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Original article

Regulation of oxidative stress inside living cells through polythiophene derivatives

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ABSTRACT

Oxidative stress stimulated by angiotensin II (Ang II) plays an important role in the progression of inflammation and cardiovascular disease. In this work, polythiophene modified with dihydropyridine groups (PTDHP) realized the control of oxidative stress induced by Angiotensin II stimulation in living cells, by inhibiting the activity of NADPH oxidase *via* DHP groups. Upon light irradiation, the PTDHP could sensitize surrounding oxygen molecules to generate reactive oxygen species (ROS). The generated ROS oxidized the pendant DHP of polythiophene into pyridine group, which inactivated the control ability of DHP to oxidative stress in living cells. Thus, PTDHP can not only control the intracellular oxidative stress effectively and suppress ROS to some degree in dark, but also regulate its anti-oxidative effect under light irradiation.

1. Introduction

Angiotensin II (Ang II) occupies a vital role in renin–angiotensin system (RAS), which acts as an endocrine system and plays a primary role in renal physiology and cardiovascular [1]. The over-activation of RAS would bring out the progression of atherosclerosis, cardiac disease, diabetes, hypertension and renovascular disorder [2–5]. Furthermore, Ang II, an essential mediator in regulation of blood pressure, could promote oxidative stress *via* increasing the reactive oxygen species (ROS) generation by interaction with Ang II receptor-1 (AT1) to activate nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) that is an essential source of ROS production in vascular cells [6]. At physiological low concentration, ROS is one kind of important second messenger in intracellular signaling and regulation [7,8]. While, high level ROS can induce intracellular oxidative stress resulting in inhibition of protein function and DNA damage [9,10]. Oxidative stress induced by Ang II is related to a significant cause of inflammation and cardiovascular disease. Besides, the over-activation of Ang II can lead to the expression of monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1) and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) which were reported to critically be involved in inflammation and ROS generation in vascular cells as well [11–13].

Dihydropyridine derivatives (DHPs) can not only regulate the influx of calcium ions but also possess the vasoprotective effects response to cardiovascular events, as DHP can depress the production of ROS effectively induced by Ang II through inhibition of the activity of NADPH oxidase [14,15]. As a result, DHP holds the potential to regulate the intracellular oxidative stress and decrease the proliferation of inflammation, and may be employed as a powerful tool to prevent the progression of heart disease [16–19].

Conjugated polymers (CPs), formed by a large number of chromic repeat units, exhibit significant optical properties and the ability of easy modification, and provide an excellent platform for imaging and detection [20–26]. Besides, CPs can harvest the energy of light efficiently and sensitize the surrounding oxygen molecules to generate ROS, thus, CPs are employed to inhibit the proliferation of cancer cells and used as antimicrobial [27–29]. Polythiophene modified with dihydropyridine (PTDHP) was reported owning ROS-scavenging ability *via* oxidizing dihydropyridine into pyridine [30]. In this work, we utilize PTDHP to realize the control of intracellular oxidative stress induced by Ang II stimulation and the light regulation through ROS generated from polythiophene.

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2. Experimental

2.1. Ang II incubation time experiment

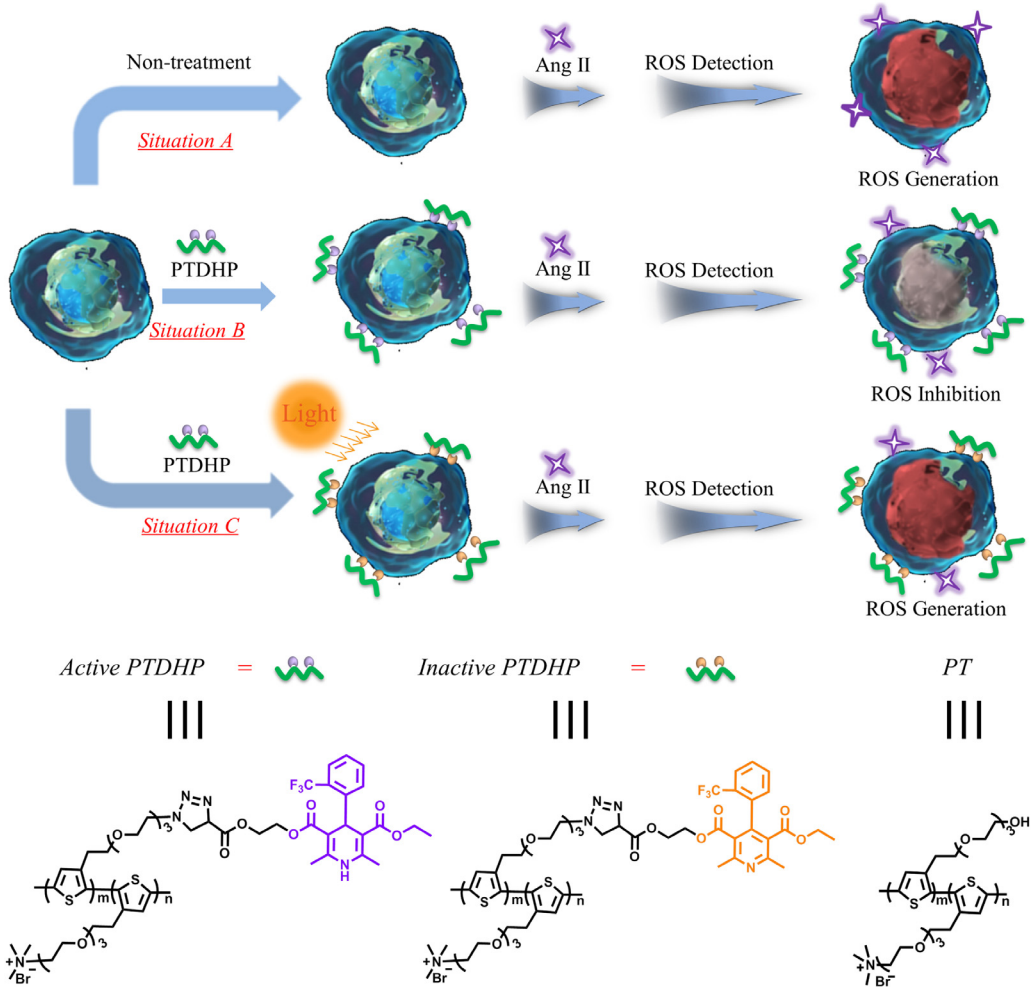
Rat aortic endothelial cells were obtained from Pricells (Wuhan, China), and cultured in medium special for primary cells. After reaching 80% confluence, cells were incubated with conditioned medium at 37 °C for 24 h before the experiment for inducing quiescence. Then new conditioned medium was exchanged for next steps. At the beginning of the experiment, rat aortic endothelial cells were incubated with Ang II (1×10^{-7} mol/L) for 0, 3, 6, 8, 10 h, respectively, in order to induce the production of ROS. As the positive control, cells were incubated with H_2O_2 (1×10^{-4} mol/L) only. Dihydroethidium (DHE) (5×10^{-6} mol/L) was applied to determinate the concentration of ROS. The cells were washed by PBS for three times and incubated with DHE in PBS for 30 min in dark. Images were obtained by CLSM with the excited wavelength of 559 nm and analyzed by scanning densitometry.

2.2. PTDHP antioxidant and light regulation experiments

Rat aortic cells were cultured as above. In the primary experiment, following incubated with PTDHP (2×10^{-5} mol/L) for 1 h and the endothelial cells Ang II (1×10^{-7} mol/L) for 8 h. For control of experimental condition, DHP (5×10^{-6} mol/L) and PT (2×10^{-5} mol/L) were incubated with cells, respectively. For blank control group, cells were incubated with Ang II only. For light group, after incubated with PTDHP, cells were exposed to white light with the dose of 0.4 mW/cm^2 for 10 min. DHE (5×10^{-6} mol/L) was applied to terrify the concentration of ROS. All cells were washed by PBS for three times and incubated with DHE in PBS for 30 min in dark. Images were obtained by CLSM with the excited wavelength of 559 nm and analyzed by scanning densitometry. Here, the white light source used in this work was equipped with a metal halogen lamp (MVL-210, Mejiro Genossen, Japan), and a radiometer (Photoelectric Instrument Factory of Beijing Normal University) was used to estimate the intensity of the incident beam.

3. Results and discussion

Scheme 1 shows the mechanism of inhibition of oxidative stress induced by Ang II *via* polythiophene and regulation of antioxidative ability by light. Stimulation of Ang II will promote oxidative stress by increasing ROS generation in rat aortic endothelial cells, and hence cells can be stained by ROS probe obviously (situation A). However this process can be prevented by PTDHP, accounting to the antioxidative ability of DHP, which have been demonstrated that DHP derivatives could reduce the generation of ROS by the inhibition of the overexpression of nicotinamide adenine dinucleotide (NADPH) oxidase in response to Ang II stimulation (situation B). When exposed to white light, the anti-oxidative potency was



Scheme 1. The regulation of Ang II-mediated ROS generation in living cells in the absence (situation A) and presence (situation B) of PTDHP, and the regulation of anti-oxidative effect under light (situation C).