Silibinin Feeding Alters the Metabolic Profile in TRAMP Prostatic Tumors: A 1H-NMR study

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Summary
Herein, we have evaluated for the first time the chemopreventive efficacy of silibinin on prostate cancer (PCa) metabolism in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model utilizing quantitative high-resolution proton nuclear magnetic resonance spectroscopy (1H-NMRs) metabolomics. Prostate tissues were obtained from 4 week-old mice which were fed control or 1% silibinin-supplemented diets for 20 weeks. Frozen tumor biopsies were extracted and analyzed by 1H-NMRS in order to establish a quantitative metabolic profile. Multivariate principle component analysis (PCA) was applied in order to: (i) cluster the samples among control (untreated) and silibinin treated groups (scores t); (ii) identify biomarkers responsible for this group clustering (plots p); (iii) distinguish metabolic markers which were responsible for group clustering in Figure 1A. Total of 14 biomarkers contributed to the group separation (Figure 1B) and were related to cellular, intracellular glucose, choline in the water-soluble fraction, glycerophosphocholine (GPC) and myo-inositol, as well as decreased values of lactate, cholesterol and three ratios from glucose and phospholipid metabolism (Figure 2). In a less extent, decreased alanine and phosphatidylcholine, as well as increased polyols, glutathione and the ratio of unsaturated fatty acids contributed to the group clustering.

Methods
TRAMP mice (C57BL/6 background) were fed either with control diet (positive control group, n=4) or with 1% silibinin-supplemented [1% silibinin (w/w) in AIN-93M purified] diet starting from 4 weeks of age till 24 weeks of age (n=4). Prostate tissue were collected, snap frozen in liquid nitrogen, and extracted with 8% perchloric acid. Hydrophilic and lipophilic prostate tissue extracts were analyzed by quantitative 1H-NMRs at a Bruker 500 MHz DRX spectrometer equipped with Bruker TopSpin software. After loading quantitative data sets into the R package software, principle component analysis (PCA) was applied in order to: (i) cluster the samples among control (untreated) and silibinin treated groups (scores t) and (ii) identify biomarkers responsible for this group clustering (plots p). Absolute individual concentrations of distinguishing biomarkers were then analyzed by ANOVA followed by Tukey’s post-hoc test to identify the groups that differed significantly.

Results
From each prostate tumor biopsy, 38 individual water-soluble and lipid metabolites as well as 4 significant metabolite ratios were quantified. Such, a total set of 42 variables was included into the multivariate data analysis. The PCA analysis allowed for precise group separation between untreated control and silibinin treated TRAMP mice (Figure 1A). In the next step of the PCA analysis, individual metabolites were distinguished, which were responsible for group clustering in Figure 1A. Total of 14 biomarkers contributed to the group separation (Figure 1B) and were related to citrate, glucose, phospholipid, osmyolate and antioxidat metabolism. The major contributors for group separations were increased concentrations for citrate, intracellular glucose, choline in the water-soluble fraction, glycercophosphocholine (GPC) and myo-inositol, as well as decreased values of lactate, cholesterol and three ratios from glucose and phospholipid metabolism (Figure 2). In a less extent, decreased alanine and phosphatidylcholine, as well as increased polyols, glutathione and the ratio of unsaturated fatty acids contributed to the group clustering.

Conclusions
In the present study, metabolic profiling of the TRAMP prostate tissues appeared to be highly sensitive to oral supplementation with silibinin. Silibinin-related metabolic changes include: (i) normalization of citrate concentrations (zinc metabolism for normal secretory functions of the gland); (ii) decrease of glucose utilization and glycolytic activity (Warburg’ effect); (iii) decrease in membrane phospholipid synthesis (Kennedy pathway); and (iv) increase in polyols and antioxidants in the prostate gland.

References

Figure 1: PCA analysis on TRAMP mouse prostate tissue

Figure 2: Silibinin-induced changes in distinguish metabolic markers