Revealing acupuncture meridian-like system by reactive oxygen species visualization

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Abstract To investigate the reactive oxygen species (ROS) distribution in living animal tissues, two ROS indicators, dichlorofluorescin diacetate (DCFH-DA) and MitoSOX™ Red were applied to visualize ROS on the frontal interior abdominal wall of living SD-rats by tail vein injection and local smearing respectively. Revealed was an unexpected ROS distribution pattern. ROS were demonstrated in a few vertical fluorescent lines, which related to neither veins nor nerves but could be almost perfectly superimposable on a standard human acupuncture meridian network. The phenomenon that cells with high ROS content should be aligned in a regular manner is interesting as well as its resemblance to meridian system.

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Reactive oxygen species (ROS) are a variety of molecular oxygen-derived molecules and free radicals playing important roles from cell signaling to oxidative stress and cellular damages [1]. As an efficient approach in intracellular ROS studies, various ROS indicators have been developed. While dichlorofluorescin diacetate (DCFH-DA) emits green fluorescent light upon interaction with ROS after being hydrolyzed into 2',7'-dichlorofluorescin (DCFH) by mitochondrial esterase [2], MitoSOX Red emits red fluorescent light when oxidized by mitochondrial superoxide [3]. In an attempt to investigate the ROS distribution in living animal tissues, those two indicators were employed to visualize ROS on the frontal interior abdominal wall of living SD-rats. An unexpected pattern of ROS distribution was revealed, on which a standard human acupuncture meridian lines could be almost perfectly superimposed.

Male SD rats of 6 weeks were used. 1 ml of DCFH-DA solution (10 mg in 1 ml dimethyl sulfoxide) was injected through the rat tail vein. 30 minutes later, the rat frontal abdominal wall was incised from over iliac crest then along the midaxillary line on both sides up to the rib bone so that the interior wall could be exposed for fluorescent imaging. On the other hand, the interior abdominal wall thus exposed was applied with MitoSOX Red reagent (100 µg in 1 ml dimethyl sulfoxide) by direct smearing with living rats without any other prior treatments.

As is demonstrated in Fig. 1, fluorescent imaging was carried out with the exciting lights, which were generated by an UltraFire MCU WF-1200L lamp through band-pass filters of 488 nm for DCFH-DA and 510 nm for MitoSOX Red reagent, respectively. The fluorescence images of the...
interior abdominal wall were collected by a NIKON (model D-80) camera equipped with band-pass filter of 525 nm for DCFH-DA and 588 nm for MitoSOX Red reagent, respectively. The experiments were repeated 6 times, and the representative data are presented here.

**Figure 2** A image of a rat with its exposed interior abdominal wall after the injection of DCFH-DA solution. The apparent highlights were the reflected light. The obtained fluorescent image of Fig. 2A is shown in Fig. 2B, in which five green fluorescent lines were revealed on the interior abdominal wall the rat. While line 1 represents the outburst of oxidative stress along the edge of the abdominal wall due to incision, lines 2–5 can be interpreted as nothing but the unusual distribution of intracellular ROS in the form of lines in the abdominal wall or the existence of lines consisting of cells under high oxidative stress. As is shown in Fig. 3B, line 6 and line 7, two broadened red fluorescent lines were revealed by direct smearing of MitoSOX on the abdominal wall of a rat in Fig. 3A, line 6 and line 7 correspond to line 4 and 5 in Fig. 2B, respectively, confirming the unusual pattern of ROS distribution. As a result of the intravenous delivery of higher dosage of DCFH-DA which interacts with more kinds of ROS, two broadened red fluorescent lines were revealed by direct smearing of MitoSOX on the abdominal wall of a rat in Fig. 3B, line 6 and line 7 correspond to line 4 and 5 in Fig. 2B, respectively, confirming the unusual pattern of ROS distribution. As a result of the intravenous delivery of higher dosage of DCFH-DA which interacts with more kinds of...
ROS than superoxide-specific MitoSOX, a stronger fluorescence was generated, which may be accounted for the better visualization in Fig. 2B than Fig. 3B. Furthermore, while ROS pattern in Figs. 2B and 3B resemble neither abdominal veins nor nerves, it is almost perfectly superimposable on the human acupuncture meridian network shown in Fig. 4 [4], with Line 2 for the spleen meridian, 3 for the stomach meridian, 4 and 6 for the kidney meridian and conception vessel, and 5 and 7 for the symmetric stomach meridian (Fig. 4).

Much more work is necessary before any conclusion can be made about the connection between the revealed ROS pattern and the acupuncture meridian. However, the fact that ROS visualization could generate meridian-like image which is more complete and relevant than most meridian images obtained by various modern approaches [5], suggests that this can be a promising new clue to meridian research. After all, regardless its possible meridian connection, it would be of great interest to elucidate the reasons behind the unusual pattern of ROS distribution in living rat, furthermore to investigate the function of the ROS containing cellular network, or to answer simple questions such as how those cells could tolerate the oxidative stress as severe as indicated by ROS imaging results.

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Conflict of interest

With the submission of this manuscript I would like to undertake that the above-mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that my Institute’s representative is fully aware of this submission. All experiments were approved by Experimental animal Ethics Committee of Fuzhou University.

I declare that there are no conflicts of interest of any kind.

References