Modulation of vascular endothelial cell function by palm oil antioxidants

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Several cardiovascular risk factors including, hypercholesterolemia and hypertension, lead to damaged blood vessels due to pathological changes in the intima (endothelium) and these recent studies also indicate that such alterations in the function and structure of the endothelium involve free radical related mechanisms(8). Therefore, in the present study, two different preparations of palm oil with various tocotrienol and tocopherol profiles, as well as a purified antioxidant fraction extracted from unrefined polished palm oil (tocotrienol-rich-factor), were tested for their ability to influence blood vessel dysfunction in the spontaneously hypertensive rat (SHR). Adult SHR was fed a synthetic diet supplemented (5% w/w) with either physiologically refined palm oil (KO), palm oil cooking oil (Nlistino; GPO) or olive oil (OO; control diet). The animals were killed after 24 weeks of feeding and the thoracic aorta was isolated for evaluation of vascular function.

Aortic ring preparation
Upon completion of the feeding period, rats were stunned, killed by decapitation and aorta from the thoracic region was carefully excised and cleared of adhering tissue. Aortic rings were then cut into eight rings, approximately 3 mm in length, and mounted under isometric conditions at a resting tension of 4g in an organ bath chamber containing oxygenated (95% N2/5% CO2) Krebs-Henselit buffer at 37°C. The composition of the buffer solution was 118.3 mM NaCl, 2.5 mM KCl, 1.2 mM KH2PO4, 1.25 mM MgSO4, 25 mM NaHCO3, 2.5 mM CaCl2, 11.2 g/l glucose and acetic acid (0.57M) in deionised water. The aortic rings were allowed to equilibrate for 60 minutes before contracting with carbachol (20 nM) to test tissue viability. The increase in tension was detected by Grass FT03 force transducers and recorded on a Graphite Linerrecorder (WPI31701) via an amplifier.

Pharmacological protocol
After establishing tissue viability with KCl concentration response curves to noradrenaline (NA; 10-9 to 10-5 M) were constructed by cumulative additions to the bath. Vascular relaxations to acetylcholine (ACh) was added in six-fold steps before contracting with carbachol (20 nM) to test tissue viability. The increase in tension was detected by Grass FT03 force transducers and recorded on a Graphite Linerrecorder (WPI31701) via an amplifier.

Statistical
Contrast responses are expressed as % contraction to KCl (20 mM) for each ring. Relaxation to acetylcholine are presented as % contraction to 10-4 M to noradrenaline which normally elicited a half-maximal response. The results are presented as Mean ± SEM. The means were compared with a one-way analysis of variance (ANOVA) followed by Tukey’s test for multiple comparisons. A p value of <0.05 was considered as statistically significant.

Results
Table 1 shows the major fatty acids of dietary oil supplements used in this study. It is clear that the two palm oil preparations differed in the proportions of palmitic (16:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. For example, physiologically refined palm oil prepared from tropical virgin palm oil contained 40.1% (12% higher) than golden palm cooking oil (GPO; Nutrolite); but a lower proportion of monounsaturated 18:1 (n-9) and polyunsaturated linoleic acid. In the GPO, this reduction in palmitic acid was offset by an increase in oleic (78%) and also in linolenic acid. Olive oil was rich in oleic acid (75%), and compared to the two palm oil supplements, contained a lower proportion of 16:0.

The oil preparations also differed in their antioxidant profiles. For instance, whilst both KO and PO oil supplements contained n-3 polyunsaturated fatty acids (PUFA), the GPO was rich in these antioxidants (approx. 450 ppm). However, the total vitamin E levels (tocopherol and tocotrienol contents) were similar between the two palm oil samples and ranged between 360-700 ppm. In contrast, PO oil contained no tocotrienol, and compared to palm oil, a lower tocopherol content (130 ppm).
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Several cardiovascular risk factors including, hypercholesterolaemia and hyperlipidaemia, lead to disrupted blood vessels and increased risk of atherosclerotic vascular disease. Recent studies also indicate that such alterations in vascular function involve free radical related mechanism(s). Therefore, in the present study, two different preparations of palm oils with various tocotrienol and tocopherol profiles, as well as a purified anti-oxidant fraction from unrefined palm oil (tocotrienol-rich factor TRF), were tested for their ability to influence blood vessel dysfunction in the spontaneously hypertensive rat (SHR). Adult SHR were fed a synthetic diet supplemented (5% w/w) with either physically refined palm oil (PO), palm-balm cooking oil (Nutrinol; GPO) or olive oil (OO; control diet). In addition, the paradoxical increase in tissue and control hypertensive vessels observed at higher doses of ACh was prevented by TRF and also by the PO and GPO diets. Although the development of thromboxane-like constriction response, after the initiation of nitric oxide in hypertensive vessels, was unaffected by test oils, both TRF and GPO feeding prevented the amplification of this unwarranted constriction by a thromboxane (7.2±0.7×10^−8 M) of noradrenaline. Results suggest a modulatory role for monounsaturated digestive oil and are in agreement with the recently reported benefits of natural antioxidants against cardiovascular diseases.

Key words: endothelium, dietary antioxidants, acetylcholine, spontaneously hypertensive rat

Introduction

The luminal surface of blood vessel is covered by a monolayer of cells commonly referred to as the vascular endothelium. The endothelium not only acts as a passive barriers to the infiltration of various molecules into the underlying tissue, but also modulates vascular tone, maintains cardiovascular homeostasis and cell growth [1]. The inflammatory and innate responses in blood vessels through the production of an array of both relaxant and constrictor factors [2]. The major endothelial cell derived relaxant factors include, nitric oxide, prostacyclin, endothelin-derived hyperpolarising factor and adenosine whilst thromboxane, free radicals, lipid hydroperoxides and the vasoreactive peptide endothelin are the main constrictor factors. Free radicals and inflammatory and innate responses in blood vessels may contribute to the development of cardiovascular diseases. For example, atherosclerosis may be influenced by the oxidative stress - oxygen derived free radicals and related products - to be an important determinant in endothelial cell dysfunction and thus increase the risk of cardiovascular diseases [3].

Such findings implicate dietary antioxidants as well as edible oils rich in endogenous antioxidants as potential candidates to extend vascular protective actions. Indeed, several recent studies, both in animal models and human subjects, have reported improvement of blood vessel function by dietary antioxidant vitamins supplementation [4]. Furthermore, a recent investigation in this laboratory also found specific dietary n-3 polyunsaturated fatty acids and several flavonoid compounds to offer vasoprotective actions against the development of vascular dysfunction in the spontaneously hypertensive rat [5].

Whilst it seems likely that cardiovascular benefits of dietary n-3 polyunsaturates mediate through favourable changes in the eicosanoid profile due to alterations in precursor/substrate fatty acid availability [6-10], it has also been reported that eicosanoid production, hence cardiovascular function, may be influenced by the anti-oxidant activity of cell membranes or by their capacity to scavenge free radicals. The influence of dietary antioxidants on the cardiovascular system may be mediated through the potentiation of membrane phospholipids by oxygen radicals and may limit the consequent functional changes of oxidative stress. In the new preparations of palm oil, (e.g. GPO, golden palm oil), polyunsaturated fatty acids have been preserved thus improving the overall composition and content of endogenous antioxidants.

Recent data also suggest that endogenous function is influenced by sustained hypertension, hypercholesterolaemia and also by aging [11]. Furthermore, data implies that an imbalance production of antioxidant and pro-oxidant factors may account for the abnormal vasoconstriction observed in these disease conditions. There is also an increasing body of evidence which indicates that oxidative stress - oxygen derived free radicals and related products - to be an important determinant in endothelial cell dysfunction [12] and thus increase the risk of cardiovascular diseases [13].

The aim of the present study was to investigate the effect of palm oil antioxidants on blood vessel function.

Materials and methods

Animals and diets

Four month old adult spontaneous hypertensive rats (SHR; N=8 per group) and normotensive Wistar-Kyoto (WKY) control rats were obtained from the colony established at this laboratory. After a stabilization period of two weeks, animals were then divided into a synthetic diet [14], based on the American Institute of Nutrition rodent diet (AIN-66). The total lipid content of the test diets was 5% (w/w). Physically refined palm oil (PO), golden palm cooking oil (GPO), palm-balm cooking oil (Nutrinol; GPO) or olive oil (OO) served as the dietary lipid source. The tocotrienol enriched diet was prepared by supplementing the base diet with 0.2% (w/w) tocotrienol-rich factor (TRF). The alpha-tocopherol level of the base diet was 0.04%. Olive oil was the source of dietary lipid for the TRF supplemented diet and the unsupplemented control diet. TRF and PO were supplied by the Palm Oil Research Institute of Malaysia whilst GPO was kindly provided by the Hai Loi Enterprise Sdn Bhd (Johore Bahru, Malaysia). OO was purchased locally.

Aortic ring preparation

Upon completion of the feeding period, rats were stunned, killed by decapitation and aorta from the thoracic region was carefully excised and cleared of adhering tissue. Aortic rings were cut into eight rings, approximately 3 mm in length, and mounted under isometric conditions at a resting tension of 4g in an organ bath containing chamber. The composition of the buffer solution was 118 NaCl. 2.5 KCl, 1.2 MgSO4, 25 NaHCO3. 25 CaCl2, 11.2 glucose and acetic acid (0.57M) in demineralized water. The aortic rings were allowed to equilibrate for 60 minutes before contracting with KCl (20 mM) to test tissue viability. The increase in tension was detected by Grass FT03 force transducers and recorded on a Graphotrace Linewriter (P3100) via an amplifier [15].

Pharmacological protocol

After equilibration, tissue viability with KCl concentration response curves to noradrenaline (NA; 10−8 M) were constructed by cumulative additions to the bath. Vascular relaxations to acetylcholine (ACh) were studied in vessels pre-contracted with NA. In brief, after repeated washing and re-equilibration for an hour, the rings were pre-contracted with NA (10−7 M) before concentration response curves to ACh (10−9 to 10−5 M) were constructed.

To study the spontaneous release of constrictor factor(s) in hypertensive blood vessels, several vascular rings were incubated with the inhibitor of nitric oxide (NO), N-Nitro-L-Arginine (NOLA; 10−5 M), for a period up to 60 minutes. Rings used for this experiment were 60 minutes before contracting with KCl (20 mM) to test tissue viability. The increase in tension was detected by Grass FT03 force transducers and recorded on a Graphotrace Linewriter (P3100) via an amplifier [16].

Statistical

Contrast responses are expressed as % contraction to KCl (20 mM) for each ring. Relaxation to acetylcholine are presented as % contraction to 10−5 M to noradrenaline which normally elicited a half maximal response at this concentration. The results are therefore, are presented as Mean ± SEM. The means were compared with a one-way analysis of variance (ANOVA) followed by Tukey’s test for multiple comparisons. A P value of 0.05 was considered statistically significant.

Results

Table 1 shows the major fatty acids of dietary oil supplements used in the present study. It is clear that the two palm oil preparations differed in the proportions of palmitic (16:0), oleic (18:1), and linoleic (18:2) fatty acids. For example, physically refined palm oil had a higher proportion of 16:0 (12% higher) than golden palm cooking oil (GPO; Nutrinol) but a lower proportion of monounsaturated 18:1 (n9) and polyunsaturated linolenic acid. In the GPO, this reduction in palmitic acid was offset by an increase in oleic (78%) and also in linoleic acid. Olive oil was rich in oleic acid (75%) and, compared to the two palm oil supplements, contained a lower proportion of 16:0.

The oil preparations also differed in their antioxidant profiles. For instance, whilst both PO and OO supplements contained no

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Figure 1 demonstrates the impaired vascular relaxation to acetylcholine (ACH) in the spontaneously hypertensive rat. It is clear that compared to the normotensive WKY control rats, the hypertensive vessels relax only partially in OO rats. For example, the dose-dependent increase in relaxation evident in the control vessels was not observed in the DS rats and the maximal relaxation achieved at the highest ACh dose amounted to only 59%. Incorporation of TRF led to significant increases in relaxation response, to 67.7% and 72.9% at 1 and 10 mM ACH, respectively, but these changes failed to achieve significance at the 5% level (Figure 2). Interestingly, the GPO diet fed animals, in contrast, TRF supplementation failed to be effective in restoring the impaired relaxation in hypertensive vessels (p<0.05 vs SHR fed OO). For example, whilst both PO and OO supplemented vessels

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11.8 ± n 75.1 ± 38.7 ± 45.7
11.2 ± 9.0 ± 14.2
0.1 ± 0.4

Table 1. Major fatty acids of dietary oil supplements.

**Fatty acid** | **PO** | **GPO** | **Nutriol** | **OO**
---|---|---|---|---
14.0 | ND | 1.0 | 1.0
16.0 | 88 | 46.1 | 34.4
18.0 | 3.1 | 3.4
18.1 ± n | 75.1 | 38.7 | 45.7
18.2 ± n | 11.2 | 9.0 | 14.2
18.3 ± n | 0.1 | 0.4

Relative proportions of fatty acids are expressed as % of total fatty acids. Oil supplements are; OO (olive oil); PO (palm oil) and GPO (golden palm cooking oil). Polyunsaturated lipid profiles were determined as reported previously. **ND:** not detected.
response was considerably reduced in animals fed different dietary experiments. For example, at the highest dose of ACh (10 mM) the contractions elicited (expressed as % KCl contraction) were PO 9.8 ± 2.1, GPO 6.4 ± 1.7. **TR 2.1 ± 0.4** (* indicates significant difference; p<0.05 vs KCl).

Figure 2 shows the time dependent release of constrictr factor(s) from the blood vessels after inhibition of nitric oxide (NO) with NG-Nitro-L-Arginine (NOLA; 10 mM). It is clear that the release of the constrictr factor(s) is a slow process as there appears to be a gradual rise in tension with time. This increase in contractile tension plateaus 45 minutes after the inhibition of NO (data not shown). None of the treatments was found to significantly modify this constrictr response in the control group (2.7 ± 0.1 mmHg). In conclusion, the release of the constrictr factors was virtually absent in the norepinephrine control animals. For example, at 60 minutes the NOLA-induced constriction was only 2.2% of the KCl contraction (data not shown).

Figure 3. Abnormal thorax-bone-like constrictr response in the SHR. After contraction with KCl (20 mM), aortic rings were incubated with an inhibitor of endothelial cell nitric oxide, N-Nitro-L-Arginine (NOLA; 10 mM), for up to 60 minutes, in order to unmask the release of constrictr factor(s). The tension developed is expressed as a percentage of maximal contractile response to KCl. Results are the mean ± SEM; p<0.05.

Both PO and GPO feeding also displayed increased relaxation to ACh compared to the control group, although this failed to reach statistical significance. Taken together, these findings tend to imply that the improvement of vascular relaxation in this model is likely to be mediated through the antioxidant components rather than the fatty acid constituents of edible oils. Both PO and GPO were rich in natural antioxidants whereas OOO was found to contain a lower vitamin E content. Data also indicate that endogenous antioxidant content of edible oils alone may not be sufficient to fully restore the impaired vascular function in diseased vessels.

It was found that the release of the constrictr factor(s) after inhibition of nitric oxide with NOLA was considerably potentiated by the presence of a threshold dose of 2.7± 0.1 mmHg (p<0.005). For example, in the control group the SHR fed the oil diet alone, presence of this low dose of NINA resulted in 68± 6% increase in the contractions at the 10 minute period (Figure 3) and over 30 at the 30 minute point following the inhibition of nitric oxide. This amplification of the NOLA induced constriction by NO is prevented by both TRF and GPO supplementation of the diet.

Discussion

The present study presents several investigations which have demonstrated that blood vessel function can be influenced by dietary lipophilic antioxidants. For instance, natural antioxidants vitamin E and β-carotene have recently been shown to restore the impaired vascular relaxation observed in hypercholesterolemia and atherosclerosis. Similarly, vitamin E and some flavonoid compounds have also been found to exert vasoprotective actions in hypertensive vessels. In the present investigation, we found that tocotrienol-rich fraction (TRF) extracted from palm oil, mimicked the previously reported effects of vitamin E (α-tocopherol) and restored the impaired vascular relaxation in the spontaneously hypertensive rat (SHR).

As in the case with the TRF supplemented diet, both PO and GPO groups prevented the paradoxical constriction to ACh which occurred mainly at the upper end of the dose-response curve. Such ACh induced constrictions have previously been observed in blood vessels from the SHR and are thought to be due to the release of endothelium derived contracting factors (EDCFs; 3.4). The candidate mediators include prostaglandin H₂ (PGH₂) and oxygen derived free radical superoxide anion (O₂⁻). The ability of antioxidant rich diets to prevent the formation of EDCF's tends to imply a role for superoxide anion in mediating the induced ACh mediated constriction. Furthermore, the effectiveness of antioxidant supplementation in restoring the endothelium dependent vascular relaxation to ACh, reported previously17 and also observed in the SHR, implies that a free radical-related mechanism is involved in causing vascular dysfunction in the SHR. This speculation is further supported by recent reports which suggest that in this model of hypertension (SHR) the main endothelium derived relaxing factor pathway (L-arginine/nitric oxide) to be functioning normally.

In the present study, the thorax-bone-like constriction response which is evident only after the inhibition of endothelial cell NO, was not influenced by antioxidant or edible oil diet. This is in contrast to earlier findings that tocotrienol-rich fraction (TRF) extracted from palm oil, mimicked the previously reported effects of vitamin E (α-tocopherol) and restored the impaired vascular relaxation in the spontaneously hypertensive rat (SHR).

References

response was considerably reduced in animals fed different experimental diets. For example, at the highest dose of AOH (10 mM) the contractions elicited (expressed as % KCl contraction) were PO 9.8±2.1, GPO 6.4±1.7, TFR 2.1±0.6 (* indicates significant difference; p<0.05 vs PO).

Figure 2 shows the time dependent release of contractile factor(s) from the blood vessels after inhibition of nitric oxide (NO) with N6-Nitro-L-Arginine (NOLA; 10-4 M). It is clear that the release of the contractile factor(s) is a slow process as there appears to be a gradual rise in tension with time. This increase in contraction normally tends to plateau at about 45 minutes after the inhibition of NO (data not shown). None of the treatments was found to significantly modify this contractile response in the normotensive hypertensive vessels (P=0.05, ANOVA and Tukey’s test for multiple comparisons). TFR dietary rats however, displayed the lowest mean levels for all time points studied. Compared to the hypertensive rats, the contractile response was virtually absent in the normotensive control animals. For example, at 60 minutes the NOLA induced contraction was only 2.2% of the KCl contraction (data not shown).

Figure 3. Effect of dietary antioxidants on norepinephrine content in tibia of Wistar-Kyoto rats. 

As in the case with the TFR supplemented diet, both PO and GPO groups prevented the paradoxical increase in NO release to Ach which occurred mainly at the upper end of the dose-response curve. Such Ach induced contractions have previously been observed in blood vessels from the same species and are thought to be due to the release of endothelium derived contracting factors (EDCFs; 3, 4). The candidate mediators include prostaglandin H2 (PGH2) and oxygen derived free radical superoxide anion (O2-). The ability of antioxidant rich diets to prevent the formation of EDCF s tends to imply a role for superoxide anion in mediating the Ach induced contractile response. Furthermore, the effectiveness of antioxidant supplementation in restoring the endothelium dependent vascular relaxation to Ach, reported previously 1,9 and also observed in the present investigation, implies that a free radical-related mechanism is involved in causing vascular dysfunction in the SHR. This specification is further supported by recent reports which suggest that in this model of hypertension (SHR) the main endothelium derived relaxing factor pathway (L-arginine/nitric oxide) to be functioning normally 10.

Discussion

The present study seeks several investigations have which have demonstrated that blood vessel function can be influenced by dietary polyphenolic antioxidants. For instance, natural antioxidants vitamin E and polyphenol antioxidants have recently been shown to restore the impaired vascular relaxation observed in hypercholesterolaemia and atherosclerosis. Similarly, vitamin E and several plant flavonoid compounds have also been found to exert vasoprotective actions in experimental hypertensive vessels. In the present investigation, it was found that tocotrienol-rich TFR diet extracted from palm oil, mimicked the previously reported effects of vitamin E (α-tocopherol) and restored the impaired vascular relaxation in the spontaneously hypertensive rat (SHR).

Both PO and GPO feeding also showed increased relaxation to Ach compared to the control group, although this failed to reach statistical significance. Taken together, these findings tend to imply that the improvement in vascular reactivity to Ach in the dietary rats is likely to be mediated through the antioxidant components rather than the fatty acid constituents of edible oils. Both PO and GPO were rich in DHA and EPA, which was found to contain a lower vitamin E content. Data also indicate that endogenous antioxidant content of edible oils alone may not be sufficient to fully restore the impaired vascular function in diseased vessels.

Acknowledgments

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