PROCEEDING

Evaluation of anti-stress nutrients in the endothelial cells with fluorescence indicator

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Abstract : Oxidative stress has emerged as an important pathogenic factor in the development of long-term complications, such as hypertension, atherosclerosis, nephropathy, and cancer. Taking many antioxidants from natural food may be effective to prevent us from those diseases. We have attempted to evaluate the effect of improvement by dietary antioxidants on the endothelial dysfunction induced by hyperglycemia. Fluorescence indicators for reactive oxygen species and nitric oxide were employed to the evaluation. The combination of those fluorescence indicators could be powerful tool to evaluate the effect of anti-stress nutrients on both oxidative stress and endothelial dysfunction. J. Med. Invest. 52 Suppl. :295-296, November, 2005

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INTRODUCTION

Oxidative stress has emerged as an important pathogenic factor in the development of long-term complications, such as hypertension, atherosclerosis, nephropathy, and cancer. Recently, huge numbers of researches have focused to discover antioxidants and to develop diet or drug containing the antioxidants to decrease oxidative stress in the body (1). Nutritional approach should be physiologically and economically reasonable to decrease oxidative stress involved in daily life and to prevent us from oxidative stress-related diseases.

For instance, oxidative stress mediated by hyperglycemia inhibits nitric oxide (NO) production as well as increase in DNA damage, inflammation and membrane lipid oxidation (2) . These adverse effects of hyperglycemia on the endothelial cells have been thought to be involved in diabetic vascular complications.

Recently, many antioxidants have been reported to decrease oxidative stress in endothelial cells and improve the endothelial dysfunctions (3) . However, many dietary antioxidants have been discovered, but the improvement activity on endothelial dysfunction is not always evident.

In this study, we evaluated the activity of dietary components on the improvement of endothelial dysfunction mediated by oxidative stress using fluorescence reactive oxygen species (ROS) indicator and NO indicator.

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METHODS

Cell culture.

Endothelial cells (BAECs) were isolated from the descending thoracic aorta of a bovine fetus by briefly digesting its intimal lining with collagenase

(4). The cells were grown in M199 medium supplemented with 20% fetal bovine serum, 50 IU/ml of penicillin, and 50 μg/ml of streptomycin, and were routinely passaged before the cells reached confluence. In all experiments, we used subconfluent BAECs that were passaged within 10 times.

Estimation of ROS and NO

APF and DAF-2DA were generally used as according to previous publications (4, 5) To estimate ROS production, BAECs were seeded on 35 mm glass bottom dishes. After 2days, they were incubated with 10 μM APF or DAF-2DA and 0.05% pluronic F127 for 30 min in Hank's balanced salt solution (HBSS), after which the dish was mounted on the inverted stage of a Leica confocal microscope (TCS-SL). The cells were excited at 488nm, and emissions at 500-540 nm were acquired every 5 min. Cells of interest (COI) were traced on the monitor and fluorescence intensity was measured using an accessory program that was built into the confocal system. Fluorescence intensity was expressed as arbitary unit (A.U.)

APF-loaded BAECs were immersed in HBSS containing 4.5 mM glucose, after 20 min, the concentration of Pi or glucose was arose from control (0.9 mM glucose) to high glucose (25 mM) , after which ROS production was estimated using APF. To determine the effect of hyperglycemia on the NO production, BAECs were pre-incubated in high glucose (25mM) or control (4.5mM glucose) medium for 48h, after which they were loaded with DAF-2DA and estimated NO concentration by adding 100 μM of ATP.

RESULTS AND DISCUSSION

After addition of high glucose (final concentration 25 mM) in the medium, the fluorescence intensity of APF was markedly increased in the BAECs. The significant increase was observed at 10 min after increase in glucose level, and the increase continued to 80min. On the other hand, in the control medium (4.5 mM glucose), the APF fluorescence level was never changed. Furthermore, no increase of APF intensity was observed under high mannitol condition (25 mM mannitol).

We also evaluated NO production in response to bradykinin in the BAECs using DAF-2DA. Pre-exposed BAECs to high glucose (25 mM) for 3days did not show increase of DAF-2 fluorescence intensity in response to 100 μM bradykinin, but control cells did. Dietary antioxidants ameliorated increase in ROS production and suppression of NO production mediated by glucose loading. Therefore, combination of those two methods that we designed can evaluate the magnitude of both oxidative stress and endothelial dysfunction. We proposed our system would be useful tools to evaluate the effect of dietary antioxidants on the endothelial dysfunction mediated by hyperglycemia and other stress factors.

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