SHORT COMMUNICATION

Pheochromocytoma and Markers of Oxidative Stress

H. TURKOVÁ[1*], O. PETRÁK[1*], J. ŠKRHA[2], J. WIDIMSKÝ Jr[1], T. ZELINKA[1]

** These authors contributed equally to this work*

[1]Hypertension Unit, Third Department of Medicine, General Faculty Hospital and First Medical Faculty of the Charles University, Prague, Czech Republic, [2]Third Department of Medicine, General Faculty Hospital and First Medical Faculty of the Charles University, Prague, Czech Republic

Received August 14, 2012
Accepted January 3, 2013
On-line March 14, 2013

Summary

High levels of catecholamines in pheochromocytoma (PHEO) are associated with risk of cardiovascular complications. In this study, we looked for potential differences in markers of oxidative stress – vitamin C, superoxide dismutase (SOD) and malondialdehyde (MDA) in PHEO before and after the operation. We studied 18 subjects with PHEO who were examined before and approximately one year after the successful tumor removal (free of disease). All subjects had elevated urinary epinephrine and/or norepinephrine levels before the operation. Vitamin C levels increased significantly after the operation from 61±27 to 77±20 μmol/l (P=0.02), and MDA decreased significantly after the tumor removal from 2.6±0.4 to 2.0±0.6 μmol/l (P=0.01). However, no changes were found in SOD activity before and after the operation. In conclusion, increased catecholamine production in PHEO is accompanied by decreased levels of vitamin C and increased levels of MDA which may indicate the activation of oxidative stress in PHEO. Successful operation was associated with lowering of oxidative stress by using both biomarkers. On the contrary, no changes in SOD activity before and after the tumor removal were noted.

Key words

Pheochromocytoma • Catecholamines • Oxidative stress

Corresponding author

Tomáš Zelinka, 3rd Medical Department of the First Faculty of Medicine and General University Hospital, U Nemocnice 1, 128 08 Prague 2, Czech Republic. Fax: +420 224 963 245. E-mail: tzeli@lf1.cuni.cz

Pheochromocytoma and functional paraganglioma (PHEO) are tumors arising from chromaffin cells either from the adrenal medulla (pheochromocytoma) or from sympathetic nervous system-associated chromaffin tissue (paraganglioma), which produce, and metabolize but not always secrete catecholamines. PHEO is manifested by various clinical symptoms and signs, e.g. sweating, headache, palpitations, hypertension, or hyperglycemia (Zelinka *et al.* 2007a).

Catecholamine excess in PHEO is also associated with different cardiovascular complications or with accelerated preclinical atherosclerosis (Holaj *et al.* 2009, Zelinka *et al.* 2012). This may be due to direct effect of catecholamines on vessel wall or due to indirect catecholamine action mediated not only by hyperglycemia or hypertension but also by other factors such as subclinical inflammation (Zelinka *et al.* 2007b).

Another factor which may contribute to accelerated atherosclerosis or to cardiovascular complications is oxidative stress, an imbalance between reactive oxygen species production and antioxidant defenses in favor of the first (Ďuračková 2010). Elevation of markers of oxidative stress was found in heart failure, which is associated with increased catecholamine levels due to activation of the sympathetic nervous system (Castro *et al.* 2003).

Germ-line mutations in eleven different susceptible genes have been identified as a cause of pheochromocytoma or paraganglioma. Mutations of some

of these genes [genes encoding subunits B, D, C, and A of mitochondrial enzyme succinate dehydrogenase (SDH), and von Hippel-Lindau (VHL) gene] may be directly involved in induction of oxidative stress (Pacák 2011).

In this study in subjects with PHEO before and after tumor removal, we have focused on markers of oxidative stress such as vitamin C, a cofactor required for the synthesis of norepinephrine from dopamine (Kvetňanský *et al*. 2009), malondialdehyde (MDA), a product of lipid peroxidation, and superoxide dismutase (SOD) which catalyzes dismutation of superoxide radicals to hydrogen peroxide.

We have studied 18 subjects with PHEO who were investigated before the operation and approximately one year after the successful operation. One subject suffered from neurofibromatosis type I. Another subject was tested positive for the mutation of the *SDHB* gene. The remaining subjects were tested negative for multiple endocrine neoplasia type 2, VHL disease and multiple paraganglioma syndromes type 1 and 4. Three subjects presented with extra-adrenal tumors (one with mutation of the *SDHB* gene) and one with bilateral adrenal tumors. Informed consent was obtained from all subjects, and the study was prepared in accordance with the Helsinki Declaration and approved by the local ethics committee.

Diagnosis of PHEO was based on elevated 24-hour urine catecholamines or metanephrines, positive imaging study either with computed tomography or less frequently with magnetic resonance and was finally confirmed by histopathological examination. Patients were treated with α-blockers (mostly doxazosine) before the operation. 24-hour blood pressure monitoring (SpaceLabs 90207; SpaceLabs Medical, Richmond, Wash., USA) was performed. Eight subjects suffered before tumor removal from diabetes mellitus defined as blood glucose >6.9 mmol/l or treatment with glucose-lowering drugs (American Diabetes Association 2011). Blood was taken in the morning after the overnight fast. Routine biochemical and hematological tests were performed using automatic analyzers. Plasma MDA (normal values 1.5-2.25 μmol/l) was determined using a fluorometric method, SOD activity in erythrocytes (expressed in units; normal values 0.7-19 U) was evaluated by a xanthine-xanthine oxidase system on the Genesys 5 spectrophotometer (Thermo Electron Corporation, Marietta, Ohio, USA). Serum for analysis of ascorbic acid (normal values 28-86 μmol/l) was treated with trichloroacetic acid and frozen until analyzed by spectrophotometry (Škrha *et al.* 2007).

Data are shown as means ± SD. Analysis of paired values (data before and after the operation) was performed by means of paired t-test or Wilcoxon rank-sum test (data with nonparametrical distribution). $P<0.05$ values were considered as significant. Data were analyzed using the statistical software package Statistica version 9.1CZ (StatSoft, Tulsa, OK, USA).

Tumor resection led to urine catecholamine normalization, to significant decrease in 24-hour blood pressure (systolic blood pressure: $P=0.005$; diastolic blood pressure: $P=0.004$), plasma glucose values ($p<0.0001$), and to increase in body mass index ($P=0.003$) in subjects with PHEO (Table 1). A significant decrease in white blood cell ($P=0.01$) and platelet ($P=0.003$) counts was detected after the operation. We have also found significantly higher levels of high-density lipoprotein cholesterol in patients with PHEO before the operation ($P=0.03$) (Table 1).

Among studied parameters of oxidative stress, successful tumor removal was accompanied with significantly increased vitamin C values ($P=0.02$), and significantly decreased MDA values ($P=0.01$), but no change in SOD activity was found (Table 1).

In this study, we have shown that PHEO removal leads to significant changes in markers of oxidative stress – increase of vitamin C levels and decrease of MDA levels. However, no change was found in SOD activity.

Decreased vitamin C levels in PHEO may be caused by its increased consumption because ascorbic acid is cofactor of dopamine β-hydroxylase for norepinephrine synthesis from dopamine (Kvetňanský *et al*. 2009). Concentration of vitamin C in adrenal glands is the highest in the body as ascorbic acid serves as cofactor for synthesis of steroids (Patak *et al.* 2004). Vitamin C is considered as a potent antioxidant which reduces both organic and inorganic radicals (Škrha *et al.* 2007). Low plasma ascorbic acid concentration was shown in diabetes mellitus and in hypertension which are typical signs of catecholamine excess in PHEO (Mandl *et al.* 2009, Stephens *et al.* 2009).

Malondialdehyde is a peroxidation product of polyunsaturated fatty acids and its level correlates with the degree of lipid peroxidation due to oxidative stress in cell membranes and their damage. MDA may be used as a marker of oxidative stress (Nielsen *et al.* 1997). It has been shown, that catecholamines in high concentrations may undergo auto-oxidation after saturation of two main

metabolic pathways regulated by monoamino oxidase and catechol-ortho-methyl transferease and so create oxidized catecholamines with their end product aminolutin. Oxidized catecholamine products may increase MDA levels showing the higher state of lipid peroxidation. Formation of oxidized catecholamines may contribute like functional hypoxia, intracellular Ca^{2+} overload, coronary spasm or increased membrane permeability to cardiac toxicity induced by high levels of circulating catecholamines (Adameová *et al*. 2009).

Table 1. Changes in baseline characteristics and markers of oxidative stress in subjects with pheochromocytoma before and after the operation.

Parameter	Pheochromocytoma		
	Before operation	**After operation**	**P**
Number of subjects	18	18	
Age, years	46±13	48±13	
Body mass index, kg.m^{-2}	24±3	26±4	0.003
24-hour systolic blood pressure, mmHg	133±18	118±9	0.005
24-hour diastolic blood pressure, mmHg	82±12	74±4	0.004
24-hour heart rate, beats/min	73±15	68±8	NS
White blood cell count, 10^9/l	8.3±2.6	6.7±1.7	0.01
Red blood cell count, 10^{12}/l	4.6±0.5	4.8±0.5	0.02
Hemoglobin, g/l	136±16	144±15	0.007
Platelet count, 10^9/l	352±119	287±84	0.003
Glucose, mmol/l	6.5±1.3	4.8±0.6	<0.001
Total cholesterol, mmol/l	5.7±1.0	5.4±1.1	0.27
Triglycerides, mmol/l	1.5±0.9	1.6±1.0	0.57
HDL cholesterol, mmol/l	1.7±0.3	1.4±0.3	0.028
Vitamin C, μmol/l	61±27	77±20	0.024
Malondialdehyde, μmol/l	2.6±0.4	2.0±0.6	0.014
Superoxid dismutase, U	0.6±0.4	0.6±0.4	0.55
24-hour epinephrine, nmol/g creatinine	382±637	17±14	<0.001
24-hour norepinephrine, nmol/g creatinine	2686±2279	166±58	<0.001

Data are expressed as means ± SD.

Superoxide dismutase is the group of metaloproteins which catalyse dismutation of superoxide radicals to hydrogen peroxide. They play an important role in endothelial and mitochondrial function by inhibiting oxidative inactivation of bioavailable nitric oxide and so protect aerobic organism against the potential damage of superoxide radicals. They are involved in compartmentalized redox signaling to regulate many vascular functions (Fukai and Ushio-Fukai 2011). Genetically decreased function of extracellular SOD has been associated with increased risk of ischemic heart disease (Juul *et al.* 2004). However, we did not find any changes in SOD activities in subjects with PHEO before and after the operation.

Other mechanism which may link PHEO with oxidative stress has been postulated by Pacák (2011). In those tumors related to the mutation of the *SDHx* gene, disruption in SDH activity is associated not only with activation of the angiogenic, hypoxic and apoptotic pathways but also with the accumulation of succinate, causing chronic oxidative stress (Ishii *et al.* 1998). Other mechanism, which may be involved in reactive oxygen species accumulation in subjects with mutation of the *VHL* gene, is the stabilization of the hypoxia-inducible factor 1α which inhibits conversion of pyruvate to acetyl-CoA and oxidative phosphorylation (Linehan *et al.* 2010). However, all but one patient included in this study were tested negative for mutation of *SDHx* or *VHL* genes. We may so only speculate, if these genetically based mechanisms play role in generating oxidative stress in

subjects without mutations of these aforementioned genes.

Oxidative stress participates in all stages of cardiovascular disease, from lipoprotein modification to plaque rupture, and biomarkers of oxidative stress predict development of coronary artery disease (Heslop *et al.* 2010). Patients with PHEO develop subclinical atherosclerosis (Holaj *et al.* 2009) and cardiovascular complications related to myocardial or cerebrovascular ischemia occur relatively frequently (Zelinka *et al.* 2012).

This study has some limitations. We have studied relatively low number of subjects with PHEO and also the number of subjects with inherited disease was rather small. Catecholamine action in PHEO leads usually to hypertension and/or to disturbances in glucose metabolism and these factors may also influence our results. In addition, the effect of antihypertensive drugs (in particular doxazosin) on measured markers of oxidative stress before surgery cannot be ruled out.

In conclusion, in subjects with PHEO, we demonstrate for the first time that successful tumor removal of PHEO with subsequent catecholamine normalization is associated with an increase of serum vitamin C levels and a decrease of MDA plasma concentration which may indicate lowering of oxidative stress in subjects with PHEO following successful removal of PHEO tumor.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This research was supported by the research programs of Charles University P25/LF1/2 and P27/LF1/1, and by Research grant of Czech Ministry of Health NT12336-4/2011.

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