first study suggests that the conjunctival lymphatic drainage was associated with the whole body's circulatory system approximately within an hour in the rat model. Here, we also report that drops to the eye can reach far extremities such as the ears, forearms, hind legs and tail.

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CONFLICT OF INTEREST

None.

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