Interaction between single nucleotide polymorphism in catalase gene and catalase activity under the conditions of oxidative stress

Short communication

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Short title: (-262C>T) catalase gene SNP affects H_2O_2-induced catalase activity

Summary

Catalase is an antioxidant enzyme the activity of which is crucial for the protection against damage caused by reactive oxygen species. The -262C>T polymorphism in the promoter region of catalase gene was found to be associated with altered catalase levels. In this study, leukocyte catalase activity was measured after H_2O_2-induced oxidative stress. C/T and T/T genotypes were associated with the decrease of catalase levels in contrast to C/C donors who had elevated catalase activity in the presence of 0.4 and 0.7 mM H_2O_2. Genotype-dependent response of catalase activity to oxidative stress might be related to the predisposition of catalase mutant allele carriers to disorders mediated by oxidative stress.

Key words: catalase, polymorphism, free radicals, H_2O_2

Catalase is an antioxidant enzyme responsible for H_2O_2 conversion into oxygen and water (Ahn *et al.* 2006). Catalase uses hydrogen peroxide as a source of electrons and is expressed in almost all types of eukaryotic cells. It is considered to be the most important regulator of H_2O_2 metabolism. H_2O_2 in high concentrations could be toxic to cells and it modulates some physiological processes such as cell proliferation, apoptosis, and platelet activation at low concentrations (Labios *et al.* 2009). Catalase gene polymorphism in promoter region (–262C>T) affects transcription factor binding and thus decreases catalase enzymatic activity leading to increased formation of hydroxyl radicals and elevated risk of breast cancer development, asbestosis, and arsenic-induced hyperkeratosis (Ahn *et al.* 2005, Mak *et al.* 2007, Franko *et al.* 2008). Another study revealed the increased frequency of mutant catalase genotype in patients with pseudoxanthoma elasticum – rare hereditary skin disorder caused by progressive alterations of connective tissue including calcification and fragmentation of elastic fibers (Zarbock *et al.* 2007). In addition, catalase gene mutation in exon 9 is associated with vitiligo development – skin disease caused by oxidative stress damage of melanocytes followed by a progressive loss of skin pigment produced by them (Casp *et al.* 2002, Schallreuter *et al.* 2012).

Catalase is not the only enzyme responsible for H_2O_2 metabolism, because thioredoxin reductase and glutathione peroxidase are also major antioxidant tools against H_2O_2-mediated stress (Schallreuter and Wood 2001). Glutathione peroxidase seems to be the major enzyme for detoxification of H_2O_2 under the normal conditions (Chabory *et al.*

2010). Catalase plays a more significant role in protecting cells against severe oxidative stress (Kinnula *et al.* 1992).

The aim of our research was to evaluate catalase polymorphism distribution in healthy voluntaries of Krasnoyarsk Territory, Siberian Federal District of Russian Federation and to estimate catalase activity in persons with different catalase genotype under the oxidative stress conditions induced by H_2O_2.

RFLP analysis The study was approved by Local Ethic Committee of Krasnoyarsk State Medical University named after Prof. V. F. Voino-Yasenetsky. Blood samples from 103 healthy adult voluntaries (78 women and 25 men) in the age ranging from 19 to 53 years were processed for DNA isolation. Genomic DNA was extracted from whole blood by DNA-sorb B isolation kit (AmpliSens, Russia). The primer sequences were CTGATAACCGGGAGCCCCGCCCTGGGTTCGGATAT-3' and 5'-CTAGGCAGGCCAAGATTGGAAGCCCAATGG-3' (SibEnzyme, Russia) constructed as described by Zarbock *et al.* (2007) for creation of the restriction site for EcoR V in wild allele (GATATC). The cycling conditions were: 95 ºC for 15 min, 40 three-step cycles (94 ºC – 1 min, 68 ºC – 1 min, 72 ºC – 1 min), followed by 72 ºC for 10 min. PCR product was digested by restriction endonuclease EcoR V for 20 h at 37 ºC. The products of digestion were separated on a 10 % polyacrylamide gel and stained with ethidium bromide. Bands were then detected by Molecular Imager ChemiDoc^{TM}XRS+ with Image Lab^{TM} Software (BioRad).

Oxidative stress modulation Human peripheral blood mononuclear cells were obtained from patients of three different genotypes (CC, C/T, T/T) and were grown in RPMI containing 10 % fetal bovine serum at 37 ºC in a 5 % CO_2 incubator. H_2O_2 was

administered to the cells in final concentrations 0.4 mM, 0.7 mM, and 1.0 mM for 60 min. Thereafter, the cells were harvested, washed twice with 0.15 mM NaCl solution and stored at -20 ºC. The density of cells at the collection time was 1×10^6 cells/ml.

Catalase activity Lysed peripheral blood mononuclear cells (0.1 ml) were added to 2 ml 0.03 % H_2O_2 solution. After 10 min, 1 ml 4 % ammonium molybdate was used to cease the reaction. Blanks consisted of distilled water. Extinction was monitored on a SF-46 spectrophotometer by changes in optical density reaction probe against blank probe at 410 nm. Catalase activity was determined by the following formula: $A = (E_b\text{-}E_s) \bullet V / v \bullet t \bullet C$ where A – catalase activity, E_b – extinction of a blank probe, E_s – extinction of a sample probe, V – total volume of the probe, v – sample volume, t – incubation time, C – extinction coefficient ($22.2*10^3$ $mmol^{-1}*sm^{-1}$).

Statistical analysis Continuous variables were analyzed by independent *t* test and were presented as means ± S.D. The allele frequency was obtained by direct gene counting. The Hardy-Weinberg equilibrium was tested by using the x^2 test. The frequency distribution at catalase single nucleotide polymorphism (SNP) was tested for deviations from Hardy-Weinberg equilibrium. Statistical analysis of catalase activity was performed using Kruskal-Wallis test followed by the Mann-Whitney U test. The differences were considered significant when $P<0.05$.

Our results indicated that 71 (69 %) individuals out of 103 persons tested had C/C genotype, while C/T genotype was found in 26 (25 %) persons, 6 (6 %) persons were classified as T/T homozygous mutants. Catalase 262C>T gene polymorphism in Russian population was shown to correlate with that in Hardy-Weinberg equilibrium (x^2=2.67, p=0.10) (Table 1). Catalase activity was different in cells derived from

persons with various genotypes. Peripheral blood mononuclear cells from C/C genotype individuals had the increased catalase activity when exposed to H_2O_2 concentrations, with a maximum at 0.7 mM. On the contrary, the decrease of H_2O_2-induced catalase activity was observed (Fig. 1) in C/T and T/T genotype individuals.

Donors with C/C genotype were found to be the most frequent, which is in agreement with the previous results received for C/C, C/T and T/T in Swedish (52 %, 31 %, 7 %) and German (59.8 %, 34.2 %, 6.0 %) populations (Forsberg *et al.* 2001, Zarbock *et al.* 2007). Peripheral blood mononuclear cells with various catalase genotypes differed in their abilities to respond to oxidative stress conditions. Mutant allele carriers had a decreased catalase activity and C/T genotype was also associated with a diminished activity of this enzyme, but it did not reach a statistical significance (Bastaki *et al.* 2006). Another study revealed a significantly lower basal catalase activity only in Caucasians individuals with C/T and T/T genotypes (Ahn *et al.* 2005). In our study significantly lower catalase activity was observed in C/T and T/T genotype carriers as compared to C/C allele carriers during H_2O_2-induced oxidative stress. Another study revealed that single polymorphism located in promoter region can affect not only catalase expression but also its activity (Liu *et al.* 2010). The explanation for that could be hypothetically based upon the evidence that catalase substrate H_2O_2 can considerably affect the enzyme activity (and) to result in catalase inactivation (Gibbons *et al.* 2006). Consequently, a decreased catalase expression in catalase mutant allele carriers may lead to further diminishing of catalase activity.

The lack of catalase activity hinders the conversion of hydroxyl radicals and peroxide into water and oxygen. Hydroxyl radicals are referred to be the most reactive species

produced in biological systems (Berlett and Stadtman 1997). Elevated levels of hydroxyl radicals have a pronounced damaging effect and account for cell injury and the development of various pathologies. The data on the influence of catalase mutant alleles on the development of pathological states are contradictory (Crawford *et al.* 2011, Jiang *et al.* 2001), but our study revealed different responses to oxidative stress in peripheral blood mononuclear cells of catalase mutant allele carriers. This fact may point to the possibility of oxidative-stress disease development (skin cancer, neurodegenerative diseases, aging etc.).

It is known that 0.5-1.0 mM concentrations of H_2O_2 induce cell apoptosis *via* the loss of mitochondrial transmembrane potential and the release of cytochrome *c* into cytoplasm (Pryor *et al.* 2006). It may be due to a decrease of catalase activity in C/C genotype donors after the incubation with 1.0 mM H_2O_2. Similar events can be associated with the decreased catalase activity in C/T heterozygotes and T/T homozygotes. The individuals with this genotype may be more sensitive to oxidative stress conditions with early apoptosis initiation. The apoptosis followed by catalase insufficiency may be a very important stage in age-related disease development.

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Table 1. Frequencies of -262C>T catalase genotypes in healthy donors from Russian population.

genotype			allele	
C/C	**C/T**	**T/T**	**C**	**T**
71	26	6	168	38
69 (%)	25 (%)	6 (%)	82 (%)	18 (%)
$\chi^2 = 2.67, \quad p = 0.10$				

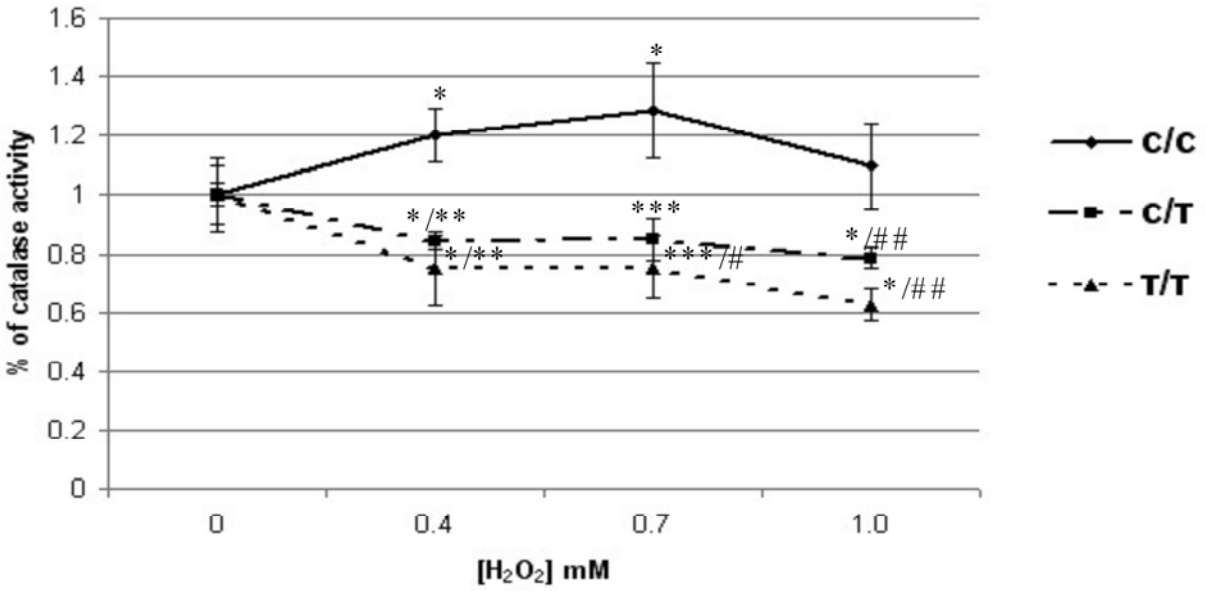

Fig. 1. Catalase gene single nucleotide polymorphism affects H_2O_2-induced catalase activity. Leukocytes from C/C genotype donors exhibit elevated catalase activity in the presence of 0.4 mM and 0.7 mM H_2O_2 for 60 min. C/T and T/T allele carriers showed decreased catalase activity in leukocytes after the incubation with H_2O_2. The results are means ± S.D. of three independent experiments. * – significant in comparison to control group (α=0.05), **, ***, $^{\#\#}$ – significant differences from C/C genotype group at the corresponding H_2O_2 concentrations (α=0.05), $^{\#}$ – significant difference from C/T genotype group (α=0.05).