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Review

Molecular mechanisms of lipid- and glucose-lowering activities of bergamot flavonoids

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ABSTRACT

Bergamot (*Citrus bergamia* Risso et Poiteau) juice has a particularly high content and a unique composition of flavonoids. Neoeriocitrin, neohesperidin, naringin, melitidin and brutieridin represent more than 95% of Bergamot Polyphenol Fraction (BPF), while rhoifolin, diosmin, poncirin and others can be found in the remaining 5%. The brilliant performance of BPF in clinical practice against as a treatment for hyperlipidemia and moderate hyperglycemia in metabolic syndrome, awaits a plausible mechanistic explanation. Considering the overwhelming scientific evidence, it is likely that flavonoid components of BPF are responsible for majority of pharmacological effects. Here, we will review the scientific evidence showing that flavonoids, in particular citrus flavonoids present in bergamot fruits, influence lipid and sugar metabolism at the molecular level. Anti-diabetic and dyslipidemia-correcting effects of bergamot polyphenols may be explained by their ability to activate AMP kinase (AMPK), which is a central regulator of glucose and fatty acids metabolism and inhibit cAMP phosphodiesterases (PDE), involved in regulation of lipolysis in adipocytes and liver. Importantly, certain polyphenols can act as 3-hydroxy-3-methlglutaryl coenzyme A (HMG-CoA) reductase inhibitors, thereby mimicking statins action. In addition, flavonoids bind and act as natural inhibitors of quinone oxidoreductase 2 (QR2/NQO2) and other enzymes with potential roles in metabolic regulation. Finally, pleiotropic and possible synergistic effects may account for enhanced nutraceutical effects of natural flavonoid mixtures, such as BPF as compared to purified flavonoids.

Contents

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1. Introduction

Metabolic syndrome (MS) is a cluster of common cardiovascular risk factors, including, atherogenic dyslipidemia, insulin resistance or glucose intolerance, visceral obesity, hypertention and endothelial dysfunction. 10–30% of individuals in industrialized countries, including Italy, suffer from this condition. MS is associated with an increased risk of accelerated atherosclerosis and cardiovascular events [1]. Cardiovascular risk factors are also represented by different dyslipidemias, such as increased cholesterol levels (hypercholesterolemia) or increased level of tryglicerides (hypertryglicedemia), that occur separately or by diabetes. Experimental and epidemiological evidence suggest that dietary polyphenols, such as flavonoids may prevent atherosclerosis by counteracting its risk factors [2–4]. Accordingly, we should expect better clinical results by increasing the dosage and quality of consumed flavonoids. Indeed, a concentrated mixture of bergamot (*Citrus bergamia* Risso et Poiteau) flavonoids, so called Bergamot Polyphenol Fraction (BPF), shows brilliant results in clinical practice. BPF derived from bergamot juice has particularly high (40%) and unique composition of polyphenols, as discussed in Section 2.

Bergamot, as the endemic plant of Calabria, occupied and continues to occupy and important place in Calabrian economy as the main source for the production essential oil used in the cosmetic industry. However, the medicinal use of bergamot derivatives, forgotten for decades now is being rediscovered. For example, bergamot juice was considered by local population of Calabria, as a remedy for "fatty arteries" and heart diseases. This inspired Mollace and co-workers to address the efficacy of bergamot juice and then its derivative BPF in experimental and clinical settings [5]. Mollace et al. showed in animal models and human studies that BPF is effective in combating several symptoms of metabolic syndrome such as increased total cholesterol (totChol), LDL-cholesterol (cLDL) and trygliceridemia (TG), increased blood glucose levels and endothelial dysfunction in a group of metabolic syndrome patients. The effects on mean cholesterol parameters were compara

ble with a moderate dose of simavastatin (20 mg daily), including a marked increase in cHDL levels. In addition, 1000 mg BPF taken for 30 days reduced moderate hyperglycemia by more than 20% [5].

The brilliant performance of BPF on MS and dyslipidemia is astonishing and needs a mechanistic explanation. Unfortunately, so far there is very limited experimental evidence proving the modulation of one or another metabolic pathway by BPF itself. However, the vast scientific literature suggests that certain individual flavonoids present in BPF are implicated in the regulation of several metabolic enzymes, expressed in the liver, blood and endothelial cells. These regulation in many cases may be direct, *i.e.* mediated by the physical interaction of a receptor (specific enzyme) and the flavonoid compound and may lead to either inhibition or activation of the catalytic function of the enzymes. In addition, natural polyphenols show less specific, antioxidant properties, that depend on the free radicals scavenging ability of hydroxyl groups linked to carbon aromatic rings [6–8]. Together with antioxidant properties, dietary flavonoids and their metabolites may modulate basic signal transduction pathways of every cell leading to anti-proliferative, anti-aging and immune responses and other beneficial effects for human health, as discussed in vast literature on the subject [6,7]. Finally, besides intracellular, molecular effects, flavonoids in cooperation with pectins, can work at the level of intestine and liver to stimulate fat excretion and reduce fat absorption, which augments the direct activity on enzymes, involved in the regulation of carbohydrate and lipid metabolism [9–11].

Here, we will review the scientific evidence showing that citrus flavonoids, present in bergamot juice and albedo may influence lipid and sugar metabolism at the molecular level *via* AMPK, PDE and other enzymes modulation. In this regard we will focus on direct molecular receptors of flavonoids, identified by crystallography and computational chemistry studies. We will also discuss a possible contribution of flavonoids to intestine and liver physiology. At the end we propose that pleiotropic and synergistic effects in natural mixtures of polyphenols defined here as "fitocomplex" may account for their superior performance *in vivo* compared to purified flavonoids.

2. Bergamot flavonoids and their metabolism

Natural phenols are vast group of compounds produced by biological sources, that contain at least one hydroxylated aromatic ring [12]. For the purpose of this review we will define polyphenols as natural compounds with at least aromatic rings. They will include: flavonoids, stilbenes, coumarins, quinones, phenolic acids and others. Bergamot juice is particularly rich in flavanones and flavones belonging to flavonoids group and is characterized by a unique profile of flavonoids, showing partial similarity to *Citrus × myrtifolia* Raf. (chinotto) [13] and *Citrus aurantium* L. (sour orange)[14].

It contains relatively rare neoeriocitrin and neohesperidin and, as recently discovered, rare brutieridin and melitidin [15]. Careful analysis of flavonoid content in 42 *citrus* species and cultivars, reported by Nogata et al. [16], leads to the conclusion that the amount of the flavonoids per volume unit of juice, or per mass unit of albedo (peels), is absolutely the highest in bergamot compared to other *Citrus* fruits. Bergamot shows the highest concentrations of flavanones: neoeriocitrin, neohesperidin, naringin, melitidin and brutieridin, and the highest content of certain flavones: rhoifolin, neodiosmin, poncirin and rutin among all 42 different *Citrus* species [16]. Bergamot juice can be further concentrated by a patented method, involving a preparative size exclusion chromatography based on polystyrene gel filtration and the eluate exsiccation to give rise to a polyphenol-enriched powder, BPF [5]. BPF contains over 40% flavonoids, carbohydrates, pectins, and other compounds, in contrast to bergamot juice powders obtained by spry-drier method that rich maximum 1% polyphenols concentration. (D. Malara, personal communication). The main polyphenol components of BPF are flavonoids and their composition basically mirrors the bergamot juice polyphenol profile (Fig. 1), with the only difference that flavonoids are over 200 times more concentrated in BPF.

95% of flavonoids present in BPF (and in bergamot juice) are flavanones, while flavone can be found in the remaining 5%. Up to date there are no published bioavailability and pharmacokinetic studies for BPF. However, absorption, metabolism and excretion parameters have been described for several individual flavonoids present in bergamot juice. It is well known that flavonoid glycosides are hydrolysed to aglycones by bacterial flora of the gut. Gut microflora hydrolysis is thought to favor flavonoid glycoside bioavailability [17]. When sugar unit is removed, the resulting aglycone can be absorbed more readily [12]. Indeed, flavonoid aglycones of diosmin, hesperidin and naringin, which are diosmetin, hesperitin and naringenin, respectively, diffuse usually easily through the plasma membrane of Caco-2 cells, in contrast to the respective glycosides. Moreover, naringin, hesperidin, rutin and poncirin are hydrolyzed to their respective aglycones by human intestinal microflora, and the resulting aglycones are absorbed better [18]. However, the bioavilability of flavonoids is low and it is estimated that only 10% of total consumed polyphenols are absorbed, although these numbers vary between species and individuals [12]. When inside the enterocytes part of