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Penetration of resveratrol into bovine aortic endothelial cells (BAEC): A possible passive diffusion

Pénétration du resvératrol dans les cellules endothéliales aortiques bovines (BAEC) : une possible diffusion passive

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ABSTRACT

Several studies have demonstrated that, in a context of oxidative stress, resveratrol, a polyphenol found in wine, could act as a protective agent on endothelial cells by various mechanisms but without showing that it could penetrate inside the cell. The aim of this study was to detect for the first time resveratrol inside bovine endothelial aortic cells and to determine which kind of transport mechanism was involved. Intracellular and membrane concentrations of resveratrol have been measured by high performance liquid chromatography after incubation of several concentrations of resveratrol with endothelial cells for 24 h. Concentrations of resveratrol in the culture media have been determined by UV spectrophotometry and experiments of transport mechanisms have been performed. Our results showed that, for the concentrations tested (1, 5, 10 and 50 μM), resveratrol was detected inside the cells and suggested that it was able to penetrate into the cells through a passive diffusion mechanism.

Mots clés :
Cellules endothéliales
Chromatographie liquide haute performance
Stress oxydant
Resvératrol

RÉSUMÉ

De nombreuses études ont montré que, dans un contexte de stress oxydant, le resvératrol, un polyphénol trouvé dans le vin, avait un rôle protecteur de la cellule endothéliale par divers mécanismes, sans toutefois prouver son passage intracellulaire. Cette étude avait pour but de détecter pour la première fois la présence de resvératrol dans la cellule endothéliale aortique bovine et de déterminer quel type de transport était impliqué. Les concentrations intracellulaires et membranaires de resvératrol ont été mesurées par chromatographie liquide haute performance après incubation des cellules avec différentes concentrations de resvératrol pendant 24 heures. Les concentrations de resvératrol dans le milieu de culture ont été déterminées par spectrophotométrie UV et des expériences de

Abbreviations: BAEC, bovine aortic endothelial cells; DMEM, Dubelcco's modified Eagle medium; FBS, fetal bovine serum; HPLC, high performance liquid chromatography; ROS, reactive oxygen species

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transport ont été menées. Nos résultats montrent que, pour les concentrations testées (1, 5, 10 et 50 μM), le resvératrol est détecté dans le milieu intracellulaire et suggèrent que le resvératrol pénètre dans la cellule par diffusion passive.

1. Introduction

Resveratrol is a natural polyphenol found in grapes [1]. Several studies have shown its protective effects on the vascular endothelium [2–4], which could be beneficial in cardiovascular diseases [5]. Over the last 10 years, cellular effects of resveratrol have been described, such as antioxidative [6], antiinflammatory [7] and antitumoral properties [8]. These effects involve different metabolic pathways, which could suggest that resveratrol is able to act both on extracellular and intracellular targets. Interaction of resveratrol with membrane receptors, such as estrogen receptor [9], leads to a series of signaling pathways, targeting for example the activation of the adenosine monophosphate-activated protein kinase (AMPK) or the silent mating type information regulation 2 homolog 1 (SIRT1), resulting in biological effects [10,11]. Antioxidative effects of resveratrol could be explained by its capacity to activate enzymatic defenses through membrane receptors, but also to directly scavenge free radicals in the cell. Resveratrol could thus be able to penetrate into the cell and to interact with reactive oxygen species (ROS) and other molecular targets [12,13].

Bioavailability of resveratrol and stilbene derivatives is currently discussed. Indeed, the hydrophobic properties of this molecule are not in favor of a good bioavailability. However, some studies conducted in humans have shown that resveratrol was detected in plasma after a moderate intake of red wine [14,15]. In these studies, resveratrol was quickly metabolized into trans-resveratrol-3-O-glucuronide and trans-resveratrol-3-sulfate that were secreted into the cell. However, only few studies have shown the presence of resveratrol in cellular models [16,17].

Thus, the protective effects of resveratrol have been described in several studies performed in vascular endothelium cells from various species in vitro, without showing if resveratrol was found inside the cell.

The aim of that study was to evaluate the ability of resveratrol to penetrate into bovine aortic endothelial cells (BAEC) in vitro after 24 h incubation, and to tentatively determine the active or passive way of transport.

2. Materials and methods

2.1. Cell culture

Bovine aortic endothelial cells (BAEC) were cultured in 75 cm^2-flasks, in Dubelcco's Modified Eagle Medium (DMEM) without phenol red (Sigma Aldrich) supplemented with 10% FBS (Invitrogen), 1% L-glutamine (Sigma Aldrich), 1% antibiotics (penicillin/streptomycin, Sigma Aldrich) at 37 °C, in a humidified 5% CO_2 incubator, until they reached 80% confluence.

2.2. Cytotoxicity assay

To evaluate the toxicity of resveratrol (purity ≥ 98%, Coger) on BAEC, a neutral red assay in 96-well plate was performed.

A 50 mM stock solution of resveratrol was prepared in ethanol (Sigma Aldrich). Cells were incubated with 200 μL of various concentrations of resveratrol (1, 5, 10 or 50 μM) diluted in DMEM (1% ethanol) for 24 h at 37 °C in a humidified 5% CO_2 incubator. One hundred microlitres of a solution of neutral red (Sigma Aldrich) were added to each well. Cells were incubated during 3 h at 37 °C in a humidified 5% CO_2 incubator. The plate was emptied by reversal. One hundred microlitres of a solution of formol-calcium (Sigma Aldrich) were distributed in each well and let in contact with the cells during 1 min. The plate was again emptied by reversal and 100 μL of a solution of ethanol with acetic acid (Sigma Aldrich) were added to each well. The plate was let under stirring during 5 min and absorbance was then measured at 540 nm on a microplate reader (MultiskanEx, Thermo Electron Corporation).

2.3. Treatments

All treatments were made in DMEM without phenol red, supplemented with 1% FBS, 1% l-glutamine, 1% antibiotics (penicillin/streptomycin), 1% ethanol. A 50 mM stock solution of resveratrol was prepared in ethanol (Sigma Aldrich). Cells were incubated with 15 mL of various concentrations of resveratrol (1, 5, 10 or 50 μM corresponding respectively at 15, 75, 150 and 750 nmoles) for 24 h. Then, they were trypsinized, washed with ice-cold phosphate-buffered saline (PAA Laboratories) by centrifugation during 10 min, at 1500 g and at 4 °C and harvested into 0.2 mL of lysis buffer (Sigma Aldrich). Supernatants were conserved and cell pellets were transferred into an Eppendorf tube before centrifugation at 3000 g during 15 min to separate the cytosolic and the membrane fractions.

2.4. Determination of total protein concentration

For each experimental condition, protein concentration was determined by Biorad DC Protein Assay kit.

2.5. Determination of resveratrol intracellular and membrane concentrations by HPLC

Measurements of resveratrol in intracellular and membrane fractions were determined by reverse-phase High Performance Liquid Chromatography (HPLC) with UV detection (304 nm) on a C18 5-μm Kromasil column (AIT)