Free Radical Research, 2002 Vol. 36 (10), pp. 1051–1058

Taylor & Francis
healthsciences

Teratogenic Actions of Thermally-stressed Culinary Oils in Rats

ANA INDART[a], MARTA VIANA[a], MARTIN C. GROOTVELD[b], CHRISTOPHER J.L. SILWOOD[b], ISABEL SÁNCHEZ-VERA[a] and BARTOLOMÉ BONET[a,*]

[a]*Facultad de Ciencias Experimentales y de la Salud, Universidad San Pablo-CEU, Madrid, Spain;* [b]*Department of Diabetes and Metabolic Medicine, Division of General and Developmental Medicine, Barts and the London, Queen Mary's School of Medicine and Dentistry, London E1 1BB, UK*

Accepted by Professor B. Halliwell

(Received 15 February 2002; In revised form 14 April 2002)

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

Lipid oxidation products (LOPs), generated in culinary oils during episodes of thermal stressing can give rise to cellular damage. The aims of this study were to determine whether orally-administered, LOP-containing thermally-stressed safflower oil exerts teratogenic actions in rats, and whether this effect could be prevented by co-administration of α-tocopherol (α-TOH). Safflower oil was heated for a period of 20 min according to standard frying practices and stored at −20°C under N_2. Four experimental groups of pregnant Wistar rats were employed; two received 0.30 ml of pre-heated oil (HO), one of which was also supplemented with 150 mg of α-TOH (HOE), and two served as controls, one treated with the non-heated oil (O) and the other without any treatment (C). The oil was administered daily by gavage from day 1 of pregnancy to day 11.5, when the animals were killed and the embryos examined. LOPs and α-TOH were determined both in the heated and non-heated oils. The percentage of embryo malformations and reabsorptions were determined in the above four experimental groups. Heating the oil substantially increased its concentration of LOPs and decreased its α-TOH content. The percentage of embryo malformations in the HO group was 21.73%, compared with 5.6 and 7% in the O and C groups, respectively. Supplementation of the pre-heated oil with α-TOH was found to decrease the percentage of malformations to 7%. The results obtained from these investigations indicate that LOPs detectable at millimolar levels in the heated cooking oils administered (e.g. saturated and α,β-unsaturated aldehydes, and/or their conjugated hydroperoxydiene precursors) exert potent teratogenic actions in experimental animals which are at least partially circumventable by co-administration of the chain-breaking antioxidant α-TOH. Plausible mechanisms for these processes and their health relevance to humans regarding diet and methods of frying/cooking are discussed.

Keywords: Oxidative stress; Congenital malformations; Neural tube defects; Aldehydes; α-Tocopherol

Abbreviations: LOPs, lipid oxidation products; PUFA, polyunsaturated fatty acids; EDTA, ethylenediaminetetraacetic acid; TBARS, thiobarbituric acid reactive substances; NMR, nuclear magnetic resonance; CHPD, conjugated hydroperoxydiene; α-TOH, α-tocopherol; BHT, butylated hydroxytoluene; s.e., standard error

INTRODUCTION

Neural tube defects are among the most devastating congenital malformations, with an incidence of 1 in 1000 pregnancies, although the incidence varies between different populations, ranging from 1/1000 to 6/1000.[1] Both genetic and environmental factors seem to play a central role in the aetiology of neural tube defects. Therefore, any information that can shed light on the environmental factors that may play a role in the development of these disorders, and also methods for their monitoring and control, may have a major impact on human health.

Subjection of glycerol-bound polyunsaturated fatty acids (PUFA) present in culinary oils to episodes of thermal stressing according to standard frying/cooking practices gives rise to lipid oxidation products (LOPs) such as α,β-unsaturated aldehydes,[2–4] compounds which are highly reactive and can give rise to cellular damage, including the covalent

*Corresponding author. Address: Department of Pediatrics, Fundación Hospital Alcorcón, Universidad Rey Juan Carlos, C/Budapest 1, 28922 Alcorcón, Madrid, Spain. Tel.: +34-1-621-95-86. Fax: +34-1-621-99-01. E-mail: bjcbonet@fhalcorcon.es

ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1071576021000006716

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

modification of DNA.[5–7] Indeed, autoxidised polyunsaturated fatty acid (PUFA)-derived aldehydes can react with DNA bases, causing mutations.[6,7] They are also major components of the clastogenic factors,[8,9] products that cause chromosomal damage.[8,9] In principle, such DNA and chromosomal damage is amenable to prevention by chain-breaking antioxidants or, alternatively, agents with the ability to render secondary LOPs (e.g. aldehydes) inactive. In fact, it has been shown that the carcinogenic effects of clastogenic factors are preventable by the administration of antioxidants.[8,10,11]

In view of the above considerations, we hypothesize that orally-administered LOPs exert teratogenic actions (α). Since both we and others have previously shown in an experimental model of increased oxidative stress (diabetes) that the administration of antioxidants decreases the rate of neural tube defects,[12–14] it is conceivable that the teratogenic potentials of LOPs are circumventable by lipid-soluble chain-breaking antioxidants.

Therefore, the aims of the present study were to determine if a typical thermally-stressed culinary oil could give rise to embryo malformations, and whether the teratogenic actions observed are inhibitable by the co-administration of α-tocopherol (α-TOH). We selected safflower oil for this purpose since, it has a very high content of peroxidisable PUFAs [77% (w/w)] and gives rise to high levels of LOPs when subjected to episodes of thermal stressing according to standard frying practices.

MATERIALS AND METHODS

Animals and Experimental Design

Female virgin Wistar rats from our own colony, weighing 190–220 g, were housed in a temperature-controlled room (22 ± 1°C) with alternating 12-h light and dark cycles, and fed a Purina Chow diet (Rat and Mouse Standard diet, Beekay Feeds, B.K: Universal S.L., Barcelona, Spain). The care and handling of the animals throughout the study followed the current animal care law of the European Community (Strasbourg, March 18, 1986). Animals were mated with untreated control animals, and the day that sperm appeared in vaginal smears represented day 0 of gestation. The animals were divided into the following experimental groups: one group was treated from day 1 of pregnancy with 0.30 ml. of untreated safflower oil (O); two groups were treated daily with the same volume of thermally-stressed safflower oil, one without any further treatment (HO), the other supplemented daily with 150 mg of α-TOH (HOE): this chain-breaking antioxidant was dissolved in the oil. In all experimental groups, oil was administered daily by gavage. A parallel group of untreated rats was also studied (C). All-rac-α-TOH was purchased from Sigma–Aldrich Chemical Company.

Rats were decapitated on day 11.5 of gestation, which corresponds to the end of the embryo period. Blood was collected on ethylenediaminetetraacetic acid (EDTA) (1 mg ml^{-1}) and plasma separated and kept at −20°C until processed. The two uterine horns were immediately dissected and immersed at room temperature in saline contained in a petri dish (100 mm). Embryos and investing membranes were torn apart with fine jewellers' forceps during visualization with a dissecting microscope (Carlzeiss Jena 212T OPM). The yolk sac was isolated from the surrounding decidua and the embryo removed. In all embryos the crown-rump length and the number of somites were determined. They were inspected under the microscope to determine whether the morphology of brain spheres, neural tube size and configuration, closure of the neural tube, and axial curvature conformed to that expected on day 11.5 of gestation. Embryos not conforming to normal morphology in any of the above structures (Fig. 1, panel A) were considered dysmorphic. Reabsorptions were considered when the decidua was present but the yolk sac or the embryo was not found.

Preparation of Thermally-stressed Safflower Oil

Safflower oil [containing 77% (w/w) polyunsaturates, 14% (w/w) monounsaturates and 9% (w/w) saturates] was purchased from a local retail outlet and heated according to standard frying practices. Briefly, a frying pan half full of this material was heated in the presence of atmospheric oxygen on a laboratory gas stove for 20 min (during this period the oil reached temperatures of 180–220°C). Thereafter, the oil was placed in sealed vials under an N_2 atmosphere at 4°C until the day of administration to the experimental animals. Once the vials were opened, they were used immediately and the remaining oil was discarded. The non-heated oil was stored under the same conditions in order to avoid oxidation during the experimental period. In the group of animals treated with heated oil supplemented with α-TOH, this antioxidant was dissolved in the thermally stressed oil, to yield a final concentration of 500 mg ml^{-1}. (equivalent to 1.161 mol dm^{-3} or 1.261 mol kg^{-1}) on the same day of administration.

Processing and Analysis of Samples

Triglycerides in plasma were determined using a commercial kit (Triglycerides Enzymatic Trinder Method; Menarini Diagnostics, Florence, Italy). The thiobarbituric acid reactivities (TBARS) of plasma specimens and culinary oils were determined by

RIGHTSLINK

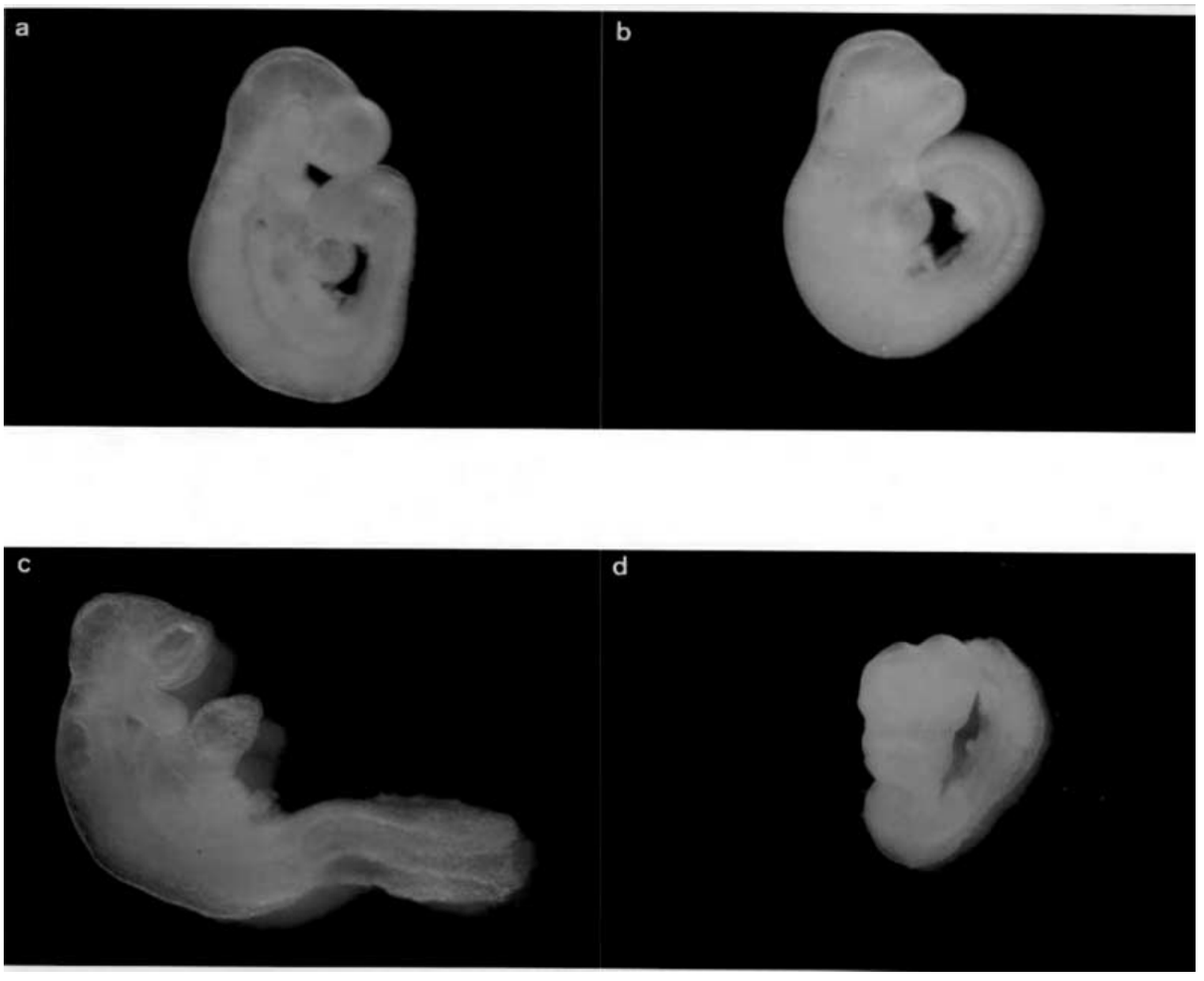

FIGURE 1 Rat embryos at day 11.5. (a) Normal embryo from a typical control animal (× 10). Embryos with neural tube defects from rats treated with thermally stressed safflower oil (× 10): (b) microcephaly; (c) and (d) severely malformed embryos, with malrotation, and open anterior and posterior neural tube.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

the high performance liquid chromatographic (HPLC) method described by Wong *et al.*[15] Although this TBA method selectively determines the red 1:2 malondialdehyde-thiobarbituric acid (MDA)-TBA adduct, it is now clear that reaction of *trans*-2-alkenals[16] and isomeric alka-2,4-dienals[17] also gives rise to the generation of this particular chromo phore under the reaction conditions employed (the latter via a two-step process), and hence in this report we shall continue to refer to this agent as TBARS.

Proton (^{1}H) nuclear magnetic resonance (NMR) measurements on control and thermally-stressed culinary oil samples were conducted on a Bruker AMX-600 spectrometer (University of London Intercollegiate Research Services (ULIRS), Queen Mary, University of London Facility, UK) operating at a frequency of 600.13 MHz and a probe temperature of 298 K. Typically, a 0.30 ml aliquot of each culinary oil was diluted to a volume of 0.90 ml with a 5.00×10^{-3} mol dm^{-3} solution of 1,3,5-trichlorobenzene in deuterated chloroform (C^2HCl_3), the former serving as an internal quantitative NMR standard ($\delta = 7.227$ ppm) and the latter providing a field frequency lock. The samples were then thoroughly mixed and placed in 5-mm diameter NMR tubes. Typical pulsing conditions were: 128 or 256 free induction decays (FIDs) using 32,768 or 65,536 data points, 72° pulses, a relaxation delay of 2.00 s and an acquisition time of 1.28 s. The spectral width was 7246 Hz, and exponential line-broadening functions of 0.20 Hz were routinely employed for the purpose of processing.

Chemical shifts were referenced to tetramethylsilane ($\delta = 0.00$ ppm, internal) and/or residual chloroform ($\delta = 7.262$ ppm). LOP resonances were assigned by a consideration of chemical shift values, coupling patterns and coupling constants as previously described (2,3). The intensities of these signals were determined by electronic integration,

RIGHTSLINK

TABLE I Thiobarbituric acid reactive substances (TBARS), *n*-alkanal, *trans*-2-alkanal, *cis*,*trans*-alka-2,4-dienal, *trans*,*trans*-alka-2,4-dienal and α-tocopherol (α-TOH) concentrations in heated and unheated safflower oil

	Heated oil	Unheated oil
TBARS ($\mu mol\,kg^{-1}$)	73 ± 2***	15 ± 1
n-alkanals ($mmol\,kg^{-1}$)	1.13 ± 0.32	ND
trans-2-alkenals ($mmol\,kg^{-1}$)	3.20 ± 0.54	ND
cis,*trans*-alka-2,4-dienals ($mmol\,kg^{-1}$)	0.49 ± 0.02	ND
trans,*trans*-alka-2,4-dienals ($mmol\,kg^{-1}$)	2.39 ± 0.22	ND
α-TOH ($mmol\,kg^{-1}$)	0.65 ± 0.16*	1.68 ± 0.08

Mean ± s.e. of the control (unheated) and heated safflower oil samples used in the experiments. For the TBARS and α-tocopherol six different oil samples were analysed and for the remaining analytes three samples were tested. (*): Denotes differences between heated and unheated sunflower oil; *: $p < 0.05$; ***: $p \leq 0.001$; ND: non-detectable.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

and the concentrations of each class of aldehyde (*n*-alkanals, *trans*-2-alkenals, and *trans*,*trans*- and *cis*,*trans*-alka-2,4-dienals) were determined by comparing their resonance areas with that of the added 1,3,5-trichorobenzene.

α-TOH in plasma and liver specimens was determined by a previously described HPLC method.[18,19]

Statistical Analysis

The mean ± s.e. values for each parameter determined (i.e. plasma triglyceride, MDA and α-TOH concentrations, and liver α-TOH content) are tabulated. The significance of differences observed between the means of the four experimental groups was determined by one-way analysis-of-variance (ANOVA) followed by the Tukey test for multiple comparisons, using the Systat program (Systat Incorporation, Evanston, IL, USA). The χ^2 test was performed to detect differences in the proportions (percentages) of malformations and reabsorptions between the different experimental groups (i.e. analysis of enumeration data in a 4×2 contingency table).

RESULTS

Subjection of safflower oil samples to thermal stressing episodes according to standard frying practices gives rise to a marked increase in its TBARS concentration, and the generation of millimolar levels of different classes of NMR-detectable aldehydes, specifically *n*-alkanals, *trans*-2-alkenals, *cis*,*trans*-alka-2,4-dienals and *trans*, *trans*-alka-2,4-dienals (Table I). With the exception of a small quantity of TBARS, all of these products were undetectable in the non-heated oil. The heating process also resulted in a substantial decrease in the oil's α-TOH content.

The embryos of the experimental animals treated with 0.30 ml of thermally-stressed oil showed a 21.73 % level of neural tube defects (Table II and Fig. 1), these being values much higher than those observed in the group C animals (8%), or in animals treated with the same concentration of unheated oil (12%). When α-TOH was added to the thermally-stressed oil, the incidence of neural tube defects decreased to 9.9%, a value similar to that of group C. No differences in the percentage of reabsorptions were observed between the different experimental groups (Table II).

In a previous investigation,[12] we have shown that administration of α-TOH alone (i.e. without an unheated or pre-heated culinary oil) to our gestating female rat colony did not exert an influence on the rates of malformations or reabsorptions observed in the untreated control (C) group.

The embryos from the HO group had a lower crown-rump length and somite number than the C or the O group (Table III). Again, the administration of α-TOH normalized the crown-rump length and

TABLE II Percentage of malformations and reabsorptions

	Total number of rats (*n*)	Yolk sacs			Embryos		
		Total (*n*)	Reabsoprtions		Total (*n*)	Malformed	
			(*n*)	(%)		(*n*)	(%)
C	18	215	15	6.97	200	16	8
O	19	232	13	5.6	219	27	12.32^{++}
HO	21	222	15	6.75	207	45	21.73^{***}
HOE	12	130	9	6.92	121	12	9.91^{++}

Abbreviations: C: control (untreated) animals; O: those treated with unheated safflower oil; HO: thermally-stressed safflower oil without any further treatment; HOE: thermally-stressed safflower oil supplemented daily with α-TOH ($500\,mg\,ml^{-1}$). (*): Denotes differences between control animals vs the different groups of rats supplemented with oil; ***: $p \leq 0.001$; and (+) the differences between rats treated with heated oil vs the animals supplemented with vitamin E or treated with non-heated oil; $^{++}$: $p \leq 0.01$.

RIGHTSLINK

TABLE III Mean ± S.E. number of somites and crown rump length in 11.5 day old rat embryos

Group	Embryos (n)	Somites (n)	Crown-Rump (mm)
C	200	26.46 ± 0.14	3.77 ± 0.03
O	219	26.60 ± 0.16	3.77 ± 0.03^{+}
HO	207	25.90 ± 0.18*	3.65 ± 0.03*
HOE	121	26.76 ± 0.16^{++}	3.74 ± 0.03

Abbreviations: C: control (untreated) animals; O: those treated with unheated safflower oil; HO: thermally-stressed safflower oil without any further treatment; HOE: thermally-stressed safflower oil supplemented daily with α-TOH (500 mg ml^{-1}). (*): Denotes differences between control animals and different groups of rats supplemented with oil; *: $p \leq 0.05$; (+) represents the differences between rats treated with heated oil and animals either supplemented with α-TOH, or treated with the unheated oil; $^{++}$: $p \leq 0.01$ and $^{+}$: $p \leq 0.05$.

the somite number. When, in the analysis of the embryos, only normal embryos were considered, no differences in the CR length and the somite number were found between any of the experimental groups (data not shown).

The weights of the animals in groups C, O, HO and HOE (mean ± s.e.) were 195.6 ± 3.0, 198.6 ± 3.9, 198.4 ± 2.8 and 197.0 ± 5.4, respectively, at day 0, and 257.9 ± 3.9, 257.0 ± 2.6, 256.1 ± 3.6 and 254.6 ± 7.9, respectively, at day 11.5 of gestation. These data represent percentage weight increases of 32.0 ± 1.9 (group C), 32.6 ± 1.5 (group O), 29.2 ± 1.5 (group HO) and 29.3 ± 1.8 (group HOE) during this time period, and there were no significant differences between these mean weight gain values (one-way ANOVA followed by further analysis using Tukey's test). Hence, there was no effect of unheated or pre-heated oil administration on the calorific intake/weight gain of the animals throughout the experimental period.

The administration of thermally-stressed oil did not lead to any significant modifications in plasma triglyceride, TBARS and α-TOH levels, nor in liver α-TOH content, when compared to the values observed in the C and O groups (Table IV). As expected, in the HOE group the levels of α-TOH in plasma and liver were much higher than that of all the other groups.

DISCUSSION

To the best of our knowledge, the results presented here, show for the first time in an experimental animal model system that administration of a thermally-stressed culinary oil containing high levels of aldehydes increases the incidence of neural tube malformations.

The teratogenic properties of LOPs are, of course, critically dependent on the rate and extent of their *in vivo* absorption from the gut into the systemic circulation. In 1970, Bergen and Draper[20] presented data consistent with the failure of conjugated hydroperoxydienes (CHPDs) to be absorbed across the gastric or intestinal epithelium. However, Grootveld *et al.*[4] have demonstrated that in rats, reactive *trans*-2-alkenal "end-products" of the lipid peroxidation process (specifically *trans*-2-pentanal and -nonenal) are indeed absorbed, then metabolised and excreted in the urine as C-3 mercapturate conjugates.

In view of their high reactivity with biomolecules, this class of α,β-unsaturated aldehyde has the capacity to exert damaging actions towards a variety of cells and tissues. Indeed, amongst other effects these agents induce the lysis of erythrocytes, potentiate human platelet aggregation,[21] are cytotoxic towards human umbilical cord vein endothelial cells,[22] Ehrlich tumour ascites cells[23] and are both genotoxic and cytotoxic to rat hepatocytes.[24] 4-Hydroxy-*trans*-2-nonenal also inactivates adenylate cyclase, 5′-nucleotidase,[25] glucose-6-phosphatase and cytochome P-450, suppresses the proliferative response to phyto-haemaglutinin and alloantigens,[26] and the expression of c-myconcogene.[27] They also may react with thiols therein such as glutathione (via a Michael addition process, involving addition of the thiol across electrophilic carbon–carbon double bonds at the C-3 position of α,β-unsaturated aldehydes) generating beta-thiyl-substituted aldehyde metabolites that serve as further source of bioactive unsaturated aldehydes, allowing the propagation and prolongation of these

TABLE IV Mean ± s.e. concentrations of thiobarbituric acid reactive substances (TBARS), α-TOH and triglycerides (TG) in plasma, and the α-TOH content of liver specimens collected from animals in the four experimental groups

		Plasma			
	n	TG (mg dl^{-1})	TBARS μmol dm^{-3})	α-TOH μmol dm^{-3})	Liver α-TOH (μg g^{-1} tissue)
C	18	129.66 ± 8.11	1.56 ± 0.11	16.17 ± 0.94	43.18 ± 1.93
O	19	104.44 ± 5.26	1.82 ± 0.14	18.57 ± 1.05	50.46 ± 1.98
HO	21	106.82 ± 7.02	1.63 ± 0.14	16.21 ± 0.72	48.21 ± 4.57
HOE	12	107.87 ± 7.83	1.64 ± 0.21	48.48 ± 3.23$^{***,+++}$	119.50 ± 7.75$^{***,+++}$

Abbreviations: C: control (untreated) animals; O: those treated with unheated safflower oil; HO: thermally-stressed safflower oil without any further treatment; HOE: thermally-stressed safflower oil supplemented daily with α-TOH (500 mg ml^{-1}). (*): Denotes differences between control animals and the different groups of rats supplemented with oil; ***: $p \leq 0.001$; (+) represents the differences between rats treated with heated oil and the animals supplemented with α-TOH, or treated with unheated oil; $^{+++}$: $p \leq 0.001$.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

dietary-derived xenobiotics.[28] These compounds can also covalently modify alternative biomolecules such as DNA base moieties; such chemically-altered DNA bases may not be recognized by their homologous bases and hence cause mutations. Furthermore, the DNA replicating systems can be inactivated by such LOP products,[29,30] which could increase the level of DNA damage. Therefore, when incorporated into cells, autoxidised PUFA-derived aldehydes can cause DNA and chromosomal damage. If these processes occur during embryo development, a period of very active cell division and differentiation, malformations may arise. The results presented here indicate that this process could occur in the neural tube of the embryos of rats treated with thermally-stressed safflower oil.

We have also demonstrated that the neural tube defects induced by the administration of LOPs contained in the thermally-stressed culinary oils can be inhibited by the co-administration of α-TOH, a powerful chain-breaking peroxyl radical scavenger. This observation indicates that, irrespective of their apparent lack of absorption from the gut into the systemic circulation *in vivo*,[20] CHPDs present in the pre-heated safflower oil are the agents responsible for the teratogenic actions of this material. However, this protective effect is not simply explicable, and α-TOH could also act by (1) prevention of the further fragmentation of safflower oil LOPs in the gut prior to absorption, decreasing the overall availability of circulating aldehydes; (2) blockage of membrane lipid peroxidation *in vivo* arising from the ability of pre-absorbed LOPs to stimulate reactive oxygen species (ROS) generation by phagocytic cells and/or (3) competitively inhibiting the absorption of aldehydes from the gut into the systemic circulation, a process that may be facilitated by the high lipophilicity of this chain-breaking antioxidant. With regard to (2) above, it should be noted that reactive α,β-unsaturated aldehydes can trigger the generation of ROS and hence promote and potentiate cellular oxidative stress. Indeed, oxidised linoleic acid markedly enhances ROS production by monocytes,[31] 4-hydroxy-*trans*-2-nonenal (HNE) and other α,β-unsaturated aldehydes induce intracellular peroxide generation in cultured hepatocytes,[32] and HNE itself induces lipid peroxidation (indicated by elevated MDA concentrations).[33]

Hence, it is conceivable that the toxic effects of thermally-stressed safflower oil could be prevented with its enrichment with antioxidants such as α-TOH or butylated hydroxytoluene (BHT), either prior to the heating process (suppressing the thermally-induced oxidation of PUFAs), or prior to its oral administration. Currently, further work is being conducted in our laboratories to determine whether the toxic effects of thermally stressed oils are preventable by enrichment with such lipid-soluble antioxidants prior to their subjection to heating episodes.

If, indeed, any culinary oil-borne CHPDs are absorbed from the gut into the systemic circulation, or, alternatively, pre-absorbed peroxidation end-products further stimulate their generation *in vivo*,[31–33] these agents may also play a role in the development of embryonic neural tube malformations. Interestingly, Graf *et al.*[34,35] found that children with neural tube defects have significantly lower levels of erythrocyte glutathione peroxidase (an enzyme involved in the clearance of CHPDs), an observation providing evidence for the involvement of oxidative stress in the development of this defect.

Interestingly, the unheated safflower oil also gave rise to a statistically significant increase in the rate of embryo malformations, but this observation is not unexpected since PUFA-rich culinary oils not subjected to thermal stressing episodes also contain detectable levels of LOPs (albeit much lower than those that are pre-heated).[2,3] Although *n*-alkanals, *trans*-2-alkenals, and *cis*,*trans*- and *trans*,*trans*-alka-2,4-dienals were not detectable in the unheated oil by ^{1}H NMR spectroscopy, this material had a TBARS level of 15 μmol kg^{-1}. Moreover, in a previous investigation we have noted a trend towards a higher rate of embryo malformations in our rat colony when treated with unheated safflower oil that had been stored at ambient temperature for prolonged periods of time, an observation consistent with the time-dependent peroxidation of PUFAs therein.

Thermal stressing of monounsaturate-rich olive oils (virgin or extra-virgin) under conditions similar to those employed here generates only low levels of selected aldehydes (*n*-alkanals and *trans*-2-alkenals)[2,3] and hence it is conceivable that, when utilized for standard frying/cooking practices, the teratogenic potential of this material is lower than that of polyunsaturated-rich safflower oil as detailed here. Indeed, monounsaturates are far more resistant to peroxidation than polyunsaturates. Moreover, the relatively high levels of antioxidants other than tocopherols present in this culinary oil may also provide further protection of the low levels of polyunsaturates present against thermally-induced peroxidation and/or the teratogenic actions of LOPs *in vivo*.

In summary, our results demonstrate that in an experimental animal model, administration of LOP-rich thermally stressed culinary oil increases the rate of neural tube defects. Hence, it is conceivable that if a similar process occurs in humans, differences in

RIGHTSLINK

cooking practices may be related to the differential incidence of neural tube defects observed amongst different populations.

Acknowledgements

This work was supported by a grant from the Ministerio de Salud (FIS 94-0398; 00-0304) and from the Universidad San Pablo CEU (22/98).

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

References

[1] Botto, L.D., Moore, C.A., Fhoury, M.J. and Erickson, J.D. (1999) "Neural tube defects", *N. Engl. J. Med.* **341**, 1509–1519.
[2] Claxson, A.W.D., Hawkes, G.E., Richardson, D.P., Naughton, D.P., Haywood, R.M., Chander, Ch.L., Atherton, M., Lynch, E.J. and Grootveld, M.C. (1994) "Generation of lipid peroxidation products in culinary oils and fats during episodes of thermal stressing: a high field ^{1}H NMR study", *FEBS Lett.* **355**, 81–90.
[3] Haywood, R.M., Claxson, A.W.D., Hawkes, G.E., Richardson, D.P., Naughton, D.P., Coumbarides, G., Hawkes, J., Lynch, E.J. and Grootveld, M.C. (1995) "Detection of aldehydes and their conjugated hydroperoxydiene precursors in thermally-stressed culinary oils and fats: investigations using high resolution proton NMR spectroscopy", *Free Radic. Res.* **22**(5), 441–482.
[4] Grootveld, M.C., Atherton, M., Sheerin, A.N., Hawkes, J., Blake, D.R., Richens, T.E., Silwood, C.J.L., Lynch, E.J. and Claxson, A.W.D. (1998) "*In vivo* absorption, metabolism and urinary excretion of α,β-unsaturated aldehydes in experimental animals. Relevance to the development of cardiovascular diseases by the dietary ingestion of thermally stressed polyunsaturated-rich culinary oils", *J. Clin. Investig.* **101**(6), 1210–1218.
[5] Yang, M.H. and Schaich, K.M. (1996) "Factors affecting DNA damage caused by lipid hydroperoxides and aldehydes", *Free Radic. Biol. Med.* **20**(2), 225–236.
[6] Chung, F.L., Chen, H.J.C. and Nath, R.G. (1996) "Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts", *Carcinogenesis* **17**(10), 2105–2111.
[7] Jenkinson, A. McE., Collins, A.R., Duthie, S.J., Wahle, K.W.L. and Duthie, G.G. (1999) "The effect of increased intakes of polyunsaturated fatty acids and vitamin E on DNA damage in human lymphocytes", *FASEB J.* **13**, 2138–2142.
[8] Emerit, I. (1994) "Reactive oxygen species, chromosome mutation, and cancer: possible role of clastogenic factors in carcinogenesis", *Free Radic. Biol. Med.* **36**, 99–109.
[9] Emerit, I., Khan, S.H. and Esterbauer, H. (1991) "Hydroxynonenal, a component of clastogenic factors?", *Free Radic. Biol. Med.* **10**, 371–377.
[10] Edeas, M.V., Emerit, I., Khalfoun, Y., Lazizi, Y., Cernjavski, L., Levy, A. and Lindenbaum, A. (1997) "Clastogenic factors in plasma of HIV-1 infected patients activate HIV-1 replication *in vitro*: inhibition by superoxide dismutase", *Free Radic. Biol. Med.* **23**, 571–578.
[11] Emerit, I., Quastel, M., Goldsmith, J., Merkin, L., Levy, A., Cernjavski, L., Alaoui-Youssefi, A., Pogossian, A. and Riklis, E. (1997) "Clastogenic factors in the plasma of children exposed at Chernobyl", *Mutat. Res.* **373**(1), 47–54.
[12] Viana, M., Herrera, E. and Bonet, B. (1996) "Teratogenic effects of diabetes mellitus in the rat. Prevention by vitamin E", *Diabetología* **39**, 1041–1046.
[13] Eriksson, U.J. and Siman, M.C. (1996) "Pregnant diabetic rats fed the antioxidant butylated hydroxytoluene show decreased occurrence of malformations in offspring", *Diabetes* **45**, 1497–1502.
[14] Sivan, E., Reece, E.A., Wu, Y.K., Homko, C.J., Polansky, M. and Borenstein, M. (1996) "Dietary vitamin E prophylaxis and diabetic embryopathy: morphologic and biochemical analysis", *Am. J. Obstet. Gynaecol.* **175**, 793–799.
[15] Wong, S.H.Y., Knight, J.A., Hopfer, S.M., Zaharia, O., Leach, Ch.N. and Sunderman, F.W. (1987) "Lipoperoxides in plasma as measured by liquid chromatographic separation of malondialdehyde-thiobarbituric acid adduct", *Clin. Chem.* **33**(2), 214–220.
[16] Kosugi, H., Kato, T. and Kikugawa, K. (1987) "Formation of yellow, orange and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid", *Anal. Biochem.* **165**, 454–464.
[17] Kosugi, H., Kato, H.T. and Kikugawa, K. (1987) "Formation of red pigment by a two-step 2-thiobarbituric acid reaction of alk-2,4-dienals. Potential products of lipid oxidation", *Lipids* **23**, 1024–1031.
[18] Cuesta, D. and Castro, M. (1986) "Simultaneous measurement of retinol and α-tocopherol in human serum by high-performance liquid chromatography with ultraviolet detection", *J. Chromatogr.* **380**, 145–150.
[19] Barbas, C., Castro, M., Bonet, B., Viana, M. and Herrera, E. (1997) "Simultaneous determination of vitamin A and E in the rat tissues by high-performance liquid chromatography", *J. Chromatogr. A* **778**, 415–420.
[20] Bergan, J.G. and Draper, H.H. (1970) "Absorption and metabolism of 1-^{14}C-methyl linoleate hydroperoxide", *Lipids* **5**, 976–982.
[21] Selley, M.L., McGuiness, J.A., Jenkin, L.A., Bartlett, M.R. and Ardlie, N.G. (1988) "Effect of 4-hydroxy-2,3-*trans*-nonenal on platelet function", *Thromb. Haemostosis* **59**(2), 143–146.
[22] Kaneko, T., Kaji, K. and Matsuo, M. (1988) "Cytotoxicities of a linoleic acid hydroperoxide and its related aliphatic aldehydes toward cultured human umbilical vein endothelial cells", *Chem.-Biol. Interact.* **67**(3–4), 295–304.
[23] Hauptlorenz, S., Esterbauer, H., Moll, W., Pumpel, R., Schauenstein, E. and Puschendorf, B. (1985) "Effects of the lipid peroxidation product 4-hydroxynonenal and related aldehydes on proliferation and viability of cultured Ehrlich ascites tumor cells", *Biochem. Pharmacol.* **34**(21), 3803–3809.
[24] Griffin, D.S. and Segall, H.J. (1986) "Genotoxicity and cytotoxicity of selected pyrrolizidine alkaloids, a possible alkenal metabolite of the alkaloids, and related alkenals", *Toxicol. Appl. Pharmacol.* **86**, 227–234.
[25] Paradisi, L., Panagini, C., Parola, M., Barrera, G. and Dianzani, M.U. (1985) "Effects of 4-hydroxynonenal on adenylate cyclase and 5′-nucleotidase activities in rat liver plasma membranes", *Chem.-Biol. Interact.* **53**(1–2), 209–217.
[26] Tessitore, L., Matera, L., Bonelli, G., Baccino, F.M. and Dianzani, M.U. (1987) "Aliphatic aldehydes inhibit the proliferative response of human peripheral blood lymphocytes to phytohemagglutinin and alloantigens", *Chem.-Biol. Interact.* **62**(3), 217–226.
[27] Barrera, G., Martinotti, S., Fazio, V., Manzari, V., Paradis, L., Parola, M., Frati, L. and Dianzani, M.U. (1987) "Effect of 4-hydroxynonenal on c-myc expression", *Toxicol. Pathol.* **15**(2), 238–240.
[28] Witz, G. (1989) "Biological interactions of α,β-unsaturated aldehydes", *Free Radic. Biol. Med.* **7**, 333–349.
[29] Wonisch, W., Kohlwein, S.D., Schaur, J., Tatzber, F., Guttenberger, H., Zarkovic, N., Winkler, R. and Esterbauer, H. (1998) "Treatment of the budding yeast *Saccharomyces cereviseae* with the lipid peroxidation product 4-HNE provokes a temporary cell cycle arrest in G1 phase", *Free Radic. Biol. Med.* **25**(6), 682–687.
[30] Catalano, C.E. and Kuchta, R.D. (1995) "Inactivation of DNA polymerase α-primase by acrolein: loss of activity depends on the DNA substrate", *Biochem. Biophys. Res. Commun.* **214**(3), 971–977.
[31] Gorog, P. (1991) "Activation of human blood monocytes by oxidized polyunsaturated fatty acids: a possible mechanism for the generation of lipid peroxides in the circulation", *Int. J. Exp. Pathol.* **72**(2), 227–237.
[32] Uchida, K., Shiraishi, M., Naito, Y., Torii, Y., Nakamura, Y. and Osawa, T. (1999) "Activation of stress signaling pathways by the end product of lipid peroxidation. 4-Hydroxy-2-nonenal

RIGHTSLINK

is a potential inducer of intracellular peroxide production", *J. Biol. Chem.* **274**, 2234–2242.
[33] Keller, J.N., Mark, R.J., Bruce, A.J., Blanc, E., Rothstein, J.D., Uchida, K., Waeg, G. and Mattson, M.P. (1997) "4-Hydroxynonenal, an aldehydic product of membrane lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes", *Neuroscience* **80**, 685–696.
[34] Graf, W.D., Oleinik, O.E., Pippenger, C.E., Eder, D.N., Glauser, T.A. and Shurtleff, D.B. (1995) "Comparison of erythrocyte antioxidant enzyme activities and embryologic level of neural tube defects", *Eur. J. Pediatr. Surg.* **5**(Suppl. I), 8–11.
[35] Graf, W.D., Pippenger, C.E. and Shurtleff, D.B. (1995) "Erythrocyte antioxidant enzyme activities in children with myelomeningocele", *Dev. Med. Child Neurol.* **37**, 900–905.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/25/11
For personal use only.

RIGHTSLINK