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Research paper

Choline-phospholipids inter-conversion is altered in elderly patients with prostate cancer

Hussain Mohamad Awwad [a, *], Carsten-Henning Ohlmann [b], Michael Stoeckle [b], Rasul Aziz [a], Juergen Geisel [a], Rima Obeid [a, c, *]

[a] Department of Clinical Chemistry and Laboratory Medicine, Saarland University Hospital, D-66421 Homburg, Saar, Germany
[b] Department of Urology, Saarland University Hospital, D-66421 Homburg, Saar, Germany
[c] Aarhus Institute of Advanced Studies, Aarhus University, Dk-8000 Aarhus, Denmark

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ABSTRACT

Background: Choline is an important source of phospholipids and methyl groups in mammalian cells. High demands for methyl and phospholipids in malignant cells suggest that choline metabolism may be disturbed in patients with cancer.

Objectives and Methods: This case-control study investigated differences in concentrations of choline metabolites between 80 elderly men (age ≥ 65 years) with prostate cancer (PCa) and 51 men with benign prostatic hyperplasia (BPH). Plasma/serum concentrations of free choline, betaine, dimethylglycine, folate, total homocysteine (tHcy), cystathionine, methylmalonic acid, S-adenosyl homocysteine (SAH), S-adenosyl methionine (SAM), and phospholipids were measured.

Results: Men with BPH and those with PCa showed no significant differences in the concentrations of free choline (median = 9.7 vs. 10.0 μmol/L), folate (17.4 vs. 19.8 nmol/L), tHcy (16.0 vs. 16.2 μmol/L), SAH (18.8 vs. 18.2 nmol/L), and phosphatidylcholine (1634 vs. 1610 μmol/L). The concentrations of methylmalonic acid were lower in men with PCa (203 vs. 228 nmol/L) but the difference was not significant after adjusting for age. Sphingomyelin species (16:0, 18:0, 18:1, 20:0, 22:0, 22; 1, 23:0, 23:1, 24:0, 24:1, and 24:2) were significantly lower in men with PCa than in the controls (6–16% differences). Multiple regression analyses showed that the presence of PCa, statin use, choline, age, cystathionine, and methylmalonic acid were significant negative determinant of sphingomyelins, whereas phosphatidylcholine was a strong positive determinant.

Conclusions: The current results support systemic alterations in phospholipids metabolism in PCa. We report on a significant decrease in plasma concentrations of sphingomyelin in elderly patients with PCa and in users of statins. The PCa-associated low sphingomyelin showed a synergy with the effect of statins. The presence of PCa was not associated with significant changes in plasma concentrations of choline or methyl metabolites. However, changes in choline absorption and tissue uptake cannot be ruled out in this study.

1. Introduction

Prostate cancer (PCa) is the most common malignancy with an annual age-standardised rate of 90–227 per 100,000 men [1]. Studying 'metabolic fingerprint' in blood samples from patients with cancer can identify novel pathways involved in cancer and may thus allow an early diagnosis, risk monitoring, or a better understanding of cancer physiology.

Imagining choline and glucose metabolism in malignant cells has shown remarkable success in recent years [2]. Metabolic studies of malignant tissues have identified phospholipid,

Abbreviations: BPH, benign prostatic hyperplasia; DMG, dimethylglycine; LDL, low density lipoproteins; Lyso-PtdCho, lysophosphatidylcholine; PCa, prostate cancer; PtdCho, phosphatidylcholine; PtdEth, phosphatidylethanolamine; PSA, prostate specific antigen; SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; SM, sphingomyelin; tHcy, total homocysteine; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry; VLDL, very low density lipoproteins.

* Corresponding authors. Saarland University Hospital, Department of Clinical Chemistry and Laboratory Medicine, Building 57, 66421 Homburg, Saar, Germany.
E-mail addresses: s9huawwa@stud.uni-saarland.de (H.M. Awwad), rima.obeid@uks.eu (R. Obeid).

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methionine, and glycine-dimethylglycine pathways that may be disturbed in PCa [3]. Some urinary metabolites failed in discriminating between patients and controls [4] or showed no association with serum levels of prostate specific antigen (PSA) [4], an established marker of PCa. Therefore, clinical studies are important to evaluate the biomarkers that have been identified through screening approaches or non-targeted metabolomics.

Abnormal choline metabolism has been reported in cancer [5,6] and higher plasma level of choline has been associated with an increased risk for PCa [7]. In a follow-up study, the intake of choline, glycerophoshocholine and phosphatidylcholine (PtdCho), but not that of sphingomyelin (SM), were associated with higher PCa risk [8]. In contrast, other studies observed a negative association between choline intake and the risk of some cancer types [9,10]. The relationship between plasma choline and cancer is not conclusive, presumably because of the diversity in choline metabolism in the body.

The nutrient choline is oxidized to betaine that is used as a source of methyl groups (Fig. 1). Choline is delivered by the diet or synthesized in the liver from PtdCho [11,12] that is produced via the methyl-dependent phosphatidylethanolamine N-methyltransferase (PEMT). SM is synthesized from PtdCho by the transfer of phosphorylcholine to ceramide through sphingomyelin synthase [13] (Fig. 1). The metabolism of choline interacts with that of folate and methionine thus reflecting the indirect role of choline in one-carbon metabolism that in turn has a significant role in DNA-synthesis and methylation. The fast growth of cancer cells demands more one-carbon units and requires accelerated conversion of choline to ceramides [14]. On the other hand, choline endogenous synthesis via PEMT requires methyl groups that can be altered in cancer cells.

The prostate gland accumulates a significant amount of choline that is considered essential for the gland's physiological functions such as production and motility of sperms. However, PCa development and cell proliferation may require more phospholipids [15]. The objective of our study was to investigate whether plasma free choline, phospholipid classes, and methyl metabolism are altered in elderly patients with PCa compared with those with benign prostatic hyperplasia (BPH).

2. Subjects and methods

Between July 2012 and March 2013, 151 elderly men (age $\geq$ 65 years) were recruited from the Department of Urology, Saarland University Hospital, Germany. The participants had either a primary PCa (n = 86) or a BPH (n = 65). Exclusion criteria were renal failure, liver disease, chronic alcohol consumption, metastases, vitamin B supplementation and methotrexate therapy. Twenty men were later excluded because of exclusion criteria: 9 patients had glomerular filtration rate (GFR) < 50 mL/min, one patient had liver cirrhosis, 2 used B-vitamins, and 8 had other co-existing cancers. The final statistical analyses included 80 patients with PCa and 51 controls with BPH.

At the day before the prostate surgery, blood samples were collected in the morning using tubes containing potassium EDTA and in those without anticoagulant. The blood specimens were immediately placed on ice, centrifuged and separated within 30 min, then stored at −70 °C until analyses. The blood measurements were performed on aliquots that were not thawed before. The results of the routine blood tests and histological biopsy were followed after the surgery. All cases with PCa were confirmed by biopsy testing. Choline and one-carbon metabolisms showed age-dependency and different associations with the disease. Therefore, men who were younger than 65 years were not included in this report.

Men were informed about the study purpose and invited to participate in the study before the prostate surgery. The study was approved by the medical ethics commission of the Saarland region

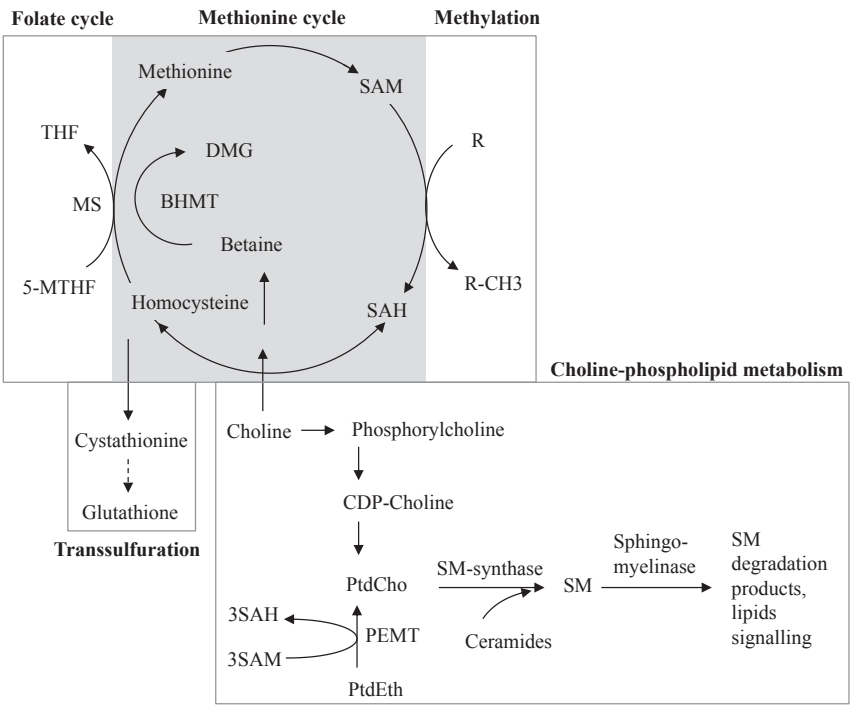

Fig. 1. Interconversion of choline and phospholipids and the interaction with folate and methionine metabolism. BHMT, betaine-homocysteine methyl transferase; DMG, dimethylglycine; MS, methionine synthase, 5-MTHF, 5-methyltetrahydrofolate; PEMT, phosphatidylethanolamine methyltransferase; PtdCho, phosphatidylcholine; PtdEth, phosphatidylethanolamine; SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; SM, sphingomyelin; THF, tetrahydrofolate.

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