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Choline: The Role in Cell Life and Potential Biomarker Molecule

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ABSTRACT

The role of choline in cellular life and its use as a biomarker of different pathologies are reviewed. Choline is an essential nutrient that is crucial for normal cellular function. It is predominantly absorbed from the small intestine. A diet is considered as a major source of choline though it can be produced by cells. Choline in the form of phosphatidylcholine is a very important cell membrane constituent. Choline and its derivatives play an important role in such events as neurotransmitter acetylcholine synthesis, cell-membrane signaling (phospholipids), lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction). Choline was considered to be an integral part of folate and methylation pathways. Myocardial and other pathologies may be associated with an alteration of blood choline level that allows considering its application as a biomarker molecule.

Key words: Choline, role in cell life, biomarker

INTRODUCTION

Different biomarkers can provide important information regarding the functional state of organs and tissues. Analyzing a single biomarker or combination of biomarkers is useful in diagnosis of pathological states, which then can be treated accordingly. Desirable characteristics of biomarker are specificity to an organ or tissue, a presence of biomarker in blood, sensitivity in its response on injury or pathology, and possibility of efficient analyses. It was demonstrated that ample number of biological substances are capable to serve as biomarkers. Choline in blood is being considered as a possible biomarker in different pathologies of myocardium, liver. In case of cardiac pathology, choline, originated from the membranes of injured cells of myocardium reacts to the injury faster, compared to other proteins of myocardium, and may be faster marker as compared to troponins I and T.

The purpose of this review is to discuss the role of choline in cell function and as potential biomarker molecule.

1. Choline contribution in cell life

Choline (vitamin/pseudovitamin B4) is an essential nutrient that is crucial for normal cellular function. Choline is predominantly absorbed from the small intestine and completely metabolized in the liver. It can be also produced by cells from methionine and serine, however a diet is considered as a major source of choline. Choline in the form of phosphatidylcholine is a very important cell membrane constituent (Parrish et al., 2008). It is a quaternaryamine having the chemical formula $(CH_3)_3N^+CH_2CH_2OHX^-$ (X^- – counterion). Choline and its derivatives play an important role in such events as neurotransmitter acetylcholine synthesis, cell-membrane signaling (phospholipids), lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction) (Penry and Manore 2008). Choline was considered to be an integral part of folate and methylation pathways (Niculescu and Zeisel, 2002). Choline involvement in phosphatidylcholine biosynthesis described long ago and reviewed lately (for review see: Gibellini and Smith, 2010). Pathologies like cancer may be associated with modulation of enzymes that control choline metabolism (Glunde et al., 2011; Stewart et al., 2012).

2. Signaling, intracellular transport

Little is known about choline implication in signaling mechanisms. Choline (0.1-50 mM) evoked dose-dependent inhibition of TNF release from endotoxin-activated macrophage-like cells, and this inhibition was associated with significant suppression of NF-kappaB activation. Intraperitoneal choline treatment (50 mg/kg) prior to endotoxin injection resulted in systemic TNF level decrease in wild-type mice but was failed to reduce it in α7 nicotinic acetylcholine receptor (alpha7nAChR) knockout (Parrish et al., 2008). Hence, choline is a selective natural alpha7nAChR agonist. It modulates TNF release, showing anti-inflammatory activity, and this process requires alpha7 subunit nicotinic acetylcholine receptor-mediated signaling (Parrish et al., 2008). Consistent to these data the inflammation, necrosis and fibrosis in the non-alcoholic steatohepatitis induced by methionine- and choline-deficient diet can be reduced by treatment of rats with anti-TNF-alpha antibody (Koca et al., 2008). It is believed that alpha7nAChR is involved in pathogenesis of Alzheimer's disease and schizophrenia (Wallace and Porter, 2011; Dinamarca et al., 2012), neuropathic pain (Loram et al., 2012), cardiovascular diseases (Liu and Su 2012) and also in the protective effects of arterial baroreflex against ischemic stroke (Liu et al., 2012) and prevention gut barrier failure after severe burn injury (Costantini et al., 2012). It may be an effective target for pharmacologic impact with agonists (Zanaletti et al., 2012).

There are three major proteins involved in choline transport in mammalian cells: high-affinity choline transporter (SLC5A7 – solute carrier family 5, member 7, or HCT1),

intermediate-affinity choline transporter-like protein (SLC44A1), and a very low affinity for choline, polyspecific organic-cation transporters (SLC22A1 and SLC22A2) (Koepsell et al., 2003; Koepsell and Endou 2004). The high-affinity choline transporter CHT, having a critical importance in maintaining cholinergic transmission, was described in cholinergic nerve terminals (for review see: Black and Rylett 2012). This transporter is considered to be important for choline delivery for acetylcholine synthesis into cholinergic nerve terminals (Black et al., 2010). CHT1 refers to the Na+/glucose cotransporter family (SLC5) and is involved in choline uptake for synthesis of acetylcholine. Ruggiero et al. (2012) suggested that ion flux changes may modulate CHT affinity to choline. CHT1 has transmembrane topology and forms a homo-oligomer on the surface of cultured cells HEK293 (Okuda et al., 2012). 3-morpholinosydnonimine [peroxynitrite ($ONOO^-$) donor] treatment of neuronal SH-SY5Y cells resulted in increase in CHT internalization that associated with decreased choline uptake (Cuddy et al., 2012). CHT inhibition mediated by proteasomal degradation and inhibitors of proteasomes attenuated choline uptake by SH-SY5Y cells. Thus, increased oxidative-nitrosative stress may alter CHT function and reduce choline availability for synthesis of acetylcholine (Cuddy et al., 2012). Ubiquitin ligase Nedd4-2 mediates ubiquitination of CHT1 in HEK293 and regulates the CHT1 expression on cell surface (Yamada et al., 2012). The inhibition of Nedd4-2 might be useful for Alzheimer's and other diseases associated with decrease in acetylcholine level (Yamada et al., 2012). Choline pretreatment inhibited angiotensin II-induced cardiac hypertrophy in vivo (0.6 mg/kg/day for 2 weeks) and cardiomiocytes in vitro (0.1 μM for 48 h) through inhibition of ROS-mediated p38 MAPK activation as well as regulation of Ca^{2+}-mediated calcineurin signal transduction pathway (Wang et al., 2012). Barwick et al. (2012) identified a mutation in gene *SLC5A7*, which encodes the CHT1, in subjects with distal hereditary motor neuropathy type VII. "Mutated" CHT1 does not have cytosolic C-terminus sequence which regulates surface transporter trafficking. The discovered mutation in *SLC5A7* may be suggested to implicate in unexplained motor neuronopathies (Barwick et al., 2012). GG allelic variant of G/T single-nucleotide polymorphism of CHT1 occurred to be associated with greater carotid intima-media thickness and plaque occurrence that may be used for assessment of atherosclerotic risk (Neumann et al., 2012). Choline acetyltransferase (ChAT) was shown to activate transcription of the CHT1 gene in human neuroblastoma cells SH-SY5Y but did not alter CHT1 expression in non-neuronal HEK293 cells (Matsuo et al., 2011). It was shown that adult (not neonatal) cardiomyocytes are able to synthesize, transport and excrete ACh in the rat heart (Rana et al., 2010). In old cardiomyocytes, ChAT expression and ACh excretion levels

down-regulated. Radiolabeled CHT inhibitors were synthesized as a tool for research needs (Gilissen et al., 2003; Zheng et al., 2007).

Therefore, choline transporters are ubiquitously expressed on cells. CHT plays a crucial role in maintaining adequate cholinergic transmission that allows considering CHT as a prospective target for cholinergic medication development. Additional efforts are needed to clear up choline implication in cellular signaling mechanisms.

3. Myocardial and other effects

Alpha7nAChR may specifically contribute to the inhibition of pro-inflammatory cytokine production through "the cholinergic anti-inflammatory pathway" by vagus nerve stimulation (Liu and Su 2012). Postconditioning with α7nAChR agonist significantly decreased the serum inflammatory variables (TNF α and high-mobility group protein B1) level following myocardial ischemia-reperfusion. Reduced infarct size and serum troponin I level were also revealed (Xiong et al., 2012). Dietary choline and betaine intakes also reduce a risk of cardiovascular diseases by decreasing inflammation and other risk factors (Rajaie and Esmaillzadeh 2011). Choline is a precoursor of a methyl group donor – betaine (trimethylglycine) which takes part in a reaction converting homocysteine to methionine (Konstantinova et al., 2008). Choline content is especially high in the brain, liver, kidney and myocardium. High plasma homocysteine is considered to be a cardiovascular risk factor (Rajaie and Esmaillzadeh 2011). Met-analysis showed that polymorphism of methionine synthase A2756G (homocysteine-metabolizing enzyme) may increase the risk of coronary heart disease for Europeans (Chen et al., 2012). Divergent associations of plasma choline and betaine with metabolic syndrome components in middle age and elderly people were found (Konstantinova et al., 2008). Plasma choline was positively associated with serum triglycerides, glucose, BMI, percent body fat, waist circumference, physical activity and inversely related to HDL cholesterol and smoking. Betaine was inversely associated with serum non-HDL cholesterol, triglycerides, BMI, percent body fat, waist circumference, systolic and diastolic blood pressure, smoking and positively associated with HDL cholesterol and physical activity. The authors believe these data may be explained by disturbed function of mitochondrial choline dehydrogenase pathway (Konstantinova et al., 2008). Chronic renal failure and uremia are also associated with high blood levels of homocysteine which may be a cardiovascular risk factor in these patients (Perna et al., 2012). 7-day choline deficiency of rats resulted in the significant increase in oxidative stress in liver, kidney, heart and brain (Repetto et al., 2010). Necrosis was also revealed in kidney and heart. Choline in combination with folic acid effectively protected lymphocytes genomic integrity during 9-day cultivation (Lu et al., 2012). Choline supplementation (1 g/d) significantly

increased the concentrations of free choline, and methyl donor, betaine, that may result in decrease in homocysteine level in plasma due to the betaine-homocysteinemethyltransferase-mediated remethylation of homocysteine (Wallace et al., 2012). Choline supplementation attenuates airway inflammation and suppresses oxidative stress in patients with asthma, in mouse model of allergic airway disease (Mehta, 2009; 2010). Dietary choline supplementation decreased in lipid peroxidation, and helped to conserve retinol and alpha-tocopherol (Sachan et al., 2005). Unexpectidly, the membrane lipid phosphatidylcholine occurred as a source of triacylglycerol in the liver which may be associated with hepatic steatosis etiology development (van der Veen et al., 2012). Consistent with authors' data, approximately 65% of the total hepatic triacylglycerol appeared to be derived from hepatic phosphatidylcholine, half of which is derived from HDL. Hyperhomocysteinemia was hypothesized as an indicator of systemic or endothelial oxidant stress but not as a primary cause of atherothrombotic disorders in the general population (Hoffman, 2011). Mild to moderate hyperhomocysteinemia depending on nutritional factors involved in homocysteine metabolism can be reduced by high intakes of choline and betaine, and also with folic acid supplements (Verhoef and de Groot 2005).

Phosphorylcholine coating of extracorporeal circuits to mimick the outer cell membrane demonstrated a favourable effect related to platelet activation (decrease in thromboxane B2, beta-thromboglobulin, postoperative bleeding, complement activation) during cardiopulmonary bypass (De Sommer et al., 2000; Marguerite et al., 2012). This approach may be useful for reducing in thromboembolic complications during surgeries.

4. Biomarker of myocardial and other pathologies

Whole-blood choline, plasma choline and serum choline may be promising biomarkers in acute coronary syndrome related to coronary plaque instability with platelet thrombus formation and ischemia (Danne et al., 2003; 2005; 2007; Wu, 2005; Body et al., 2009; Danne and Möckel 2010). Choline is reported to be prognostic in patients with acute coronary syndromes via its release from ischemic cell membranes. It may be faster marker as compared to troponins I and T.

Serum choline concentration is a predictive variable for myocardial infarction, and moreover, it may help to distinguish between high- and low-risk patients with a positive troponin test (LeLeiko et al., 2009; Danne and Möckel 2010). Whole blood choline and plasma choline may be useful and independent predictors of cardiac complications in admitted patients with suspected acute coronary syndromes, particularly if troponin data are negative (Danne et al., 2003; 2007). Both variables were predictive for cases of tissue ischemia and, moreover, whole blood choline differentiated risks associated with coronary plaque instability. A good prognostic value had a combination of serum choline and free F2-isoprostane, biomarker of membrane lipid

peroxidation, in predicting of 30-day cardiac events (LeLeiko et al., 2009). The cutoff points of serum choline and F(2)-isoprostane levels were 30.5 micromol/L (r = 0.76) and 124.5 pg/ml (r = 0.82), respectively. Positive predictive values were 44% for choline and 57% for isoprostane while a negative predictive value was similar about 90% for both biomarkers. Authors suggested that biomarker combination of troponin, choline, and free F(2)-isoprostane might be useful in identifying patients at greater risk of future events in acute coronary syndrome (LeLeiko et al., 2009). Body et al. (2009) measured plasma choline and whole blood choline in patients with suspected cardiac chest pain within the previous 24 h. Plasma choline was not a diagnostic marker of acute myocardial infarction, however predicted it within 6 months whereas whole blood choline was predictive in major adverse cardiac events (MACE: cardiac death/arrest, heart failure, non-fatal MI, unstable angina, congestive heart failure, CHF, requiring admission, urgent PCI and CABG). It should be noted that events such as surgery, childbirth, and traumatic head injury result in significant decrease in serum choline while a pregnancy is characterized by an elevated level of serum choline (Ulus et al., 1998).

Plasma free choline level reflects the phosphatidylcholine synthesis in the liver and may be also a potential diagnostic marker for early-stage non-alcoholic steatohepatitis (Imajo et al., 2012). The hypercholinemic response to hypoxia was revealed in old rats (Jenden et al., 1996). Plasma choline metabolites may predict metabolic stress among overweight men: overweight subjects had significantly lower betaine and betaine to choline ratio compared with normal men (Yan et al., 2012). Choline intake was considered to be a risk factor for cancer, especially for prostate cancer (Awwad et al., 2012; Richman et al., 2012). 18F-fluoromethylcholine-PET imaging may be effective replacement of ^{3}H-2-deoxyglucose for differentiation of cancer and chronic inflammation in lung and brain (Kubota et al., 2006). Another diagnostic possibility may be connected with phosphorylcholine antibodies. Low level of phosphorylcholine antibodies, which are involved in atherosclerosis, in female patients showed fairly strong association with higher risk for cardiovascular disease and the incidence of stroke (Fiskesund et al., 2010). Hence, whole-blood, plasma and serum choline may be considered as presumable biomarkers of myocardial and other pathologies. Blood choline alterations studies in different pathologic conditions are needed to exclude a false diagnosis.

5. Choline and exercise

Taking into account choline involvement in synthesis of neuromediatoracethylcholine many works were aimed to study choline supplementation effect on exercise resistance. Trained athletes after a 26 km marathon demonstrated 40% decrease in blood choline concentration (from 14.1 to 8.4 microM) (Conlay et al., 1992). In top level triathletes the 2 x 2-hour bicycle

exercise resulted in 17% decrease in blood plasma choline, however it was unchangeable in adolescent runners possibly because of exercise duration (von Allwörden et al., 1993). When lecithin (phosphatidylcholine, 90%) supplementation was used a significant elevation of plasma choline concentration without exercise and unchangeable level after exercise were found (von Allwörden et al., 1993). These data indicated the reduction of acetylcholine release from the neuromuscular junction that might affect endurance of athletes (Conlay et al., 1992). However, no blood choline depletion, fatigue time and work performance were found in trained cyclists after exercise under either test conditions (brief and prolonged tests at a power output equivalent to approximately 150% and 70% of VO_2max, respectively; +/- choline bitartrate) though choline ingestion led to its increase in blood (Spector et al., 1995). No significant effects were observed on run time-to-exhaustion and squat tests in volunteers after choline citrate supplementation compared to placebo (4-hour strenuous load carriage treadmil exercise) (Warber et al., 2000). Using the similar exercise test Deuster et al. (2002) also failed to find differences between placebo and choline experimental groups in physical or cognitive performance after exercise. Interestingly, choline deficiency impaired choline transport in mitochondria due to down-regulation the choline transporter SLC44A1, reduced phosphatidylcholine synthesis with no change of degradation and resulted in triacylglycerol accumulation in the form of lipid droplets due to reduced triacylglycerol metabolism (Michel et al. 2011). Thus, it may be assumed that only very heavy and prolonged loads are able to decrease choline content in blood. Cited data remain to be open the question about the effectiveness of choline administration to increase exercise endurance performance though choline deficiency may affect muscle metabolism.

6. Analysis, sample preparation and stability

Conditions used for collection, storage and preparation of samples are critical for measurement of choline. Kind of anticoagulants (plasma), freeze-thaw cycle (whole blood), storage at ambient temperature (plasma and whole blood) may significantly influence on result of measurements (Yue et al., 2008). As an analytical method for choline determination in plasma and whole blood the liquid chromatography tandem mass spectrometry analysis (LC-MS/MS) after extraction was successfully applied (Bruce et al., 2010; Griffith et al., 2010). Plasma concentrations for free-choline ranged from 9.8 to 18.5 micromol/L (15.2-66.3 micromol/L for betaine) within the range of the other reports. Homogeneous choline chemiluminescent assay adopted for 96-well microplate format was also described (Adamczyk et al., 2006). The method was validated for plasma and whole blood, it required 4 microL sample volume, very short time for analysis and demonstrated good correlation with LC-MS/MS ($r > 0.97$).

7. Conclusion

Choline is an essential for ensuring membrane integrity, neurotransmission and different methylation pathways of cell. Blood choline level changes occur in various situations, including surgeries, childbirth, traumatic head injuries, cardiac events, exercises. Whole-blood, plasma and serum choline may be useful for prediction of cardiac events, particularly in combination with troponins and other cardiac markers.

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