Pharmacokinetic study of natural polyphenols and new insights on the mechanism of their action

Zherebker A.¹, Orlov A.A.¹, Semenov S.¹, Bugrova A.², Rukhovich G.¹, Brzhozovskiy A.¹, Kononikhin A.S.¹, Fedoros E.I.³, Nikolaev E.N.¹

¹Skolkovo Institute of Science and Technology, Moscow, Russia, a.zherebker@skoltech.ru

²Institute of Biochemical Physics of RAS, Moscow, Russia

³N.N. Petrov National Medical Research Center of Oncology, Saint Petersburg, Russia

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Molecular complexity of natural polyphenols prevents description of molecular mechanisms of their biological action and hampers pharmacokinetic studies. They are composed of thousands of unknown compounds and routinely, tandem MS experiments are hardly applicable. Still, FTICR MS combined with chemical and isotopic labeling can be used to obtain various structural information. Additionally, incorporation of deuterium tags enables to explore tissue distribution of majority of compounds. Molecular and structural information can be further elucidated by using chemoinformatics approaches. Here, we report a first successful attempt to apply a comprehensive approach consisted of determination of individual components in mouse liver, structural characterization and full-structure generation by deep learning and application of chemical similarity network analysis (CSNAP) to predict exact molecular targets.

An algorithm has been developed for fully automatic calculation of the series of isotopic or chemical labeling. For each reaction, a set of heuristic rules was formulated which were taken into account in the computer code to remove possible analysis artifacts. Applied to liver extracts it resulted in detection of 600 and 450 components after intravenous and oral administration, respectively. For the determined components 100 thousand relevant structures were generated and recorded in the form of SMILES. The distribution of Bemis-Murcko scaffolds was calculated. The most common scaffolds of compounds corresponded to known natural compounds: flavonoids, coumarins, and steroids. Application of CSNAP method was adapted to create a weighted graph with vertices corresponding to joints and edges corresponding to chemical similarities between them. The generated CSNAP topology was used to find probable targets for compounds action using Schwikowski and Hishigaki scores. As a result, for 657 compounds there was at least one structural analogue found in ChEMBL. A total of 11 target proteins involved in the pathogenesis and progression of non-alcoholic fatty liver disease (NAFLD) were predicted. From the predicted biological targets, the three most probable were selected that would be associated with NAFLD and structures that would be active in relation to these diseases: Kelch-like ECH-associated protein 1 (KEAP1), which is responsible for binding to Nrf2, Peroxisome proliferator-activated gamma receptor (PPARγ) and AMP-activated protein kinase (AMPK). To examine the predicted results top-down proteome analysis was performed. Accordingly, alkylation of KEAP1 in the presence and absence of phenol mixture was performed followed by tryptic digestion and LC-MS analysis. The significant decrease of alkylation degree of three cysteine residues supported results of CSNAP prediction. Additionally, fraction enriched with the liver-detected compounds was obtained and tested against NAFLD model on mice showing normalization of biochemical blood parameters and increase of survival.

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