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DIETARY VITAMIN E DECREASES ESR SIGNAL INTENSITY IN HEPATIC MICROSOMAL PREPARATIONS FROM MALIGNANT HYPERTHERMIA SUSCEPTIBLE PIGS

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On incubation with the spin trap α-(4-pyridyl-1-oxide)-N-*tert*-butylnitrone (4-POBN), a characteristic electron spin resonance (ESR) signal was produced at a greater rate in hepatic microsomal fractions from malignant hyprthermia susceptible (MHS) pigs compared with resistant (MHR) pigs. This was accompanied by increased formation of thiobarbituric acid reactive substances (TBARS). Supplementation of diets for six weeks with 235 mg α-tocopherol acetate/kg significantly increased microsomal vitamin E content of both pig types. Moreover, the rate of formation of TBARS and ESR signal height of incubated microsomes from supplemented MHS pigs was decreased to that of MHR pigs. Elevated pyruvate kinase activities and TBARS concentrations in plasma of MHS pigs were also moderated by dietary vitamin E. Vitamin E supplementation may decrease the peroxidative events associated with MH.

KEY WORDS: Malignant hyperthermia, vitamin E, electron spin resonance, lipid peroxidation.

INTRODUCTION

Malignant hyperthermia (MH) is triggered in susceptible animals and humans by exposure to volatile anaesthetics such as halothane. Stresses such as transportation, mating and parturition also induce an MH response in certain breeds of pigs. The syndrome, characterised by a limb rigidity and a rapid and fatal rise in body temperature, may result from the uncontrolled release of Ca^{2+} to the cytosol. Such disruption of skeletal muscle Ca^{2+} homeostasis may reflect a defect in the antioxidant defence system which leads to free radical-mediated damage to cell membranes.[1] The nature of the antioxidant abnormality in MH is unclear. However, on incubation with the spin trap α-(4-pyridyl-1-oxide)-N-*tert*-butylnitrone (4-POBN) microsomal preparations from MH susceptible (MHS) pigs show enhanced formation of a spin adduct compared with those from MH resistant (MHR) pigs.[2] The present study has assessed whether the increased production of unstable free radicals in microsomes from MHS pigs is affected by supplementation of diets with vitamin E.

MATERIALS AND METHODS

At ten weeks of age, British Landrace pigs homozygous for the present (n = 10) or

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absence ($n = 10$) of the halothane gene[3] were individually housed. Five pigs of each type were offered, *ad libitum*, a standard ration containing the recommended amount of 10 iu vitamin E/kg.[4] The remainder were offered the ration with an increased amount of 235 iu vitamin E/kg (as α-tocopherol acetate; BASF, W. Germany). The selenium content of the diets was 0.15 mg/kg. After five weeks 6 ml of blood were removed from the jugular vein of each pig into heparinised "vacutainers" (Becton Dickinson, Cowley, Oxford, U.K.). The plasma (centrifugation; 10 min, 1500 × g, 4° C) was stored at −40° C. One week later, the pigs were killed by captive bolt. The livers were removed and immediately frozen in liquid nitrogen.

Experimental procedures were the same as previously described.[2] In brief, liver microsomal preparations were incubated at 37° C with 4-POBN and aliquots removed at intervals for recording of ESR spectra with a Varian E104 X-band spectrometer (9.5 GHZ microwave frequency, 100 KHZ modulation frequency, 10 mW microwave power, 0.2 mT modulation amplitude). Formation of thiobarbituric acid reactive substances (TBARS) by the microsomal incubations was also determined.[2] Plasma vitamin E and TBARS concentrations, plasma pyruvate kinase activities and microsomal glutathione peroxidase activities were also measured.[1] Microsomal vitamin E and vitamin A content was determined by HPLC.[5]

Data were subjected to analysis of variance. Individual group comparisons were made by Student's t-test using the residual mean square from the analysis of variance to estimate the standard error of the difference between group means.

RESULTS

Vitamin E supplementaion produced a significant twofold increase in plasma vitamin E in both the MHS and MHR pigs ($P < 0.001$). MHS pigs had a greater plasma pyruvate kinase activity and TBARS concentration ($P < 0.001$ and $P < 0.02$ respectively). Supplementation with vitamin E produced a marked decrease ($P < 0.01$) in pyruvate kinase and TBARS in the MHS pigs (Table I).

Hepatic microsomal vitamin E content was increased to a similar extent in both MHS and MHR pigs by dietary vitamin E supplementation ($P < 0.001$). Glutathione peroxidase activities were unaffected by either pig type or vitamin E. However,

TABLE I

Concentrations of vitamin E and thiobarbituric acid reactive substances (TBARS) and pyruvate kinase activity in plasma from malignant hyperthermia susceptible (MHS) and resistant (MHR) pigs fed a diet supplemented with 235 i.u. vitamin E/kg (+ E) or unsupplemented (10 i.u./kg) for 5 weeks

Parameter	Group				RSD	Pig type effect	Vitamin E effect	Interaction
	MHS	MHS + E	MHR	MHR + E				
Vitamin E (μg/mg protein)	0.88	2.16	0.88	2.29	0.30	NS	****	NS
TBARS (nM/ml)	4.42	2.53	2.74	2.35	0.72	**	***	*
Pyruvate kinase (mU/ml)	650	1176	252	214	194	****	***	**

5 animals per group. Residual standard deviations (RSD) obtained from analysis of variance.
NS – Not significant; *$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.001$

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TABLE II

Vitamin E and vitamin A concentrations, glutathione peroxidase (GSHPx) activities and production of thiobarbituric acid reactive substances (TBARS) of microsomes from malignant hyperthermia susceptible (MHS) and resistant (MHR) pigs fed a diet supplemented with 235 i.u. vitamin E/kg (+E) or unsupplemented (10 i.u./kg) for 6 weeks

Parameter	Group				RSD	Pig type effect	Vitamin E effect	Interaction
	MHS	MHS + E	MHR	MHR + E				
Vitamin E (μg/mg protein)	0.18	1.59	0.16	1.37	0.04	NS	***	NS
Vitamin A (μg/mg protein)	1.79	2.89	1.04	2.80	0.94	NS	**	NS
GSHPx (U/mg protein)	33.8	36.8	26.8	38.4	9.32	NS	NS	NS
TBARS (nM/mg protein/180 min)	1.25	0.74	0.88	0.45	0.10	*	**	NS

5 animals per group. Residual standard deviations (RSD) obtained from analysis of variance.
NS - Not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

vitamin A content of the microsomes was increased in line with the vitamin E ($P < 0.01$) irrespective of pig type (Table II).

The characteristic[2] ESR spectra obtained from the microsomal incubations consisted of a triplet of doublets (A(^{14}N),1.57 mT: A(^{1}H),0.26 mT). By 120 min signal height for incubated microsomes from MHS pig was greater than for MHR pigs ($P < 0.05$). However, this difference was not apparent in the preparations from the vitamin E supplemented MHS pigs (Figure 1). Moreover, the significantly greater rate of formation of TBARS by microsomal suspensions of the MHS pigs ($P < 0.05$) was reduced by dietary vitamin E (Table II).

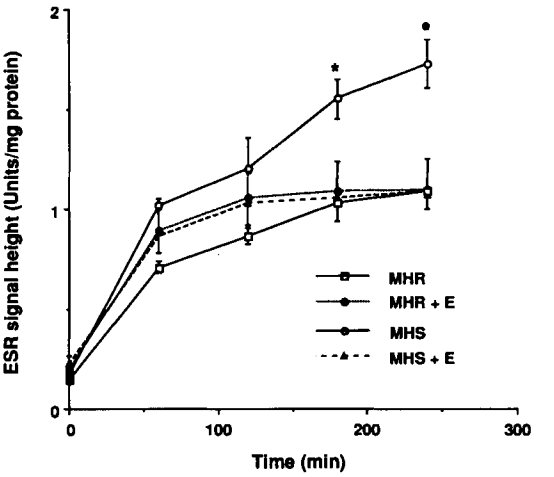

FIGURE 1. The increase of ESR signal height with time in incubations of liver microsomal suspensions from MHS and MHR pigs. +E denotes supplementation with 235 iu α-tocopherol acetate. At incubation times of 180 and 240 min, there is a significant effect of pig type ($P < 0.05$) and vitamin E supplementation ($P < 0.05$).

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DISCUSSION

The increased plasma pyruvate kinase activities and TBARS concentrations in MHS pigs corroborate previous suggestions[1] that there is cell membrane damage involving peroxidation of polyunsaturated fatty acids. The improvement in cell membrane integrity and reduction in indices of lipid peroxidation by vitamin E supplementation suggest that the MHS pig has an inadequacy in its antioxidant defence system which can be modified by increasing the α-tocopherol content of cell membranes. For example, the enhanced formation of ESR signals and TBARS by microsomal preparations from the MHS pigs is reduced in vitamin E supplemented animals and is associated with a 7–10 fold increase in microsomal vitamin E. The nature of the ESR signal also suggests that the antioxidant abnormality is associated with the membrane component of the preparations. The free radical/4-POBN adduct was identical to that previously reported in microsomes from MHS pigs.[2] Such spectra are similar to those obtained from microsomes of vitamin E deficient rats[6] and may represent a pentadienyl adduct of linoleic acid.[2]

There was no difference in glutathione peroxidase activities in the microsomes from the four groups. However, independently of pig type, increased microsomal vitamin E content was associated with increased microsomal vitamin A concentration. As the vitamin A content of all the diets was the same, it is not clear whether the relationship between the microsomal content of the two vitamins reflects an interaction at the cellular level or at the site of absorption in the gut.

Several studies indicate that MHS pigs have an impaired antioxidant defence system and membrane defects in a wide range of tissue including erythrocytes, monocytes, liver, heart and skeletal muscle.[1,7,8] Whether the enhanced free radical activity and increased peroxidation in membrane preparations are a prime cause or a secondary consequence of the MH syndrome is uncertain. Rapid peroxidation of cell membranes in skeletal muscle could lead to an uncontrolled increase in myoplasmic Ca^{2+} and result in the limb rigidity that occurs during the MH response. Alternatively, specific faults in mechanisms of cellular Ca^{2+} homeostasis such as the voltage sensitive ryanodine receptor[9] could lead to similar increases in myoplasmic Ca^{2+}. Subsequent Ca^{2+}-mediated tissue damage[10] may then cause increased intracellular free radical production. Although the biochemical lesion responsible for MH remains unclear, dietary supplementation with vitamin E decreases the peroxidative events associated with the syndrome.

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