Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Snežana B. Pajovic
Laboratory for Molecular Biology and Endocrinology,
Vinca Institute of Nuclear Sciences, Belgrade, Yugoslavia

FAX: 38-1-11-455-561
e-mail: pajovic@rt270.vin.bg.ac.yu

J. Med. Sci., 2 (5-6): 234-236
September-December, 2002

Activities of Superoxide Dismutase and Catalase in Cancerous and Non-cancerous Human Kidney Tissues

Snežana B. Pajovic, [1]Gordana Cavric, Jelena Kasapovic, Ana Todorovic and Snežana Pejic

Superoxide dismutase and catalase activities were measured in tissue extracts obtained from cancerous and corresponding non-cancerous tissues of patients with renal adenocarcinoma. The activities of cytosol superoxide dismutase (CuZnSOD) and mitochondrial superoxide dismutase (MnSOD) were decreased significantly ($P \leq 0.02$) in cancerous tissues in comparison to non-cancerous tissues, while the activity of catalase (CAT) was increased significantly ($P < 0.02$) in cancerous tissue at stage III-IV. These results suggest that human kidney cancer progression is accompanied by altered antioxidant status of both superoxide dismutase (SOD) and catalase activities.

Key words: Superoxide dismutase, catalase, kidney, cancer, human

ANSInet
Asian Network for Scientific Information

Laboratory for Molecular Biology and Endocrinology,
Vinca Institute of Nuclear Sciences, Belgrade, Yugoslavia
[1]Surgery "Ngaka", Independence Avenue-881, Gaborone, Botswana

Introduction

An increasing amount of experimental and epidemiological evidence implicates the involvement of oxygen-derived radicals in the pathogenesis of cancer development (Mates *et al.*, 1999). Oxygen-derived radicals are able to cause damage to membranes, mitochondria and macromolecules including proteins, lipids and DNA. Accumulation of DNA damages has been suggested to contribute to carcinogenesis. It would, therefore, be advantageous to pinpoint the effects of oxygen-derived radicals in cancer development (Portakal *et al.*, 2000). Reactive oxygen species (ROS), represented by superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH), have been implicated in many diseases including cancer. ROS have been known to play an important role in the initiation and promotion of multistep carcinogenesis. The cellular antioxidants play a crucial role in protection against neoplastic disease (Ray *et al.*, 2000; Bostwick *et al.*, 2000).

In our previous study, investigated the correlation between chromosome aberrations, micronuclei and activity of superoxide dismutases (SOD) in human lymphocytes after irradiation *in vitro*. The indirect role of intracellular SODs on the yield of radiation-induced DNA damage was observed (Joksic *et al.*, 2000). Related to that, further defined the correlation between SODs activities and intrinsic radiosensitivity induced by radiotherapy in breast cancer patients (Pajovic *et al.*, 1998; Pajovic *et al.*, 2000).

Homma-Takeda *et al.* (2000) examined the enzyme activity and protein content of superoxide dismutase isozymes in human renal cell carcinoma. Immunoblot analysis showed that the loss in enzyme activity in cancerous tissue was greater than the decrease in protein content for either isozyme. The selective decrease in CuZnSOD activities in cancerous tissue observed in that study suggests that the cytoplasmic defense system against free radical damage appears to be reduced in renal cell carcinoma. Since the oxidant–antioxidant balance within tissues is thought to contribute to the development and progression of cancer.

The aim of the study was to evaluate whether the kidney cancer progression is accompanied by changes in the activity of antioxidant enzymes such as manganese superoxide dismutase, copper-zinc superoxide dismutase and catalase.

Materials and Methods

This study was performed on 7 patients with renal adenocarcinoma (mean age 59 years), hospitalized at Department of Urology, Clinical Center of Serbia, during January to December 2001. After nephrectomy and histopathological evaluation, tissues with III-IV stage of malignancy were selected as well as non-malignant tissues of the same kidneys were used as control.

Cancerous and non-cancerous kidney tissue homogenates were prepared according to slightly modified methods of Rossi *et al.* (1983) and DeWaziers and Albrecht (1987). Tissues were homogenized in 0.05 M KH_2PO_4, 0.1 mM EDTA, pH 7.8. Homogenates were vortexed 30 sec several times with intermittent cooling on ice and left frozen at -70°C for 20 h. The homogenates were than defrosted and centrifuged at 35000 rpm for 100 min. Cytosols were kept at -20°C until use. Protein concentration in the cytosol was determined by the method of Lowry *et al.* (1951).

Enzyme activity of SOD was determined by the method of Misra and Fridovich (1972) before and after the inhibition of CuZnSOD with KCN (Geller and Winge, 1983). This method is based on the ability of SOD to inhibit the auto-oxidation of adrenaline to adrenochrome in 0.05 M Na_2CO_3, 0.1 mM EDTA, pH 10.2, which was monitored spectrophotometrically at 480 nm. The enzyme activity was expressed in units per mg protein. One unit of SOD was defined as the amount of protein, which caused 50% inhibition of conversion rate of adrenaline to adrenochrome between the third and the fourth minute of auto-oxidation.

CAT activity was assayed as suggested by Beutler (1982) and expressed as μmol H_2O_2 min^{-1} mg^{-1} protein. The method is based on the rate of H_2O_2 degradation by the action of CAT contained in the samples, monitored spectrophotometrically at 230 nm, in 5 mM EDTA, 1 M Tris-HCl, pH 8.0.

The significance of differences between groups was determined by Student's t-test for dependent variables.

Results

The activity of mitochondrial MnSOD (C: 4.01 ± 1.29; NC: 9.17 ± 1.49), P=0.02) as well as the activity of cytosol CuZnSOD (C: 19.06 ± 4.83; NC: 34.95 ± 5.56, P<0.02) was significantly reduced in cancerous (C) kidney tissue in comparison to non-cancerous (NC) tissue (Fig. 1). On the contrary, the activity of CAT was significantly (P < 0.02) increased in neoplastic tissue in comparison to corresponding controls (C: 20.41 ± 4.25; NC: 14.55 ± 2.47) (Fig. 2).

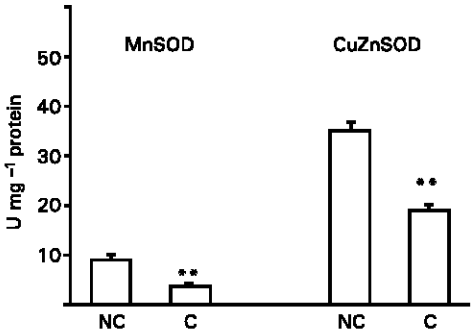

Fig. 1: The activities of MnSOD and CuZnSOD in non-cancerous (NC) and cancerous © kidney tissues of 7 subjects. Data are means ± SE **: P ≤ 0.02

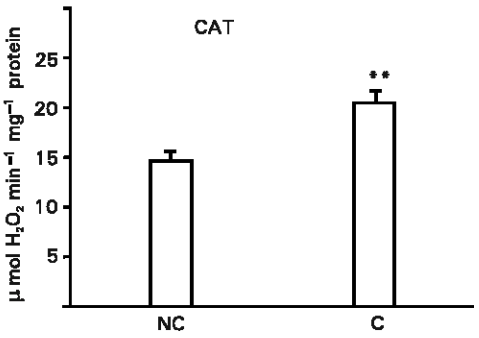

Fig. 2: The activity of catalase (CAT) in non-cancerous (NC) and cancerous (C) kidney tissues of 6 subjects. Data are means ± SE **: P < 0.02

Discussion

Results suggest that antioxidant enzyme activities are significantly altered the human renal adenocarcinoma at stage III-IV. Segura-Aguilar *et al.* (1994), Durak *et al.* (1997) and Homma-Takeda *et al.* (2000) showed that human renal cell carcinomas have significantly reduced the activities of CuZnSOD and glutathione peroxidase (GSH-Px) and non significant variation of MnSOD activity when compared to corresponding non-cancerous renal tissue. Reduced activity of CuZnSOD, in this study (Fig. 1), is in accordance with these findings. The differences with respect to MnSOD activity and increment of CAT activity are in agreement with Oberley *et al.* (1994) and Oberley and Oberley (1997). They reported that human renal cell carcinomas show greatly altered MnSOD level (either elevated or depressed), which depends on heterogeneity of cell types and number of mitochondria, and the high level of CAT. Also shown that relevant variations of GSH-antioxidant enzyme system exist in the different stages of renal cell carcinoma (Lusini *et al.*, 2001), which together support the hypothesis that

oxidative stress plays an important role in renal carcinoma growth and progression. Namely, growing evidence suggest that $O_2^{\cdot -}$ and H_2O_2 mediate the mitogenic signaling of protein kinase activity in transformed cells, as well as the growth factor induction of proto-oncogene expression (Finkel, 1998). Also, it can not be excluded that increment of ROS in malignant cells may be caused by accumulation of DNA damages related to antioxidative defense system.

Free radicals and antioxidant enzymes may play a critical role in cell proliferation and in the resistance of malignant cells against cytotoxic drugs and radiation (Kahlos *et al.*, 2001). Data suggest that enhanced ability in scavenging free radicals by antioxidant enzymes contribute to the resistance of cancer during chemo- and radiotherapy (Ahmed *et al.*, 1999; Suzuki *et al.*, 2000). Therefore, examination of antioxidant status in cancer tissues seems to be necessary for enhancing the efficacy of cancer therapy.

This study indicates that the development of neoplasia in the human kidney is accompanied by significant changes in the activity of superoxide dismutases and catalase. In conclusion, antioxidative disbalance might have a functional role in human kidney carcinogenesis. These findings are used to propose new cancer therapies based on modulation of cellular oxido-redox state.

Acknowledgments

The authors wish to thank Prof. Stevan Matijaševic and Dr. Nevena Ribarac-Stepic for help in organization of the experiments.

References

Ahmed, M.I., S.T. Fayed, H. Hossein and F.M. Tash, 1999. Lipid peroxidation and antioxidant status in human cervical carcinoma. Dis. Markers, 15: 283-291.

Beutler, E., 1982. Red cell metabolism, a manual of biochemical methods. Catalase. In: Beutler, E. (Ed.) Grune and Stratton Inc., New York, pp: 105-106.

Bostwick, D.G., E.E. Alexander, R. Singh, A. Shan, J. Qian, R.M. Santella, L.W. Oberley, T. Yan, W. Zhong, X. Jiang and T.D. Oberley, 2000. Antioxidant enzyme expression and reactive oxygen species damage in prostatic intra epithelial neoplasia and cancer. Cancer, 89: 123-134.

DeWaziers, I. and R. Albrecht, 1987. The effects of vitamin A, nutritional status on glutathione, glutathione transferase and glutathione peroxidase activities in rat intestine. Experientia, 43: 394-395.

Durak, I., Y. Beduk, M. Kavutcu, S. Ozturk, O. Canbolat and S. Ulutepe, 1997. Activities of superoxide dismutase and glutathione peroxidase enzymes in cancerous and non-cancerous human kidney tissues. Int. Urol. Nephrol., 29: 5-11.

Finkel, T., 1998. Oxygen radicals and signaling. Curr. Opin. Cell Biol., 10: 248-253.

Geller, B.L. and D.R. Winge, 1983. A method for distinguishing Cu, Zn-and Mn-containing superoxide dismutases. Anal. Biochem., 128: 86-92.

Homma-Takeda, S., A. Sasaki, M. Kikushima, Y. Kumagai, K. Uchida, K. Kawai, H. Akaza and N. Shimojo, 2000. Enzyme activity and protein content of superoxide dismutase isozymes in human renal cell carcinoma. Res. Commun. Mol. Pathol. Pharmacol., 108: 49-55.

Joksic, G., S.B. Pajovic, M. Stankovic, S. Pejic, J. Kasapovic, N. Calonghi, L. Masotti and D.T. Kanazir, 2000. Chromosome aberrations, micronuclei, and activity of superoxide dismutases in human lymphocytes after irradiation *in vitro*. Cell. Mol. Life Sci., 57: 842-850.

Kahlos, K., Y. Soini, R. Sormunen, R. Kaarteenaho-Wiik, P. Paakko, K. Linnainmaa and V.L. Kinnula, 2001. Expression and prognostic significance of catalase in malignant mesothelioma. Cancer, 91: 1349-1357.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Lusini, L., S.A. Tripodi, R. Rossi, F. Giannerini, D. Giustarini, M.T. del Vecchio, G. Barbanti, M. Cintorino, P. Tosi and P. Di Simplico, 2001. Altered glutathione anti-oxidant metabolism during tumor progression in human renal-cell carcinoma. Int. J. Cancer, 91: 55-59.

Mates, J.M., C. Perez-Gomez and I. Nunez de Castro, 1999. Antioxidant enzymes and human diseases. Clin. Biochem., 32: 595-603.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Oberley, T.D. and L.W. Oberley, 1997. Antioxidant enzyme levels in cancer. Histol. Histopathol., 12: 525-535.

Oberley, T.D., J.M. Sempf, M.J. Oberley, M.L. McCormick, K.E. Muse and L.W. Oberley, 1994. Immunogold analysis of antioxidant enzymes in human renal cell carcinoma. Virchows Arch., 424: 155-164.

Pajovic, S.B., G. Joksic, J. Kasapovic, S. Pejic, J. Stankovic and Lj. Radoševic-Jelic, 1998. Correlation between superoxide dismutase activities and intrinsic radiosensitivity induced by radiotherapy in breast cancer patients. 35th Annual Meeting of Oncology, Institute for Oncology and Radiology of Serbia, Health Protection Suppl., pp: 59-60.

Pajovic, S.B., G. Joksic, J. Kasapovic, S. Pejic and D.T. Kanazir, 2000. Role of antioxidant enzymes in radiosensitivity of human blood cells. J. Environ. Pathol. Toxicol. Oncol., 19: 325-331.

Portakal, O., O. Ozkaya, M.E. Inal, B. Bozan, M. Kosan and I. Sayek, 2000. Coenzyme Q 10 concentrations and antioxidant status in tissues of breast cancer patients. Clin. Biochem., 33: 279-284.

Ray, G., S. Batra, N.K. Shukla, S. Deo, V. Raina, S. Ashok and S.A. Husain, 2000. Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast Cancer Res. Treat., 59: 163-170.

Rossi, M.A., G. Cecchini and M.M. Dianzini, 1983. Glutathione peroxidase, glutathione reductase and glutathione transferase in two different hepatomas and in normal liver. IRCS Med. Sci., 11: 805.

Segura-Aguilar, J., A. Cremades, A. Llombart-Bosch, E. Monsalve, L. Ernster and F.J. Romero, 1994. Activity and immunohistochemistry of DT-diaphorase in hamster and human kidney tumours. Carcinogenesis, 15: 1631-1636.

Suzuki, Y., Y. Kondo, S. Himeno, K. Nemoto, M. Akimoto and N. Imura, 2000. Role of antioxidant systems in human androgen-independent prostate cancer cells. Prostate, 43: 144-149.