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 (^{1}H-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 ^{1}H-NMRS in order to establish a quantitative metabolic profile. Multivariate principle component analysis (PCA) was applied for group separation (control vs. silibinin treated) and biomarker identification. In the present study, comparative metabolic profiling indicated that the antitumor effect of silibinin is accompanied by alteration of the metabolic profile of the TRAMP prostatic tumors as indicated by a 6 fold (P=0.016) increase in the glucose content of the prostatic tissue along with a significant 48% (P=0.015) reduction in the lactate levels. The increase in citrate utilization by prostate tissue was also reversed with silibinin, as indicated by a 3 fold (P=0.01) increase in citrate levels in the silibinin-fed group. Also a 61% and a 50% (P<0.01, for both) decrease in the levels of cholesterol and phosphatidylcholine (Ptd-Cho), respectively, was observed with silibinin feeding. This will help identify the specific metabolic biomarkers altered during the course of treatment, which when detected in clinical biopsies or non-invasive MRS studies can help monitor the effectiveness of silibinin against PCa malignancy.

Intorduction

Recent studies have shown that the metabolic profile of various cancerous /non-cancerous tissues can be correlated with cell growth and death, specific tumor type as well as the pathological stage of tumor [1]. In this regard, the metabolomics of prostatic tissues using magnetic resonance spectroscopy (in both *in vivo* and *in vitro* conditions) has also helped to identify and establish the metabolic profiles specific to prostate cancer (PCa) malignancy [2]. We have recently reported the inhibitory effect of silibinin (flavonolignan isolated from the seeds of milk thistle), which has shown promising chemopreventive and anticancer effects in various *in vitro* and *in vivo* studies, on prostate tumor progression in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice [3]. We utilized quantitative high-resolution proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) to assess the metabolic profile of the silibinin treated TRAMP prostatic tissue so as to complement the histopathological and proteomics data generated in the earlier studies. The working hypothesis was that, upon silibinin treatment, specific metabolic biomarkers will sensitively detect changes in prostate gland tissue.

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 MHX 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_i) and (ii) identify biomarkers responsible for this group clustering (plots p_i). Absolute individual concentrations of distinguished 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, osmolyte and antioxidant metabolism. The major contributors for group separations were increased concentrations for citrate, 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.

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

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3.Raina K, Blouin MJ, Singh RP, et al. *Cancer Res* 67: 11083-11091 (2007).

Figure 1: PCA analysis on TRAMP mouse prostate tissue

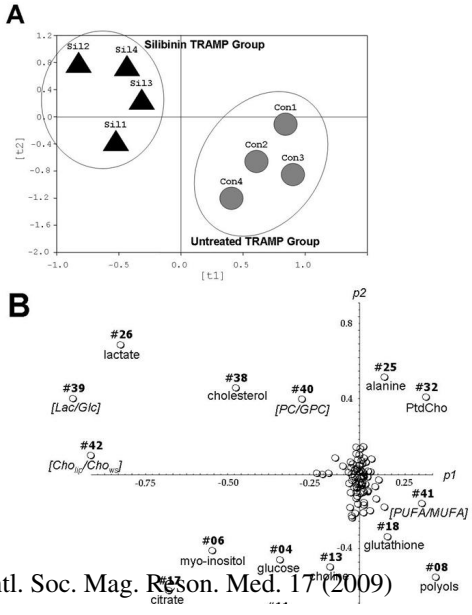

Figure 2: Silibinin-induced changes in distinguish metabolic markers