A rapid and highly predictive chemiluminescent cell-based assay to monitor intracellular xanthine oxidase activity for the screening of natural inhibitors in living endothelial cells

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Abstract

Xanthine oxidase (XO), an enzyme expressed at high levels in vascular endothelium, catalyses the oxidation of hypoxanthine to uric acid, linked to gout and cardiovascular diseases. Testing the inhibition of XO is crucial to identify potentially effective natural products that could be used to treat several diseases. Herein, we report the development of a fast, reliable and highly predictive chemiluminescent (CL) bioassay to determine XO activity into living endothelial cells and its use to obtain quantitative information on the XO inhibiting activity of natural products of great interest in the nutraceutical field. The method is based on the intracellular CL emission of luminol, caused by XO-derived H2O2, employing Fe2+-EDTA complex as catalyst in 0.1M borate buffer (pH=10.3). The assay, which could be completed in less than 30 minutes and displayed a limit of detection of 0.4μU/mL, allowed measuring intracellular XO activity, which was assessed to be (1.3±0.3)×10-7mU/cell in human vascular endothelial cells (HUVEC). Next, to demonstrate the applicability of the CL cell-based assay to screen potential XO inhibitors, HUVEC cells were treated with different natural extracts, utilizing oxypurinol, the active metabolite of the drug allopurinol, as standard (IC50 152±76 ng/mL). The capability of several natural products to inhibit XO activity was evaluated, obtaining an IC50 of 28±4μg/mL and 14±3μg/mL for *Ganoderma Lucidum* and *Cordyceps Sinesis fungi*, respectively; 0,86±0,07μg/mL and 1,27±0,08μg/mL for ultrasonic-assisted extracted (UAE) and Naviglio®-assisted extracted (NAV) winemaking by-products, respectively; 120±50ng/mL and 150±40ng/mL for *yeast* fermented papaya (FPPA) and *Lactic Acid Bacteria* fermented papaya (FPPB), respectively. Since the bioavailability of the compounds, especially the ability to cross cell plasma membranes, is an important issue to take into consideration for nutraceutical applications, this method, which employs whole cells, is highly representative and predictive to study intracellualr XO inhibitors *in vitro*.

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