Interaction of milk thistle flavonolignans with human fecal microbiota

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Milk thistle flavonolignans are known to possess low oral bioavailability, and their phase II conjugates are mainly excreted via the bile. Therefore, it is likely that considerable flavonolignan quantities reach the colon where gut microbiota reside [1]. Human gut microbiota are known to possess an enormous diversity as well as the capacity to metabolize many plant constituents. Therefore, the aim of this study was to assess the ability of human gut microbiota to metabolize milk thistle flavonolignans.

Silymarin (final concentration 0.21 mg/ml) was incubated with 10 % fecal suspension from the feces of a healthy donor for 24 h at 37 °C under anoxic conditions. Samples were taken at 0.5 h, 4 h, and 24 h, and analyzed for metabolic profile changes by UHPLC-HRMS analysis.

A significant reduction of all main Silymarin constituents was observed over incubation time, indicating that milk thistle flavonolignans are metabolized by human fecal microbiota. Furthermore, three different types of newly formed flavonolignan metabolites were detected and tentatively identified on the basis of their HRMS data, namely demethylation products, metabolites formed by cleavage of the dioxane ring (resp. tetrahydrofurane ring in case of silychristin), and products of demethylation plus ring cleavage.

To fully elucidate the structures of the major metabolites, a large scale incubation with silibinin (an 1:1 mixture of silybin A and B) was performed under the same conditions, and two major metabolites were isolated from the fecal incubate. They were assigned as demethylsilybin and 2-{4-[2-(3,4-dihydroxy-phenyl)-1-hydroxymethyl-ethoxy]-3-hydroxy-phenyl}-3,5,7-trihydroxy-chroman-4-one on the basis of 1D and 2D- NMR spectroscopic data. A subsequent incubation experiment with pure silybin A and silybin B allowed to assign their stereochemistry. Moreover, this experiment showed that silybin A is only demethylated by fecal microbiota, while silybin B is metabolized via demethylation plus dioxan ring cleavage, indicating a high stereoselectivity of the occurring metabolic reactions.

In order to identify and characterize the microorganisms involved in silybin A and B metabolization, fecal bacterial cultures capable of silibinin degradation were plated on agar plates, however, no silibinin-degrading microorganisms could be isolated. Subjecting liquid cultures to different selection strategies eventually led to enrichment of *Eggerthella* species that are obviously involved in silibinin demethylation. Isolation and identification of the silibinin- metabolizing bacteria is still under progress.

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References:

1. Tvrdý et al, Med Res Rev 2021, 41 (4): 2195-2246.