Oleic Acid Reduces Brain Injury by Oxidative Stress Induced by Some Anticancer Drugs in Rat Brain

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Abstract

Background: Oleic acid (OA) is a monounsaturated compound with many health-benefitting properties such as obesity prevention, increased insulin sensitivity, antihypertensive and immune-boosting properties, etc. Within the untold benefits of this fatty acid is the novelty of anticancer property. OA can interact with cancer cell proteins and increase cancer cell proliferation-inhibiting factors; thus, turning down their potentials to spread to other tissues.

Purpose: The proposal in the present work was to carry out a factual analysis on how oleic acid (OA) and some anticancer drugs offset nitropropionic acid-induced oxidative stress .

Methods: Thirty Wistar rats were recruited and divided into five groups A, B, C, D and E; and made to take anticancer drugs in combination with OA in the following forms: Group A, (control) 0.9% of sodium chloride (NaCl); group B, oleic acid (OA) only ; group C, cyclophosphamide (CPP) + OA; group D, daunorrubicine (DRB) + OA; and group E , dexrazoxane (DXN) + OA. Every treatment was by intraperitoneal route and the administration was every 24 h for 5 days. The measurement of lipid peroxidation products, reactive oxygen species, sodium-potassium pump, antioxidants and amines were performed from brain extracts of the animals using TBARS, H_2O_2 , Na^+, K^+ ATPase activity, GSH and dopamine (DA) respectively. Glucose hemoglobin and triglycerides were measured in blood.

Results: In cortex, GSH increased in all groups, except in group B . Group C showed the highest increase of this biomarker. TBARS decrease and dopamine increase in all regions of groups C, D and E. H_2O_2 increased only in cerebellum/medulla oblongata of group D and E. ATPase expression decreased in striatum of group C. Glucose increased in group E and hemoglobin increased in groups C and D.

Conclusions: The boost in the amine (DA) and the antioxidant (GSH) generated by OA administration depicts that brain damage due to anticancer drugs could be ameliorated when jointly given with OA.

1.1 Background

Worldwide increase in obesity prevalence across age groups has remained unabated in different countries [1, 2]. In Mexico, this tendency has continued to rise until date, affecting both young and old [2, 3]. Metabolic syndrome (MS) is a group of conditions characterized high abdominal fat accumulation, high systolic and diastolic blood pressure, high density lipid cholesterol, and high blood glucose and triglycerides [2,4]. Irrational consumption of fatty foods and meats has been linked with oxidative stress with its concomitant adverse health effects and as an important risk factor for the development of cancer [2,5]. In Mexico, oncological diseases rank second as the principal cause of death in the general population. The toll of cancer mortality in the country had been witnessing an increase since 1953 in such way that by 1998, it had registered an accumulated rise of 68%, a figure that may have increased today. Likewise, in recent years, malignant tumor morbidity has gone up as depicted in the ´90s report where it was shown that malignant tumor in adult females and males of 30 years old rose to 63.5% and 35.3% respectively [2,6]. Besides, malignant tumors have been reckoned as the main cause of death in pediatric population under 15 years old [2,7]. The treatment most widely used in cancer therapy focuses on drugs that target on fast growing and dividing cancerous cells and most of these drugs have been associated to an increase in monoamine concentrations in the brain [2,8]. In any case, these drugs eliminate these cells in specific points of cell cycle [2,9]. One of the principal side effects of chemotherapeutic drugs is MS [2,10]. In Mexico, childhood obesity is today a preoccupying problem of health [2,11]. In our country, chemotherapy protocols employed in childhood cancer treatments generally includes anticancer agents [2,12]. It has been reported that exogenous polyunsaturated fatty acids such as oleic and linoleic acids can temper the cytotoxic activity of anti-cancer drugs [2,13], and dexrazoxane has been used to protect the heart against cardiotoxic side effects of anthracyclines [14].

The anti-cancer properties of OA have been demonstrated. OA can interact with cancer cell proteins and increase cancer cell proliferation-inhibiting factors; thus, turning down their potentials to spread to other tissues. However, the administration of OA may generate nitric oxide (NO) [2,15]; in the form of nitro-oleic acid (OA-NO2); a nitric oxide-derived endogenous lipid product - likely via its anti-inflammatory and antioxidant properties [2,16]. NO is a neuromodulator and in high concentrations leads to cell damage by the formation of nitroso-glutathione (NOGSH) or by oxidative stress [2,17]. Oxidative stress due to nitric oxide is the main cause of alterations in the mitochondrial ultrastructure and DNA damage [18,19]. This damage is the primary event in 3-nitropropionic acid toxicity.

Free radicals (FR) are deleterious to cell components [2,20], especially the lipid bilayer of the plasma membrane [21]. The CNS not only mediates in the regulation of appetite and free radicals generation but also actively takes part in the control of the metabolic functions of these [2,22].

Cell membrane alterations dependent on mechanical changes that principally involve the lipid bilayer are known to influence different biological processes [2,23], and modulate energy and glucose homeostasis via the activation of dopaminergic systems of the hypothalamic neurocircuits and higher brain circuits [2,24]. The lipid bilayer of the neurons contains structural proteins that are contiguous with brain plasma membrane phospholipids [2,25]. This permits ionic interchange between these two structures, fueled by the stimulating action of Na+, K+ ATPase that boosts Na+ and K+ flows through the bilayers [2,26]. The main effect of Na+, K+ ATPase inhibition is the release of excitatory amino acid which can be injurious to the CNS [2,27].

Based on this background, this work aims to analyze the effect of oleic acid and some anticancer drugs as cyclofosfamide, daunorrubicine and dexrazoxane (a cardioprotector) against NPA-induced oxidative stress and the levels of dopamine in rat brain.

2.1 Materials And Methods

Thirty Wistar rats – gender male; age 3 months and average weight 100g - were recruited for the study and equally divided into five groups A, B, C, D, and E. The treatments given to the animals consist of anticancer drugs in combination with OA. Group A (control), received NaCl 0.9%; group B, received only oleic acid (OA) (1.5ml/rat); group C was given cyclophosphamide (CPP) (20mg/rat) + OA; group D was administered daunorrubicine (DRB) (4mg/rat) + OA; and group E was treated with dexrazoxane (DXN) (50mg/rat) + OA. Every treatment was by intraperitoneal route and the administration was every 24 h for 5 days. The animals were procured from Bioterium of Metropolitan University of Mexico City and housed six per cage in clean plastic cages and allowed to acclimatize in the room environment for 1 day. Animals were maintained in a mass air displacement room with a 12-h light:12-h dark cycle at 22 ± 2°C with a relative humidity of 50 ± 10%. Balanced food (Rodent diet 5001) and drinking water were given to the animals *ad libitum*. On the last day of the treatments, blood samples were obtained to measure glucose hemoglobin and triglycerides. After that, the rats were sacrificed by decapitation and their brains were extracted and sectioned into cortex, striatum, and cerebellum/medulla oblongata (CMO). We do not use anesthetics because they could alter our results. The sections were immediately immersed in NaCl at 0.9% and kept at 4°C. 3ml of tris-HCl 0. 05M pH 7.2 was employed in the homogenization of every section of the brain and used to assay the peroxidation of lipids (TBARS), H_2O_2, Na+, K+ ATPase activity, glutathione (GSH), and dopamine using previously validated methods. To avoid the degradation and loss of integrity of the samples, they were stored at −20°C until analyzed. Animal management and care was conducted in accordance to the international guidelines for animal care and to the Mexican Guidelines ZOO-062, and that allowed by the Laboratory of Animal Care Committee of The National Institute of Pediatrics.

Triglycerides, blood hemoglobin and glucose were measured at the end of the treatments. Glucose and triglyceride concentrations were measured with two sets of 20μl of volume non-anticoagulant tail-end blood samples using Accu- Chek glucose reactive paper (Roche Mannheim, Germany) and reported in mg/dl.

2.1.1 Measurement of Dopamine (DA)

DA concentrations were measured from a 9000 rpm 10 min centrifuged HCLO4 homogenized supernatant tissue. The centrifugation was done in a microcentrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany), and the measurement was carried out based on Calderon et al [2,28] method using FL Win Lab version 4.00.02 software. The values were deduced from a previously standardized curve and reported as nM/g of wet tissue.

2.1.2 Glutathione (GSH) measurement

To measure GSH levels, the homogenized tissue supernatant obtained prior to centrifugation for 5 min period at 9000 rpm (Mikro 12-42, Germany centrifuge) using Hissin and Hilf modified method [2,29].

2.1.3 Lipid peroxidation measurement

The approach employed in the measurement of TBARS was Gutteridge and Halliwell modified technique [21]. A 1 ml of the tris-HCl0. 05M pH 7.4 brain homogenate was mixed with 2ml thiobarbaturic acid (TBA) that contains 1. 25g TBA, 40g trichloroacetic acid (TCA) and 6. 25ml of concentrated chlorhydric acid (HCL) diluted in 250ml of deionized H_2O. The resulting mixture was heated to boiling point for 30min (Thermomix 1420) and cooled for 5 min in an ice bath. This was subjected to a centrifugation at 700g for 15min (Sorvall RC-5B Dupont). The floating tissue absorbances were spectrophotometrically (Helios-α de UNICAM) read in triplicate at 532nm. The concentration of reactive substances to the thiobarbaturic acid (TBARS) was expressed in μM of Malondialdehyde/g of wet tissue [30].

2.1.4 Total ATPase determination

ATPase activity was assessed based on Calderón et al method [31]. One mg (10%) w/v of the tris-HCl0. 05M pH 7.4 tissue homogenate was subjected to an incubation for 15 min in a solution containing 3mM MgCl2, 7mM KCl and 100mM NaCl. 4 mM tris-ATP was added to the mixture and the incubation was repeated for another 30 min at 37°C in a shaking water bath (Dubnoff Labconco). The reaction was detained using 100 μL 10% trichloroacetic acid w/v. The samples were final centrifuged at 100 g for 5 min at 4O °C [32]. Inorganic phosphate (Pi) was measured in duplicates using one supernatant aliquot as reported by Fiske and Subarrow [33]. The reading of the absorbance of the supernatant of each sample was carried out spectrohpotometrically at 660 nm in a Helios-α, UNICAM and expressed as mM Pi/g wet tissue per min.

2.1.5 H_2O_2 determination

H_2O_2 measurement was performed with Asru [34] and Hernandez [35] techniques after modification. The brain regions (cortex, striatum, CMO) were separately diluted in 3 ml of tris-HCl 0. 05M pH 7.4 buffer and homogenized. 100μl of the resulting homogenate of each brain region was mixed with1ml of potassium dichromate solution (K2Cr2O7). The mixture was heated to boiling point for 15min (Thermomix 1420) and cooled for 5 min in an ice bath. Then, the samples were subjected to a centrifugation at 3,000g for 5min (Sorvall RC-5B Dupont). The reading of the floating absorbances was spectrophotometrically done in triplicate at 570nm (He?ios-?, UNICAM). H_2O_2 concentration was expressed in μMoles [30].

2.1.6 Statistical analysis

For statistical analysis, the fisher estimation test (ANOVA) and the nonparametric test of Kruskal-Wallis were used with their corresponding contrasts and previous to variance homogeneity comparison. Statistical significance was put at p-values of <0.05 [2,36]. JMP Statistical Discovery Software, version 8 .0.0 from SAS was employed in the statistical tests [2].

3.1 Results

3.1.1 GSH

In the cortex, GSH was observed to increase in all the groups except in the group treated with OA alone. The increase in this bioamine was highest in the group that received CPP + OA when compared with the control group and all other groups. On the other hand, a decrease was appreciated in GSH levels in the striatum of the animal group treated with OA. However, this event showed a significant difference only with the group that received CPP + OA where a greater decrement was observed. In CMO region, GSH increased in the groups that were administered DRB + OA and CPP +OA with this having statistically significant difference on comparing it with the groups of OA and CDX + OA (table 1).

3.1.2 TBARS

The peroxidation of lipids witnessed a significant decrease in the cortex, striatum, and cerebellum/medulla oblongata (CMO) of the treated groups. Data analysis depicted that the reduction in TBARS levels was significant when the groups that received OA, CDX + OA, CPP + OA and DRB + OA were compared with the control group. The same significant effect was observed on comparing CDX + OA, CPP + OA and DRB + OA groups with the group that received only OA. In this brain region, a significant difference in TBARS levels was also observed between CDX + OA group vs. DRB + OA group, which showed the least lipid peroxidation (table 1).

3.1.3. Dopamine

The concentration of this neurotransmitter increased ten folds in the groups treated with drug combinations CDX + OA, CPP +OA and DRB + OA in the three brain regions studied with respect to control and OA groups. Additionally, statistically significant differences were appreciated on comparing the group CDX + OA vs the group DRB + OA in the cortex and DRB + OA vs. CPP + OA in the striatum. In none of the regions were differences observed between the control group and OA group (table 1).

3.1.4 H_2O_2

With respect to H_2O_2, data analysis revealed significant changes consisting of an increase in the levels of this indicator in the CMO brain region attributable to the treatments with CDX + OA and DRB + OA with respect to the group of control and the OA group (table 1).

3.1.5 ATPase

ATPase activity was only seen to be affected in the striatum regions where a significant

reduction in the enzyme activity was observed in the CPP + OA group in comparison with the control group and the group treated with CDX + OA. The levels of glucose showed significant increase in the group that was administered the combination of CDX + OA (table 2). Hemoglobin increased in CPP + OA and DRB + OA groups. Although this indicator increased in the group treated with CDX + OA, this was not significant when compared with the control group (table 2). With respect to triglycerides, differences between the control group and the rest of the treated groups were not observed (table 2).

4.1 Discussion

Drugs that are used in the treatment of cancer usually have adverse impacts on brain functions. The neurological side effects of these drugs have been widely reported in peripheral neuropathy and encephalopathy [2,8]. In this study, the results indicated that CDX, CPP and DRB increased dopamine levels in cortex, striatum and CMO in the animals. However, in the group treated with OA, the biomarkers evaluated in this study did not witness any changes. This may be explained either by alterations in the metabolic processes of OA or that the deacylation/acylation reactions of this acid were inhibited and this triggers an increase in the markers of oxidative stress and dysfunction of the mitochondria in dopaminergic neurons [2,37]. Hemoglobin levels increased in the groups treated with CPP and DRB in combination with OA, except in the group treated with CDX. Probably this may be owed to the fact that the antioxidant actions of CDX are totally dependent on its metabolism that yields hydrolyzed open-ringed product or because CDX does not have any effect on the iron-independent oxygen free radical production [38], and on the increase in the levels of glucose. ATPase activity increased in striatum region in the group treated with CPP + OA. Similar results were found in the antioxidant enzymes of animals treated with CPP and OA / linoleic acids, suggesting a reduction in the oxidative stress [39]. This finding coincides with the studies of Park et al [40], who suggested that fatty acids (linoleic acid, oleic acid and palmitic acid), inhibit the increase in [Ca2+], ROS generation and apoptosis with a similar potency, and as consequence may ameliorate toxicity of the anticancer drugs. Nitro-oleic acid (OA-NO2) is an electrophilic nitroalkenyl fatty acid with several attractive signaling properties. In healthy human blood, the concentration is found to be ~0.6 μM [41], and its increased production is demonstrated during inflammatory and metabolic stress [42]. Baraldi et al [43], which suggests that conjugated linoleic acid (CLA) reduces the accumulation of body fat and increases body metabolism. In the groups that received oleic acid, lower lipid accumulation was found to be reversed aided by the increase in PPAR gamma content. In the same way, high ROS generation was reversed in this group; thus, improving the oxidative status. Studies have shown that the percentage of cell membrane content of free fatty acid is lower than 5% and that any excess is rapidly incorporated into phospholipids [2,44]. These findings are supported by Hilvo et al [45], who suggested that the alterations in OA levels in the blood in patients with breast cancer are associated with their response to chemotherapy [2]. In the case of oncological agents as DRB and CPP as well as CDX, a cardioprotector, GSH levels increased in the cortex, and CMO when rats were treated with CPP + OA [35]. Besides, there was a decrease in lipoperoxidation levels in cortex, striatum and medulla oblongata with the combination of oleic acid.

The blood-brain barrier, formed by the tight junctions within the capillary endothelium of the vertebrate brain, discriminates the access of several molecules to the brain tissue [2,25]. The brain is the most consumer of oxygen in the body and therefore has a high level of oxidant production; however, its protective antioxidant mechanisms is weak. Oxidative stress hampers lipid metabolism at various levels ranging from benign lipid storage to so-called second hit of inflammation activation, and thereby induces lipid overload [2,46]. Hence, substances that can modulate the prooxidant-antioxidant balance would provide a therapeutic option to enhance neuroprotection in response to oxidative stress [2,47] produced by nitropropionoic acid [48].

Conclusion

Oleic acid, as depicted by the results of this work, increases dopamine and antioxidant defense; thus, it can mellow down the oxidative side effects of oncological agents and in this way, can protect the brain from the injurious impact of these agents.

In the light of the above, food supplements such as OA could be a potential strategy to offset the adverse effects of anticancer drugs during chemotherapy in pediatric population. Nevertheless, more studies with different animal models are needed to demonstrate OA neuroprotective mechanism and potential, not only during treatment with anticancer drugs but also in other health conditions.

Abbreviations

CNS. Central Nervous System

DA. Dopamine

DNA. Deoxyribonucleic acid

FR. Free radicals

GSH. Glutathione

NOGSH. nitroso-glutathione

3-NPA. 3-nitropropionic acid

L-DOPA. L-3,4-dihydroxyphenylalanine

OPT. ortho-phthaldehyde

RNS. Reactive nitrogen Species

ROS. Reactive Oxygen Species

TBARS. Thiobarbituric acid reactive substances

Declarations

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Tables

Table 1. Levels oxidative biomarkers and domamine in brain regions of rats treated with oleic cid (OA) alone or with ciclophosphamide (CPP), daunorrubicine (DRB) and dexrazoxane (DXN).

Region	Treatment	GSH		TBARS		Dopamine		H_2O_2		ATPase	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cortex	Control	336.849	58.55	11.082	0.96	166.98	71.85	0.028	0.012	63.32	19.91
	OA	415.958	61.05	8.920**	2.19	270.36	101.40	0.019	0.004	58.09	18.20
	CPP + OA	569.57*a	98.92	2.125*a	0.30	2276.04*a	372.11	0.026	0.005	50.36	14.78
	DRB + OA	542.24*	95.81	1.772*a	0.16	2112.67*a	294.20	0.028	0.008	49.10	13.06
	CDX + OA	557.79*	95.47	2.017*a	0.19	2715.99*a,b	414.40	0.031	0.004	46.85	10.21
Striatum	Control	769.527	167.28	15.017	1.34	272.85	103.96	0.020	0.003	128.02	41.14
	OA	606.155a	106.93	14.303	2.81	360.33	112.39	0.029	0.011	109.72	27.75
	CPP + OA	855.508	105.18	3.906*a	0.58	3343.38*a	776.09	0.026	0.004	75.01*,a	13.32
	DRB + OA	701.385	111.82	3.080*a	0.36	2562.25*a,b	485.31	0.030	0.006	104.79	32.12
	CDX + OA	778.960	111.11	3.551*a	0.54	3299.42*a	360.68	0.032	0.011	117.24	25.83
CMO	Control	476.879	46.98	16.076	1.31	324.00	94.74	0.022	0.009	66.71	4.36
	OA	446.223	118.15	11.439*	1.56	386.17	92.29	0.027	0.009	115.73	67.78
	CPP + OA	672.299a	91.39	4.011*a	0.54	2733.62*a	540.52	0.031	0.004	82.41	24.18
	DRB + OA	637.982	157.03	3.209*a,b	0.60	2223.63*a	556.74	0.041*a	0.007	91.85	18.54
	CDX + OA	480.647	152.33	5.463*a	1.02	2511.37*a	572.24	0.040*a	0.006	80.64	24.10

CMO:Cerebellum/medulla oblongata, SD: Standard Deviation

Glutathione (GSH)

Cortex: Anova   F=6.8774 p=0.0006. * p<0.005 vs control, [a]p=0.03 vs OA

Striatum: Anova   F=3.9468 p=0.0124. [a]p=0.007 vs CPP + OA

CMO: F=4.4578 p=0.0071. [a]p<0.05 vs OA and CDX + OA

Lipid peroxidation (TBARS)

Cortex: Anova (Welch) F=86.7878 p<.0001. *p<0.0001 vs control. ** p=0.016 vs control, [a]p<0.0001 vs OA

Striatum: Anova (Welch) F=78.3615 p<.0001. *p=<.0001 vs control, [a]p<0.0001 vs OA

CMO: Anova F=156.7193 p<.0001 *p=0.001 vs control, [a]p<0.0001 vs OA [b]p<0.002 vs CDX + OA

Dopamine

Cortex: Anova (Welch) F=152.1796 p<0.0001. *p<0.0001 vs control, [a]p<0.0001 vs OA, [b]p=0.01 vs DRB + OA

Striatum: Anova (Welch) F=144.5489 p<.0001. *p=<.0001 vs control, [a]p<0.0001 vs OA, [b]p=0.04 vs CPP + OA

CMO: Anova (Welch) F=67.3055 p<0.0001. *p=<.0001 vs control, [a]p<0.0001 vs OA

H_2O_2

Cortex: Anova   F=2.2322 p=0.0931

Striatum: Anova   F=2.0085 p=0.1228

CMO: Anova F=8.2568 p=0.0002. *p=<0.001 vs control, [a]p<0.01 vs OA

ATPase

Cortex: Anova F=1.0989   p=0.3780

Striatum: Anova   F=3.4580 p=0.0215. *p=0.03 vs control, [a]p=0.04 vs CDX + OA

CMO: Anova F=1.4778 p=0.2376.

Table 2. Levels of glucose, hemoglobin and triglycerides in blood of rats treated with oleic acid (OA) alone or with ciclophosphamide (CPP), daunorrubicine (DRB) and dexrazoxane (DXN).

	Glucose (mg/dL)		Hemoglobin (g/dL)		Triglycerides (mg/dL)	
Treatment	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Control	120.25	6.90	23.46	2.72	106.75	18.79
Oleic Acid	122.40	8.05	23.28	3.80	102.40	4.88
CPP + Oleic acid	110.63	16.13	39.02*	3.79	99.75	17.25
DRB + Oleic acid	109.14	13.07	31.90*a	4.66	88.57	7.07
CDX + Oleic acid	145.86*	13.55	28.50	0.97	101.86	26.30

Glucose: Anova F=9.2322   p<0.0001. *p<0.03 vs all treatments.

Hb: Anova F=22.2349 p<0.0001. *p<0.004 vs all treatments. [a]NS vs CDX + OA

Triglycerides: Anova F=0.948 p=0.45

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklistfillable.pdf