Free Rad. Res. Comms., Vol. 4, No. 1, pp. 21–25

Printed in Great Britain

TISSUE DAMAGE IN VITAMIN E DEFICIENT RATS IS NOT DETECTED BY EXPIRED ETHANE AND PENTANE

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(Received November 4th 1986)

Despite evidence for tissue damage in vitamin E deficient rats, no discernible difference in ethane and pentane production was apparent compared with controls. It is suggested that other sources of hydrocarbons may mask hydrocarbon production from peroxidation of polyunsaturated fatty acid components of cell membranes, or that tissue damage occurs before peroxidation of the polyunsaturated fatty acids.

KEY WORDS: Vitamin E deficiency, tissue damage, lipid peroxidation, ethane, pentane, pyruvate kinase, creatine kinase.

INTRODUCTION

Vitamin E deficient animals have elevated plasma activities of muscle enzymes such as pyruvate kinase and creatine kinase[1,2,3] indicating tissue damage. Such damage may arise through increased free-radical mediated peroxidation of polyunsaturated fatty acid components of cell membranes.[4] Of the resulting production of numerous byproducts,[5] determination *in vitro* of some such as conjugated dienes, lipid hydroperoxides and malonaldehyde have been used to assess susceptibility to lipid peroxidation.[6] It is unlikely that any single method can give an accurate measure of lipid peroxidation.[7] Measurement of expired hydrocarbons, mainly ethane and pentane arising from decomposition of ω-3 and ω-6 fatty acids, respectively, is frequently used to assess lipid peroxidation which is occurring *in vivo*.[8]

The present study provides evidence that marked tissue damage may occur in vitamin E deficient rats which is not detected by the measurement of ethane and pentane in the expired breath.

MATERIALS AND METHODS

Male Hooded Lister rats (n = 48) of the Rowett strain were weaned onto a semisynthetic diet containing 5% lard[9] and either 200 or < 1 mg vitamin E kg^{-1} as α-tocopherol acetate (Sigma, London). They were housed in plastic cages with stainless steel grid floors and tops. Water and diet were available *ad libitum*. After 12 weeks, twelve rats from each diet were used for experimentation. Of the remainder, 6 from each group were offered diets with the lard replaced by cod liver oil B.P. (Boots, London) for a further 2 weeks, the remaining 6 from each group being maintained as before.

After overnight starvation, individuals were transferred to glass desiccators (volume 4.71) for determination of expired ethane and pentane as based on Bolt *et al.*[10] After flushing with hydrocarbon free air (Air Products Ltd., Aberdeen) for 10 min, the chambers were sealed. Respiratory CO_2 was absorbed by soda lime and replaced by hydrocarbon free oxygen. Samples of 0.5 ml of gas were removed by glass "pressure-lock" syringes (Precision Sampling, Louisiana, U.S.A.) every hour for 6 hours and injected into a Photovac 10A10 photoionization gas chromatograph (Centronics Ltd., Croydon, UK) with a 0.75 m column (Carbopack BHT 100 40/60). Peak height (ethane) and peak area (pentane) were compared against known standards (Phasesep, Clwyd, UK). Both ethane and pentane increased linearly over time. After substracting the volume of rate and soda lime from the chamber volume, their production was calculated as nMoles 100 g rat^{-1} h^{-1}. Teflon tubing, bungs and connectors were used throughout the gas collection system. No loss of standards injected into the system was detected over 8 hours.

After ether anaesthesia, blood was removed from rats by cardiac puncture into heparinised vacutainers and centrifuged at 4000 g at 4°C for 20 min. Plasma creatine kinase and pyruvate kinase activities were determined with test combination kits from Sigma (London) and Boehringer Mannheim (Lewes, East Sussex) respectively. Liver and gastrocnemius muscle were excised, frozen in liquid N_2 and stored at -40°C prior to analysis. Plasma and tissue vitamin E were determined as described by Taylor *et al.*[11] and spontaneous and iron stimulated malonaldehyde production by homogenates of liver and gastrocnemius muscle was estimated by the measurement of thiobarbituric acid reactive substances.[3]

Data are presented as means $\pm$ S.E. The statistical significance of differences between means was assessed by Student's t-test.

RESULTS

Table 1 presents the measured variables for the six experimental groups. Plasma and tissue vitamin E levels in those animals on vitamin E deficient diets were barely detectable ($< 0.1\ \mu g\ g^{-1}$). In vitamin E sufficient animals plasma pyruvate kinase activity did not significantly differ with age or cod liver oil supplementation. Vitamin E deficiency for 12 or 14 weeks resulted in significantly elevated ($P < 0.05$) pyruvate kinase activity compared with corresponding controls and was further elevated by cod liver oil supplementation. Plasma creatine kinase activities did not significantly differ between the vitamin E supplemented and deficient groups. There was no significant difference in ethane production between vitamin E deficient rats and corresponding controls but a similar and statistically significant ($P < 0.05$) two fold elevation occurred in both groups when given a cod liver oil supplement. Pentane production did not differ between vitamin E deficient and corresponding control groups. Both spontaneous and iron-induced tissue malonaldehyde production were markedly elevated in the vitamin E deficient rats.

DISCUSSION

The increased plasma pyruvate kinase activity in vitamin E deficient rats is indicative of damage to skeletal muscle.[3] These data illustrate the greater sensitivity of plasma

TABLE I
Indices of vitamin E status and tissue damage, and ethane and pentane expiration in control and vitamin E deficient rats.

Parameter	*Control at 12 weeks (12)*	*−E at 12 weeks (12)*	*Control at 14 weeks (6)*	*−E at 14 weeks (6)*	Control + c.l.o. (6)	−E + c.l.o (6)
Weight (g)	350 ± 8	339 ± 9	412 ± 17	389 ± 10	416 ± 6	400 ± 6
Vitamin E						
plasma ($\mu g\,ml$)	6.2 ± 1.5	0.1 ± 0.1*	5.4 ± 0.3	0.1 ± 0.1*	5.4 ± 0.3	0.1 ± 0.1*
liver ($\mu g\,g^{-1}$)		n.d.	6.7 ± 0.8	n.d.	5.5 ± 0.2	n.d.
muscle ($\mu g\,g^{-1}$)	13.7 ± 0.5	n.d.	15.1 ± 0.6	n.d.	13.7 ± 0.5	n.d.
Pyruvate kinase ($mU\,ml^{-1}$)	109 ± 16	1125 ± 215*	73 ± 6	1200 ± 164*	50 ± 11	3040 ± 98*
Creatine kinase ($mU\,ml^{-1}$)	192 ± 7	236 ± 58 *	73 ± 8	138 ± 36	231 ± 115	352 ± 136
Ethane ($nM\,100\,g^{-1}\,h^{-1}$)	12.8 ± 2.3	12.0 ± 1.4	11.5 ± 1.9[a]	13.8 ± 2.0[b]	23.5 ± 4.1[a]	19.1 ± 2.9[b]
Pentane ($nM\,100\,g^{-1}\,h^{-1}$)	1.89 ± 0.4	1.43 ± 0.26	1.95 ± 0.61	2.23 ± 0.35	0.82 ± 0.29	0.87 ± 0.27
Malonaldehyde ($mM\,mg\,protein^{-1}$)						
liver spontaneous	–	–	–	–	0.06 ± 0.02	0.39 ± 0.08*
induced	–				0.01 ± 0.01	0.64 ± 0.10*
muscle spontaneous	–				0.27 ± 0.40	0.52 ± 0.05*
induced	–			–	0.48 ± 0.10	3.55 ± 0.11*

The values are means ± S.E. for the number of animals shown in parentheses. n.d. is not detectable. * indicates a significant difference ($P < 0.05$) of parameter in vitamin E deficient rats (−E) from the corresponding control. Values with corresponding superscripts denote a significant difference ($P < 0.05$) between the rats maintained on the lard based diet for 14 weeks and those with the lard replaced by cod liver oil (c.l.o.) 2 weeks previously.

pyruvate kinase as an indicator of subacute muscle damage than plasma creatine kinase activity.[3] Further increases in plasma pyruvate kinase activity caused by substitution of the lard based diets by cod liver oil based diets indicated exacerbation of muscle damage. Damage caused in tissues by administration of polyunsaturated fatty acids has been attributed to the free radical–mediated degradation of fatty acids which compromises cell membrane function and thus cell viability.[4] Increased malonaldehyde formation in tissue homogenates of vitamin E deficient animals supports the contention that damage to polyunsaturated fatty acids occurred during the onset of tissue damage and shows, at least, that muscle is susceptible to such damage.

Increases in ethane and pentane expiration have been demonstrated previously in vitamin E deficient animals given diets rich in polyunsaturated fatty acids from weaning[12,13] thus implicating lipid peroxidation as a cause of tissue damage. However, we have indications of muscle damage in vitamin E deficient rats on a lard based diet in the absence of increased ethane and pentane output. The origins of these apparently conflicting observations are not clear. One possibility is that ethane and pentane are minor end-products in the metal ion catalysed breakdown of fatty acid hydroperoxides.[7] The peroxidation could be initiated with cell damage but without the subsequent increased production of hydrocarbons.

Elevated plasma pyruvate kinase activity without comparable increases in creatine kinase activity suggests that the tissue damage arising from the vitamin E deficient regime is relatively minor.[3] The possibility that the initial cause of tissue damage results from an increased oxidative stress damaging non-polyunsaturated membrane components such as sulphydryl containing proteins cannot be excluded. The peroxidative damage to lipids may merely be a secondary process and of insufficient magnitude in the present experiments to result in the production of significantly increased amounts of hydrocarbons. The additional tissue damage after cod liver oil supplementation may arise through the cod liver oil providing a source of free radicals but not necessarily via direct damage to the polyunsaturated fatty acid components of cell membranes. Ethane production in both vitamin E deficient and sufficient rats may depend on the greater availability of peroxidisable substrate which need not be an integral part of the cell membrane.

The possibility that production of ethane and pentane from exogenous sources such as fat stores[14] may be masking endogenous production from peroxidation or alternatively that these hydrocarbons are being metabolised to CO_2[15] must also be considered. Attempts to alter the production of hydrocarbons by use of fat free diets and starvation were unsuccessful (unpublished observations) but this does not eliminate the possibility of ethane and pentane being produced from other non-dietary lipid sources such as gut bacteria.

The results of the present study indicate that, under the dietary conditions employed, ethane and pentane expiration did not correlate with the tissue damage monitored by changes in plasma pyruvate kinase activity. The possibility that damage to non-lipid components is an integral event in the deterioration of cell membranes may need to be considered.

Acknowledgements

Our thanks to BASF, West Germany for funding this work and to Dr. Peter Hoppe for advice.

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Accepted by Dr. J.V. Bannister