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The Chronic Influence of Carbon Monoxide Intoxication on Lipid Peroxidation

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Abstract

Carbon monoxide is a known notorious gas with a high preponderance of causing either morbidity or mortality depending on the concentration inhaled or administered. The study was aimed to determine whether chronic CO intoxication could stimulate lipid peroxidation. A total of twenty albino rabbits of same sex, age and weight constituted the sample size as validated by the Mead's formula. The study was divided into four groups of five rabbits each; the control, 10 days, 20 days and 30 days respectively. Except the control group, others were exposed daily to thirty minutes of 200 ppm CO intoxication. The statistical analysis was done with the aid of SPPS 20 version, using One-way Anova (Post Hoc-LSD). Biochemical parameters indicative of lipid peroxidation were analysed using blood and vitreous humor samples. The serum and vitreous chemistry revealed a significant decreased ($p<0.05$) in the concentrations of total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein, uric acid, Glutathione Reductase and Superoxide Dismutase. On the contrary, the concentration of serum and vitreous MDA increased significantly ($p<0.05$). The findings revealed that chronic CO intoxication could provoke lipid peroxidation.

Keywords: Free radicals; Malondialdehyde; Superoxide dismutase; Catalase; Uric acid

Introduction

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process usually proceeds by a free radical chain reaction mechanism. The reaction consists of three major steps: initiation, propagation, and termination. The chemical products of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs) [1].

Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA [2]. More severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis [3,4].

Carbon monoxide (CO) is a poisonous, colourless, tasteless, odourless and non-irritant gas produced by incomplete combustion of organic material due to insufficient oxygen supply to enable complete oxidation to carbon dioxide (CO2) [5]. It utilizes hypoxia in exerting its nefarious effect on the entire body system by making oxygen inaccessible.

Carbon monoxide poisoning is the inhalation of large quantity of CO that is deleterious to the body. It could be acute or chronic depending on the concentration inhaled. Chronic CO poisoning involves the inhalation of abnormal concentration of CO that cannot lead to immediate death over a period of time exceeding one month.

Lipid peroxidation is studied with the aid of biochemical parameters that are affected or that cushion the effects of free radicals. Such parameters include malonaldehyde, glutathione reductase, superoxide dismutase, catalase, uric acid, and lipid profiles. These biochemical parameters to a large extend could be altered as a result of lipid peroxidation.

The effect of lipid peroxidation is not new in biomedical science. The alterations arising from the above stated biochemical parameters by acute CO intoxication is very revealing as posited by Agoro et al. [6]. In carbon monoxide poisoned patients, an altered balance between reactive oxygen species and antioxidant concentrations has been reported [7]. Also it has been observed that free radicals and oxidative stress are among factors involved in pathogenesis of acute carbon monoxide poisoning [8]. Another author held a strong relationship between acute carbon monoxide poisoning and free radical and [9]. Egwurugwu et al. did a work on gas flaring in the Niger Delta of Nigeria using human subjects and revealed that serum concentrations of triglycerides, low density lipoprotein (LDL) and very low density lipoprotein

(VLDL) were elevated as compared to the control, whereas high density lipoprotein (HDL) decreased [10].

Nigerians are exposed to varying degrees of CO due to the high demand of CO-producing machines and equipment. A deliberate study of the effect of chronic exposure to CO on lipid peroxidation parameters will in no measure shaping the perspective to several diseases linked to lipid alterations. The products of this study could be useful in policy enactment in combating chronic diseases, preventing avoidable epidemics and enhancing medical well-being.

Materials and Methods

Study area

The animal intoxication was carried out in the animal science laboratory of the Niger Delta University. Similarly, the biochemical analysis took place at the Chemical Pathology Laboratory of the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State.

Animals

New Zealand white albino rabbits (male, 6-8-mo-of-age, 1.5-2.0 kg) were obtained from Imo State in Nigeria. Rabbits used were apparently healthy and active as confirmed and approved by a university veterinarian. Each was housed in an individual cage in a specific pathogen-free facility maintained at 25°C with a 60% relative humidity and a 12 hrs light/dark cycle. All rabbits had ad libitum access to standard rabbit chow bought in Yenagoa, Bayelsa State and filtered water. Any rabbit showing signs or symptoms of illness prior to exposures were excluded from the research. Ultimately, a total of 20 rabbits were used for the exposures/analyses.

After two weeks of acclimatization, the rabbits were randomly allocated into four groups. The first group was designated controls that were exposed to air only (n=5). The remaining three groups of rabbits were exposed daily whole-body to CO for 30 min for 10, 20, or 30 days (n=5/group). All animals were exposed in a CO chamber. The source of the CO was a portal Sumac generating set. All CO levels were constantly monitored using a Carbon Monoxide Meter Gasman-CO (PCE Instrument, UK). Exposure levels were never greater than 200 ppm in the chamber. This level was selected based on earlier studies of Goldstein and Struttmann et al. wherein 200 ppm was found to reflect CO concentration that will not lead to immediate mortality and morbidity [11,12].

At 30th minutes after the final exposure, CO-treated rabbits and their matched controls were euthanized mechanically. Bio-samples (i.e., vitreous humor and blood) were then collected for analyses (see below).

All protocols were approved by the Ethics Committee of the Bayelsa State Ministry of Health and the template of the Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered.

Collection of vitreous humor

Vitreous humor samples were collected by the method of Coe [13]. In brief, within 30 min of euthanization, samples were collected by insertion of a 27-G needle into the cantus of the eye and ≈ 1 ml was then retrieved into a plane tube. Only clear liquid free of any tissue contaminants/fragments was acceptable for analyses. Each isolated vitreous samples was centrifuged at 2050 rpm (10 min, 25°C) and resultant supernatants were collected for the analyses.

Collection of cardiac blood

At necropsy, blood samples were collected from the heart using the method of Ness [14]. In brief, within 30 min of euthanization, samples were collected by insertion of a 27-G into the left side of the chest, through the diaphragm, from the top of the sternum. Blood was then slowly withdrawn into a plane tube. The samples were allowed to clot at room temperature and then serum was collected by centrifugation at 2000 rpm for ten minutes at 25°C for use in biochemical analyses.

Bio-analyses

The vitreous or serum CAT activity was assayed according to the method of Aebi [15]. The activity of serum superoxide dismutase (SOD) was assayed by the method of Xin et al. as contained in Randox commercial kit leaflet [16]. Lipid peroxid analyses was carried out by determining the concentration of MDA formed using the method of Varshney and Kale [17].

The concentration of glutathione was determined according to the method of Habig et al. [18]. Vitreous or serum uric acid concentration was estimated quantitatively by Uricase Method using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous or plasma total cholesterol, triglyceride, HDL concentrations were estimated quantitatively using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous or plasma LDL and VLDL concentration was derived mathematically by the formular as stated by Carl and Edward, and Friedewald et al. [19,20] respectively.

Statistical analysis

All data were reported as means ± Standard Deviation. A one-way analysis of variance [ANOVA] followed by a Fisher's Least Significant Difference (LSD) post-hoc test was used to compare values for each biomarker across the study groups. All data were analyzed by Statistical Package for Social Sciences program v.18-21 (SPSS Inc, Chicago, IL, USA).

Results and Discussion

Table 1 shows a multiple comparison of serum lipid profile (Mean ± SD) in the four groups of the chronic CO intoxication using one way-Anova (LSD's post hoc test). Serum total cholesterol, HDL and VLDL significantly decreased ($p<0.05$) as the days and duration of chronic CO exposures increases. On

the contrary, serum HDL significantly increased ($p<0.05$) at the 10th day of CO intoxication and later significantly declined ($p<0.05$) as the days and duration of chronic CO exposures increases. However, serum triacylglycerol exhibited a significant increase ($p<0.05$) as days and duration of chronic CO exposures increases.

Table 2 shows that total cholesterol, triacylglycerol, HDL, LDL and VLDL significantly decreased ($p<0.05$) as the days and duration of chronic CO exposures increases. **Table 3** shows that Serum uric acid significantly decreased ($p<0.05$) as the days and duration of chronic CO exposures increases. However, serum catalase and melonaldehyde exhibited a significant increase ($p<0.05$) across the chronic study groups. Serum glutathione and superoxide dismutase showed significant decrease ($p<0.05$) as the intoxication duration and day's increases.

Table 1: A multiple comparison of serum lipid profile on the basis of duration of chronic CO intoxication.

Parameters	Control	Duration of CO Exposure			f-value	p-value
		Day 10	Day 20	Day 30		
TC (mmol/L)	3.28 ± 0.20	3.10 ± 0.29	2.03 ± 0.25^{ab}	1.89 ± 0.17^{ab}	37.89	0.00
TG (mmol/L)	1.12± 0.09	1.62± 0.23^{a}	1.54 ±0.03^{a}	1.53 ±0.31^{a}	3.05	0.07
HDL (mmol/L)	0.91± 0.18	0.59± 0.18^{a}	0.31± 0.01^{ab}	0.20± 0.09^{ab}	22.83	0.00
LDL (mmol/L)	0.39± 0.16	1.36± 0.75^{a}	0.28 ± 0.24^{b}	0.20± 0.16^{b}	6.77	0.01
VLDL (mmol/L)	1.99± 0.18	1.15± 0.44^{a}	1.45±0.29^{ab}	1.48± 0.30^{a}	4.80	0.02
Legend: TC- Total Cholesterol; TG-Triacylglycerol; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein; VLDL-Very Low Density Lipoprotein.						
Symbols- a: P < 0.05 vs control, b: P < 0.05 vs Day 10, c: P < 0.05 vs Day 20						
Data are expressed as mean ± SD; Significant at 0.05 Confidence (p < 0.05)						
Concentration of acute CO intoxication= ≤ 200 pm						

Table 2: A multiple comparison of vitreous lipid profile on the basis of duration of Chronic CO intoxication.

Parameters	Control	Duration of CO Exposure			f-value	p-value
		Day 10	Day 20	Day 30		
TC (mmol/L)	1.44 ± 0.05	0.85 ± 0.13^{a}	0.67 ± 0.17^{a}	0.55 ± 0.13^{ab}	38.33	0.00
TG (mmol/L)	0.76 ± 0.06	0.23 ± 0.04^{a}	0.19 ± 0.03^{a}	0.16 ± 0.05^{ab}	171.04	0.00
HDL (mmol/L)	0.39 ± 0.04	0.12 ± 0.02^{a}	0.10 ± 0.01^{a}	0.13 ± 0.01^{a}	144.58	0.00
LDL (mmol/L)	0.71 ± 0.05	0.63 ± 0.10^{a}	0.49 ± 0.15^{a}	0.38 ± 0.10^{ab}	7.33	0.01
VLDL (mmol/L)	0.35 ± 0.03	0.11 ± 0.02^{a}	0.090 ± 0.01^{a}	0.08 ± 0.02^{a}	153.49	0.00
Symbols- a: p<0.05 vs. control, b: p<0.05 vs. Day 10, c: p<0.05 vs. Day 20.						

Table 4 shows vitreous uric acid significantly decreased ($p<0.05$) as the days and duration of chronic CO exposures increases. a multiple comparison of some vitreous oxidative stress parameters of the chronic study groups. Vitreous glutathione and melonaldehyde exhibited a significant increase ($p<0.05$) across the study groups. However, vitreous catalase and superoxide dismutase showed significant decrease ($p<0.05$) as the intoxication duration and days increase

The finding of this study showed significant decrease ($p<0.05$) in concentrations of serum and vitreous lipid profile parameters except for triacylglycerol **(Tables 1 and 2).** The scientific basis for the alterations observed in this study is multifaceted. The plasma and vitreous lipid profile which were lower (except serum TG) could be attributed to massive infiltration of lipids into cells occasioned by the consistent presence of CO. Some gases are known to pass through cell membranes irrespective of the discriminatory barriers. This could be the basis for the easy access of lipids into cells. The movement may be responsible for the decrease in lipid concentration in extracellular fluids, and increase in cells. This research is of the view that CO enhances the permeability of cell membranes to the infiltration of lipids. The main mechanism by which CO causes cardiac collapse is basically by hypoxia. The infiltration mechanism as postulated in this research is in accordance with arrays of studies that observed similar findings in terms of increased cholesterol accumulation in cells [21-23]. Studies in rabbits and monkeys have reported that exposure to CO produced accumulation of cholesterol in the aorta, coronary arteries, and endothelial damage [24]. Widlansky et al. also showed an endothelial dysfunction occasioned by cholesterol infiltration [25]. Oxidative stress is a

condition characterized by elevated concentrations of intracellular reactive oxygen species (ROS). The deprivation of lipids of its normal configuration resulting from the effect of reactive oxygen species (ROS) is termed lipid peroxidation. Free radicals are highly reactive and capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrate, and nucleic acids) [19,26,27]. Free radicals generated during CO intoxication have the propensity of distorting and/or degrading lipids in the systems. This may be the basis of the lipid peroxidation cascade which in turn reflected the reduction in concentration of studied lipid profiles. An increase in serum triacylglycerol was observed in this study. Triacylglycerol increase is closely related to gluconeogenesis.

Table 3: A multiple comparison of serum oxidative stress biomarkers on the basis of duration of chronic CO intoxication.

Parameters	Control	Duration of CO Exposure			f-value	p-value
		Day 10	Day 20	Day 30		
GRT (µ/mg)	28.01 ± 0.69	21.44 ± 0.96^{a}	22.30 ± 0.70^{a}	20.98 ± 1.23^{a}	51.17	0.00
SOD (µ/mg)	53.25 ± 2.75	24.31 ± 0.90^{a}	25.26 ± 1.02^{a}	22.84 ± 1.75^{a}	272.55	0.00
CAT (µ/mg)	45.5 ± 1.29	52.87 ± 1.38^{a}	59.19 ± 0.70^{ab}	60.26 ± 1.15^{ab}	137.23	0.00
MDA (mmol/mg)	1.82 ± 0.70	5.07 ± 0.22^{a}	5.66 ± 0.44^{ab}	5.57 ± 0.41^{ab}	128.88	0.00
Uric Acid (µmol/L)	74.25 ± 18.01	59.00 ± 6.02	45.01 ± 7.00^{a}	30.06 ± 6.24^{ab}	12.36	0.00
Legend: GRT= Glutathione Reductase; SOD = Superoxide Dismutase; CAT= Catalase; MDA = Malondialdehyde.						
Symbols- a: p<0.05 vs. control, b: p<0.05 vs. Day 10, c: p<0.05 vs. Day 20.						

Table 4: A multiple comparison of vitreous oxidative stress biomarkers on the basis of duration of chronic CO intoxication

Parameters	Control	Duration of CO Exposure			f-value	p-value
		Day 10	Day 20	Day 30		
GRT (µ/mg)	21.98 ± 1.06	21.85 ± 1.07	23.11 ± 1.55	23.95 ± 0.85^{ab}	2.93	0.08
SOD (µ/mg)	5.81 ± 0.23	5.60 ± 0.21	5.89 ± 0.11^{ab}	5.63 ± 0.16	4.8	0.02
CAT (µ/mg)	62.99 ± 2.17	57.82 ± 1.21^{a}	54.43 ± 0.99^{ab}	57.10 ± 4.25^{ab}	23.49	0.00
MDA (mmol/mg)	2.44 ± 0.22	5.43 ± 0.31^{a}	5.58 ± 0.28^{a}	5.68 ± 0.38^{a}	107.46	0.00
Uric Acid (µmol/L)	56.50 ± 5.80	55.00 ± 7.79	44.00 ± 7.44^{ab}	27.50 ± 4.36^{abc}	16.99	0.00
Symbols- a: p<0.05 vs. control, b: p<0.05 vs. Day 10, c: p<0.05 vs. Day 20.						

It is an established fact that any phenomenon that could distort normal utilization of glucose usually lead to an increase in triacylglycerol as an alternative source of energy. Increase in serum TG is a medical fact of the pathophysiology of diabetes mellitus [28]. This could be due to the inhibition of glycogeneolysis pathway by CO. Alternatively; the increase could be due to the propensity of chronic CO intoxication to causing diabetes mellitus.

The results of the chronic study further showed explicitly both in the serum and the vitreous that oxidative stress processes are exacerbated by carbon monoxide intoxication **(Tables 3 and 4).** The result of this study showed a significant increase (p<0.05) in concentration of malondialdehyde (MDA) both in the serum and vitreous as the duration of CO exposures increased. The production of this aldehyde is used as a biomarker to assess the extent of oxidative stress in an organism [29]. The observed increase in MDA concentrations could be due to the massive production of ROS due to the inhalation of CO. This finding has further reaffirmed that one of the mechanisms utilized by CO in propagating its deleterious effect is through oxidative stress. Similar findings have been reported by Muhammad and Fredrick, and Ismail though the severity of exposure could not be ascertained [30]. On the contrary, it contradicted the reports of Thom et al. and Guan et al. [31,32].

Superoxide dismutase (SOD) is an important indicator in assessing the level of oxidative stress. The decrease in concentrations of SOD both in the serum and vitreous humour observed in this study showed that the production of ROS is higher than the compensatory roles of SOD. The result of the study concurred with those of Ismail et al. and Patel et al. which reported a decrease in concentration of SOD in comparison to the control with a significant negative correlation with COHb concentration [9,33]. However, the findings of this study disagreed with that of Piantaosi et al., Hamed et al. and Kavakli et al. [7,34,35].

Furthermore, catalase activities exhibited a significant increase in serum and a decrease in the vitreous. The abnormality observed could be due to compartmental difference, accessibility or portal of entry difference.

Accessibility in pharmacology is crucial in determining dosages of drugs. Carbon monoxide is a volatile gas and could cross lipid bilayers without much hindrance. Also, the difference could be due to the magnitude of free radical generated and the adequacy of catalase presence.

Glutathione concentration decreased in the serum and increased in the vitreous. The increase as observed in the vitreous could be attributed to the abundance of glutathione in the eye. Glutathione is utilized in vision and other photo-actions in eye and also crucial in oxidative stress prevention [9,36]. The increase is due to actions to counteract the debilitating roles of ROS in the eye. Also, the decrease as seen in the serum could be due to the insufficiency of glutathione in the blood to counteract ROS produced by the CO.

The decrease in concentrations of serum and vitreous uric acid could be attributive to the toxicity potentials of CO on one hand and the other hand the anti-oxidant attribute of uric acid. Uric acid formation is an enzymatic process which involves arrays of enzymes. The step wise process yields uric acids at the end of the pathway. The product is the target in therapeutics and toxicology. Sevelamer and similar drugs for the management of renal dysfunction, arthritis and gout management are designed to inhibit uric acid production [37]. The decrease observed could be as a result of the inhibition of one of the enzymes in the uric acid pathway. Alternatively, the decrease could also be seen from the perspective of uric acid activity as an anti-oxidant. Uric acid is a known marker of oxidative stress and may have a potential therapeutic role as an antioxidant [38,39]. The decrease could be due to utilization of uric acid in mopping up free radicals generated from the ravaging CO concentrations. The findings of this study disagreed with that of Baillie et al. that stated that exposures to acute CO resulted to hyperuricemia, though in high concentration [40].

Study Limitations

This study had some limitations. The most important handicap is the impossibility and the ethical implication of using humans as research subjects. Others are pausitiy of funds and lack research articles in the area of vitreous chemistry of carbon monoxide intoxication.

Conclusion

The biochemical parameters studied are indicative of oxidative stress which is defined based on the level of lipid distortion by free radicals. The findings of the study showed that chronic CO intoxication could provoke lipid peroxidation phenomenon which is a hallmark of a lot of chronic and acute diseases. Hence, lipid peroxidation could serve as a hallmark of chronic CO intoxication and diseases linked to it.

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