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Effect of vitamins E+C and taurine on the oxidative state of DNA in the liver of growing pigs*

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ABSTRACT

Growing pigs (n 21) at an initial liveweight of 30 kg were divided into three groups of 7 animals each and housed in individual cages for 100 days. The pigs were fed with similar diets (13.5 MJ ME and 178 g crude protein/kg) but with different additions of vitamins C+E and taurine.

The antioxidative vitamins E and C supplied to diets with high energy concentrations (containing lard) can act as pro-oxidants on liver DNA in growing pigs. Taurine decreased the concentration of products from nucleotide oxidation, but in the presence of vitamin E and C, promoted hepatocyte lesions.

KEY WORDS: pigs, vitamin E, vitamin C, taurine, DNA oxidation

INTRODUCTION

A high concentration of energy and protein in the diet can enhance the rate of energy metabolism, activity of electron transport in the mitochondrial respiratory chain and cause an increase in the synthesis of Reactive Oxygen Species (ROS) (Weindruch, 2002). ROS enhance the destruction of lipids, proteins, carbohydrates and DNA. Oxidative DNA damage leads to formation of 8-oxo-2-deoxyguanosine, an adduct that occurs in DNA and causes mutagenesis (Helbock et al., 1998). Vitamin E prevents lipid peroxidation while vitamin C reduces the tocopheryl radical to its active form, tocopherol. It can be expected that a complex antioxidative system requires an antioxidant affiliated with DNA and therefore taurine, mainly taurine's amino group, which is strongly associated with DNA, can be one of the potential antioxidative agents protecting DNA. However,

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unlimited supply of antioxidants can activate pro-oxidative processes resulting in an imbalance in redox homeostasis. The objective of this study was to determine the effects of vitamins E and C and taurine on the oxidative state of DNA in relation to antioxidative/oxidative indicators of redox homeostasis in growing pigs as a model for humans.

MATERIAL AND METHODS

Pigs (30 kg BW; 3 groups × 7 animals) were housed in individual cages for 100 days, and fed with similar diets (13.5 MJ ME and 178 g crude protein/kg) containing, %: barley, 63; wheat, 10.0; soyabean meal, 13.5; meat-and-bone meal, 5.0; lard, 7.0; L-lysine, 0.12; DL-methionine, 0.018; vitamin-mineral mixture, 0.5, but with different additions of vitamins: C (natrium ascorbinate) 0.1; E (α-tocopherol acetate), 0.2, and taurine, 0.5.

At the end of the experiment the animals were fasted for 12 h and blood was sampled from the heart. Thiobarbituric acid reactive substances (TBARS) were measured with 1.2.3.3-tetraethoxypropane (TEP) as the standard by a colorimetric method. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were analysed by Randox Laboratories Ltd. kits at 37°C in a COBAS FARA II analyser. 8-oxo-2'-deoxyguanosine (8-oxo-dG) and 2'-deoxyguanosine were determined using a Dionex HPLC with electrochemical (at 350 mV) and UV (at 254 nm) detectors and a 250×4.6 mm Supelcosil LC-18-S column (5μm grain). The amount of 8-oxo-dG in DNA was calculated as the number of 8-oxo-dG molecules/10^6 unmodified dG molecules. Albumin, total bilirubin, glucose, asparagine transferase (AST) and alanine transferase (ALT) were determined using Vitros DT II (Johnson and Johnson, USA).

The results were subjected to statistical analysis by monofactorial ANOVA and Duncan's range test, using the Statgraphic 4.1 Plus software package.

RESULTS AND DISCUSION

The addition of antioxidants increased the activity of SOD. This enzyme catalyses the dismutation of the superoxide radical ($O^{\cdot}_2$) which is produced by single electron reduction of oxygen, especially in the mitochondrial respiratory chain (Sohal et al., 1995). Dietary antioxidants support the system of defence against ROS in cells, which could lead to SOD protection.

Serum albumins belong to major antioxidants. The elevated serum albumin concentrations and decreased $NO^{\cdot}_3$ concentrations in pigs receiving antioxidants may suggest that the degradation of the cell structure was associated with the water phase and thereby protecting a protein fraction. However, feeding diets supplemented with antioxidants E and C (group II) significantly affected the oxidations of

2-deoxyguanosine (dG) at the C-8 position, which led to an increased concentration of 8-oxodG in liver DNA. These results confirm earlier results (Niemiec and Sawosz, 2004) regarding the pro-oxidative function of vitamin C in rats. Vitamin C can increase generation of $^{\cdot}OH$ in a superoxide anion-driven, redox active Fe^{2+} catalysed, Fenton reaction. DNA is highly resistant to $O^{\cdot}_2$ or H_2O_2, while sensitive to the hydroxyl radical ($^{\cdot}OH$). However, it is likely that reduced transition metal ions, mainly Fe (II) and Cu (II) can inhibit the activities of 8-oxo-2'deoxyguanosine 5'triphosphate pyrophospho-hydrolase (8-oxo-dGTPase), (Kasprzak et al., 1999). This enzyme termed an antimutagenic, "sanitizes" the cellular nucleotide pool (Mo et al., 1992) and prevents incorporation of 8-oxo-dG into DNA. The presence of 8-oxodG in the DNA template was observed in liver disease (Kitada et al., 2001) and may stimulate mutagenesis and cancerogenesis (Shibutani et al., 1991).

The addition of taurine (Group III) could counteract the negative effects of vitamin C because the concentration of 8-oxodG in liver DNA was significantly lower than in Group II, and was similar to Group I. The amine group of taurine has a high affinity to DNA. Redmond et al. (1998) demonstrated that taurine protected DNA against ROS generated by respiratory bursts in neutrophils. However, the activities of ALT and total bilirubin in the serum of pigs receiving vitamins E+C and taurine (Group III) increased, which may be indicative of hepatocyte lesions.

TABLE 1

Concentration of 8-oxo-2'-deoxyguanosine in the liver and biochemical parameters in peripheral blood of rats

Parameters	Group			ANOVA	
	Control I	CE II	CE + T III	SEM	P
8-oxo-2'-deoxyguanosine, 8 oxod G/10^6Dg	7.79^a	10.30^b	8.16^a	0.580	0.0133
Thiobarbituric acid reactive substances, μmol/L	1.97	1.78	1.91	0.547	0.0547
$NO^{\cdot}_2$, μmol/L	20.5^a	3.98^b	5.73^b	4.271	0.0303
Superoxide dismutase, U/gHb	329^a	436^b	435^b	22.7	0.0480
Glutathione peroxidase, U/gHb	49.2	60.6	49.2	4.58	0.1577
Albumin, g/L	40.1^a	43.0^b	43.9^b	0.930	0.0289
Total bilirubin, μmol/L	7.0^a	8.3^a	15.3^b	0.73	0.0000
Glucose, mmol/L	4.6	5.4	5.3	0.53	0.5218
Asparagine transferase, U/L	36.7	43.6	63.9	9.42	0.1346
Alanine transferase, U/L	57.0^a	70.4^{ab}	83.6^b	6.65	0.0362

^{ab} means with different superscripts are significantly different ($P<0.05$)

CONCLUSIONS

Antioxidative vitamins E and C supplied to diets with a high concentration of energy (containing lard) can act as pro-oxidants on DNA in growing pigs. However,

taurine decreases the concentration of oxidative stress-related compounds involving products from nucleotide oxidation, but in the presence of vitamins E and C may contribute to liver damage.

REFERENCES

Helbock H.J., Beckman K.B., Shigenaga M.K., Walter P.B., Woodall A.A., Yeo H.C., Ames B.N., 1998. DNA oxidation matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. Proc. Nat. Acad. Sci. USA 95, 288-293

Kasprzak K.S., Bal W., Porter D.W., Białkowski K., 1999. Studies on oxidative mechanism of metal-induced carcinogenesis. In: Dizdaroglu, Karakaya (Editors). Advances in DNA Damage and Repair. Kluwer Academic Plenum Publishers, New York, pp. 193-207

Kitada T., Seki S., Iwai S., Yamada T., Sakaguchi H., Wakasa K., 2001. In situ detection of oxidative DNA damage 8-hydroxydeoxyguanosine in chronic human liver disease. J. Hepatol. 35, 613-618

Mo J.Y., Maki H., Sekiguchi M., 1992. Hydrolytic elimination of a mutagenic nucleotide 8-oxo-dGTP by human 18-kilodalton protein: Sanitization of nucleotide pool. Proc. Nat. Acad. Sci. USA 89, 11021-11025

Niemiec T., Sawosz E., 2004. Effect of addition of L-ascorbic acid megadoses to the diet on the antioxidative/oxidative state in the rats. Proceedings of European Society of Veterinary and Comparative Nutrition. Budapest, pp. 258-259

Redmond H.P., Stapleton P.P., Neary P., Bouchier-Hayes D., 1998. Immunonutrition: The role of taurine. Nutrition 14, 599-604

Shibutani S., Takeshita M., Grollman A.P., 1991. Insertion of specific based during DNA synthesis past the oxidation-damage base 8-oxodG. Nature 349, 431-434

Sohal R.S., Sohal B.H., Orr W.C., 1995. Mitochondrial superoxide dismutase and hydrogen peroxide generation. Protein damage and longevity in different species of flies. Free Radical Biol. Med. 19, 499-504

Weindruch R., 2002. Caloric intake, oxidative stress and aging. Free Radical Biol. Med. 33, Suppl. 2, S169

STRESZCZENIE

Wpływ dodatku witamin E+C oraz tauryny na utlenienie DNA w wątrobie rosnących świń

Rosnące świnie (n=21, o m.c. 30 kg) podzielono na 3 grupy po 7 sztuk i utrzymywano w indywidualnych klatkach przez 100 dni. Tuczniki otrzymywały izoenergetyczną i izobiałkową dietę z udziałem różnych dodatków: witamin E i C oraz witamin E+C i tauryny.

Witaminy antyoksydacyjne dodawane do diety o wysokiej koncentracji energii (dodatek smalcu) wykazywały prooksydacyjne działanie w stosunku do DNA wątroby świń. Tauryna wpłynęła na zmniejszenie koncentracji produktów oksydacji nukleotydów w wątrobie, wraz z witaminami E i C spowodowała uszkodzenie miąższu wątroby.