

Anti-atherosclerotic Ingredients of Foods Regulate Y2 Receptor Gene Expression

Hidesuke Kaji^{a, b}, Masayo Nagai^b

a) Division of Pathophysiology, Kobe Women's University, Kobe 650-0046, Japan

b) Division of Physiology and Metabolism, University of Hyogo, Akashi 673-8588, Japan

Corresponding author:

Hidesuke Kaji, M.D., Ph.D.

Address: 4-7-2, Minatojima-nakamichi, Chuo-ku, Kobe 650-0046, Japan

TEL: 81-78-303-4939

Abstract

It has been reported that chronic stress, combined with high-fat/high-sugar diet, causes metabolic syndrome by neuropeptide Y (NPY) through NPY Y2 receptor (Y2R). We have previously reported the causal relation between serum HDL-cholesterol levels and single nucleotide polymorphisms (SNPs) on the regulatory region of Y2R gene. This study aims to investigate whether drug or ingredients of foods with anti-atherosclerotic functions influence Y2R gene expression through which NPY might promote atherosclerosis in HepG2 and prevent it in macrophages. Effect of varying ingredients of foods or drugs on mRNA levels of Y2R in cultured HepG2 or on mRNA levels of ABCA1, ABCG1 and Y2R in cultured THP-1-derived macrophages was examined by using real time PCR methods. Chlorogenic acid (CGA) and zeaxanthin significantly upregulated, and isoflavone and astaxanthin significantly downregulated Y2R mRNAs in HepG2. CGA and protocatechuic acid (PCA) (microbiota metabolite of anthocyanin) significantly upregulated mRNA levels in macrophages.

Isoflavone, astaxanthin, anthocyanin/PCA and CGA might prevent atherosclerosis from metabolic syndrome under chronic stress, combined with high-fat/high-sugar diet especially in subjects having Y2R gene promoter with SNPs able to transcribe in hepatocytes or macrophages.

Keywords: Neuropeptide Y, Y2 receptor, HepG2, macrophage, mRNA, anti-atherosclerotic ingredients

1. Introduction

It is well known that stress causes catecholamine release from sympathetic neuron and cortisol secretion from adrenal cortex. It has been reported that chronic stress, combined with high-fat/high-sugar diet, shifts sympathetic signaling toward neuropeptide Y (NPY) and leads to obesity and metabolic syndrome through one of the NPY receptors, Y2 receptor (Y2R) (Kuo LE et al. 2007, 2008). We have previously reported that single nucleotide polymorphism (SNP)s of Y2R gene upstream are correlated with serum high-density lipoprotein-cholesterol (HDL-C) levels in healthy subjects (rs6857530; GG<CA<AA or rs6857715; TT<TC<CC) (Takiguchi E et al. 2010). Transcriptional activity could be achieved by transiently transfecting the pGL3 luciferase reporter vector inserted by Y2R gene promoter including SNPs rs6857530GG plus rs6857715TT but not those including rs6857530AA plus rs6857715CC in cultured human hepatocyte HepG2 (Okada M et al. 2015). These transcriptional activities could not be observed

in the cell lines of adipocytes, macrophages, and endothelial cells of blood vessels. We have previously reported the effect of Y2R specific antagonist BIIE0246 on gene expression in HepG2 assessed by a microarray analysis, suggesting that Y2R may be involved in the promotion of cholesterol synthesis pathway and suppression of HDL metabolic pathway in HepG2 (Kaji H et al. 2016).

On the other hand, transcriptional activity could be achieved by transiently transfecting the pGL3 luciferase reporter vector inserted by Y2R gene promoter including SNPs rs6857530AA plus rs6857715CC but not those including rs6857530TT plus rs6857715GG in cultured THP-1-derived macrophages (Okada M et al. 2015). These transcriptional activities could not be observed in the cell lines of adipocytes, hepatocytes, and endothelial cells of blood vessels. We have previously reported the effect of BIIE0246 on gene expression in macrophages assessed by a microarray analysis, suggesting that Y2R may be involved in leptin signal suppression in macrophages (Kaji H et al. 2016). Leptin is known to inhibit reverse cholesterol transfer via activation of acyl coenzyme A: cholesterol acyltransferase 1 (ACAT1) in macrophages (Hongo S et al. 2008), so NPY/Y2R may promote reverse cholesterol transfer via suppression of leptin induced ACAT1 activation in macrophages.

This study aims to investigate whether ingredients of foods (Amarowicz R et al. 2017, MarcoMatteo C et al. 2013, Kishimoto Y et al. 2016, Yamagata K. 2019, Qin Y et al. 2009, Xia M et al. 2005, Du C et al. 2015, Wu C et al. 2014) or drug (Jiang M et al. 2017, Ogata M et al. 2009, Ozawa H et al. 2011) with anti-atherosclerotic functions influence hepatic or macrophage Y2R gene expression through which NPY might promote or prevent atherosclerosis from metabolic syndrome under chronic stress, combined with high-fat/high-sugar diet.

2. Materials and Methods

Human hepatocyte HepG2 and human monocyte THP-1 were obtained from JCRB Cell Bank, National Institute for Medical Science and Health and Nutrition, Japan. The nucleotide sequence of Y2R gene upstream region in each cell heterogeneously included SNPs for cell-specific Y2R gene transcription (Kaji H et al. 2016a, 2016b). HepG2 cells and macrophages were cultured in Dulbecco's Modified Eagle Medium containing 10% fetal calf serum (FCS) and in RPMI1640 medium containing 10% FCS at 37 °C under 5% CO₂, respectively. Macrophages were differentiated from THP-1 by phorbol ester. Effect of various anti-atherosclerotic ingredients of foods and drug on Y2R as well as ATP-binding cassette A1 (ABCA1) and ATP-binding cassette G1 (ABCG1) was compared with the vehicle-alone control. ABCA1 and ABCG1 are transporters involved in reverse cholesterol transfer from macrophages to HDL. Primers for Y2R, ABCA1, ABCG1, and β -actin were synthesized (Biological Co., Japan) to amplify each mRNA by real time PCR:

Y2R (forward) ATCTTGTTTCCGCGTCTCC, (reverse) TTCCACCTTCATTTCTTCCAC, ABCA1 (forward) GGCCTACCAAGGGAGAACT, (reverse) TGTCATCACATGTCACAT CCA, ABCG1 (forward) GTGTCCGCACATCTGAAGC, (reverse) AGCGCTGTCAGTATC TCGTTG, β actin (forward) CCAACCGCGAGAAGATGA, (reverse) CCAGAGGCGTACAGG GATAG.

Real Time ready Cell Lysis kit was used for cell lysis. Transcriptor universal cDNA Master was used for reverse transcription. Fast Start Essential DNA Green Master was used for real time PCR. Each kit was purchased from Roche Diagnostics, USA. Real time PCR was performed using Light Cycler Nano (Roche Diagnostics, USA). β -Actin was used as an internal control. Anti-atherosclerotic ingredients and drugs were obtained from Wako Pure Chemical Industries, Japan, and Tokyo Chemical Industry, Japan. Statistical significance of the differences was analyzed by Mann Whitney U or Kruskal Wallis test using SPSS (IBM, Co, Japan). $P < 0.05$ was considered significant.

3. Results and Discussion

3.1. Y2R gene modulation by ingredients in hepatocytes

Chlorogenic acid (CGA) (a phenolic acid among polyphenols), and zeaxanthin (a carotene), at a dose of $10\mu\text{M}$ significantly upregulated Y2R mRNA levels in cultured HepG2 cells, while isoflavone (a flavonoid) and astaxanthin (a xanthophyll) significantly downregulated Y2R mRNA levels (Table 1).

Among anti-atherosclerotic ingredients, CGA and zeaxanthin might enhance, while isoflavone and astaxanthin might degrade atherosclerotic NPY action through Y2R in hepatocytes. These results suggest that isoflavone and astaxanthin might prevent atherosclerosis from metabolic syndrome under chronic stress, combined with high-fat/high-sugar diet especially in subjects with Y2R gene promoter SNPs (rs6857530GG plus rs6857715TT).

3.2. ABCG1/ABCA1 gene modulation by ingredients or Y2R agonist in macrophages

Anthocyanin (a flavonoid among polyphenols) is absorbed and then metabolized to protocatechuic acid (PCA) by gut microbiota [Wang D et al. 2012]. PCA at a dose of $10\mu\text{M}$ significantly increased ABCG1 but not ABCA1 mRNA levels in THP-1-derived macrophages. The result was partly consistent with the previous other's report that PCA has the anti-atherosclerotic effect by enhancing reverse cholesterol transfer from macrophages on the vascular endothelium via transporters ABCA-1 and ABCG-1 (Xia M et al. 2005). Ten μM CGA, the other ingredient known to enhance reverse cholesterol transfer [Wu C et al. 2014], failed to change mRNA levels of ABCA1, and ABCG1 (Fig. 1). A specific agonist of Y2R, peptide YY (3-36) (PYY3-36), failed to change mRNA levels of ABCA1 and ABCG1 at doses of up to 200nM (data not shown). The result was consistent with our previous report using microarray analysis (Kaji H et al. 2016). Y2R independent upregulation of ABCG1 expression might be involved in the anti-atherosclerotic function of PCA.

3.3. Y2R gene modulation by ingredients or drug in macrophages

The effect of $10\mu\text{M}$ CGA and PCA on Y2R mRNA levels was examined in THP-1-derived macrophages. Both PCA and CGA significantly increased Y2R mRNA levels, in which the effect of PCA was much more prominent than CGA (Fig. 2). We have previously reported that BIIE0246 stimulates leptin signaling pathway in macrophages assessed by microarray (Kaji H et al. 2016). It has been reported by others that leptin inhibits ACAT1 and reverse cholesterol transfer (Hongo S et al. 2008). Taken together, NPY-Y2R inhibits leptin signaling pathway with ACAT1 activation, thereby stimulates reverse cholesterol transfer. Based on these previous and

present findings, PCA and CGA with anti-atherosclerotic function might enhance anti-atherosclerotic NPY action through Y2R gene upregulation in macrophages. These results suggest that PCA and CGA might prevent atherosclerosis from metabolic syndrome under chronic stress, combined with high-fat/high-sugar diet especially in subjects with Y2R gene promoter SNPs (rs6857530AA plus rs6857715CC).

Thiazolidinedione (agonist of peroxisome proliferator activated receptor γ : PPAR γ) at a dose of 5 μ M failed to change mRNA levels of ABCA1, ABCG1 and Y2R in THP-1-derived macrophages (data not shown). The result was consistent with the recent report (Jiang M et al. 2017) but not with the previous reports that PPAR γ agonist increased ABCA1 and ABCG1 expression (Ogata M et al. 2009, Ozawa H et al. 2011). An amelioration of insulin resistance may be mainly involved in the anti-atherosclerotic mechanism of thiazolidinedione.

In conclusion, CGA and zeaxanthin upregulated, while isoflavone and astaxanthin downregulated atherosclerotic Y2R gene expression in HepG2. Isoflavone and astaxanthin might prevent atherosclerosis from metabolic syndrome under chronic stress, combined with high-fat/high-sugar diet especially in subjects having Y2R gene promoter with SNPs (rs6857530GG plus rs6857715TT). PCA and CGA upregulated anti-atherosclerotic Y2R gene expression in THP-1-derived macrophages. Anthocyanin/ PCA and CGA might prevent above mentioned atherosclerosis in subjects having Y2R gene promoter with SNPs (rs6857530AA plus rs6857715CC). Further in vivo study is required to confirm these findings and apply to personalized preemptive medicine.

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Table 1 Effect of varying anti-atherosclerotic ingredients on relative Y2R mRNA levels in cultured human hepatoma cells HepG2.

	Mean	SD	P-value vs. control
control	1	0.99	
chlorogenic acid	62.1	63.6	0.009
zeaxanthin	40.6	52.8	0.021
isoflavone	0.56	0.78	0.023
astaxanthin	0.018	0.035	0.002

Representative result among 3 determinations was shown. Statistical significance of difference was assessed by Kruskal Wallis test: $P < 0.05$ was considered as significant.

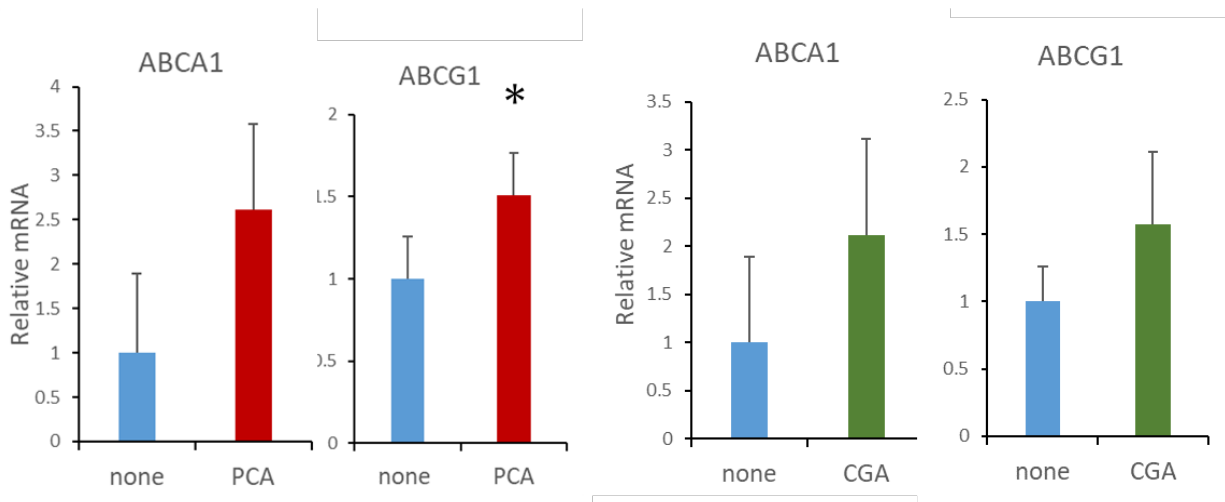


Fig. 1 Effect of protocatechuic acid (PCA) or chlorogenic acid (CGA) on ABCA1 or ABCG1 mRNA levels in cultured THP-1 derived macrophages. Each bar represents mean±SD (n=5). Representative result among 3 determinations was shown. *: P < 0.05 vs. control (none): Difference of significance was statistically analyzed by Mann Whitney U test.

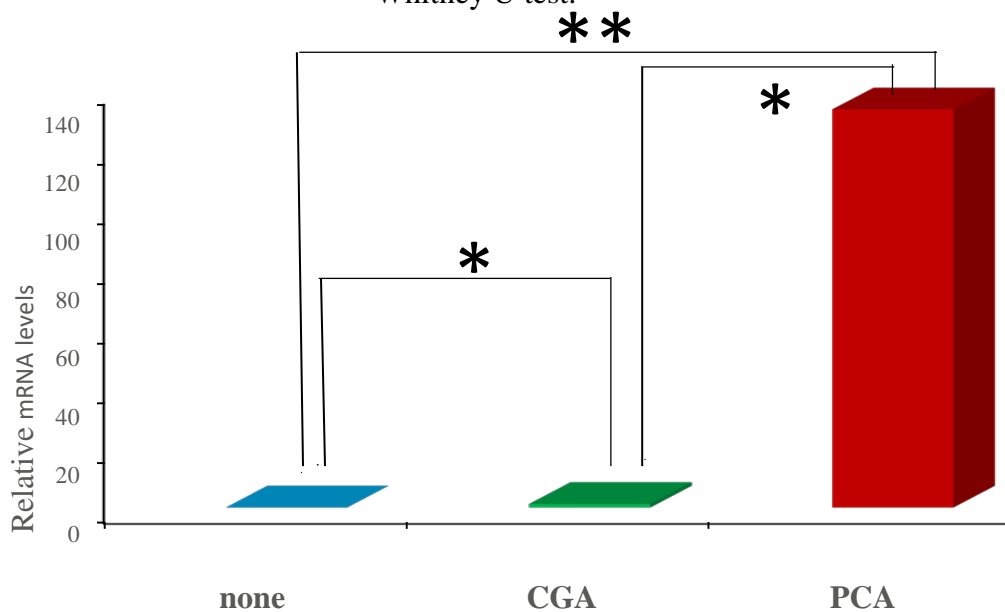


Fig.2 Effect of protocatechuic acid (PCA) or chlorogenic acid (CGA) on Y2R mRNA levels in cultured THP-1 derived macrophages. Each bar represents mean (n=5). Representative result among 3 determinations was shown. * P < 0.05, **: P < 0.01: Statistical significance of difference was analyzed by Kruskal Wallis test.