Original Paper

Co-induction of Regenerative DNA, Proteins and Glucose-6-phosphatase Activity after Treatment of Partially Hepatectomized Rats with Nigerian Light Crude Oil

Ibiba F Oruambo

ABSTRACT

The effect(s) of two doses of Light Crude Oil (LCO) on the concentrations of regenerative DNA, total protein and glucose-6-phosphatase activity, as molecular indices of potential carcinogenicity was determined in liver homogenates of partially-hepatectomized and non-hepatectomized (normal) rat liver.

Rats were treated with intraperitoneal injection at six hours, and sacrificed twenty-four hours post-partial hepatectomy (pph); control rats were partially hepatectomized but not treated; while reference rats (with normal liver) were non-hepatectomized and not treated.

Regenerative DNA was partially purified from liver homogenates and quantified by the diphenylamine method; total protein concentration was determined directly in the homogenates by the Biuret method; and glucose-6-phosphatase activity by a modification of the Fiske-Subbarow method.

Results showed a 21.5% increase in glucose-6-phosphatase activity in partially-hepatectomized rat liver over non-hepatectomized controls, 59.3% and 9.8% increases in total homogenate protein concentration at 2.5 and 5.0ml/kg body weight (bw) LCO respectively; 68.2% and 46.0% increases in glucose-6-phosphatase activity at 2.5 and 5.0 ml/kg bw over the control, respectively. Increases in partially-purified regenerative DNA concentrations also occurred at 13.7% and 20.5% over the controls at 2.5 and 5.0 ml/kg bw, respectively. Nigerian light crude oil (LCO) apparently induced increases in both regenerative DNA and protein syntheses at the first wave of synthesis (24hrs.pph) at the two dose levels tested, while also inducing increases in the bio-transformation by, or perhaps synthesis of, microsomes (cytochrome P450 and 448 detoxification enzymes) as judged by the increased level of the marker enzyme glucose-6-phosphatase.

These results may shed more light on the probable molecular mechanism of LCO's potential carcinogenicity and/or toxicity.

Key Words: Partial-hepatectomy; Regenerative-DNA; Light crude oil; Glucose-6-phosphatase

Introduction

Light Crude Oil (LCO) is the major foreign exchange earner for Nigeria and is exclusively produced in the Niger-Delta region. Its popularity and preference abroad especially in the United States and Europe is due in part to its near-zero sulfur content ('sweet') which complies with environmental pollution legislation as regards the need for zero sulfur petrol; and its high content of the low molecular weight aliphatic hydrocarbons ('light': 20/80 ratio of polyaromatic hydrocarbons - the 'heavy fraction' to aliphatic hydrocarbons - 'light fraction').

Oil spillages, mostly of LCO, are chronic in the Niger-Delta region of Nigeria which constantly threaten aerial, terrestrial and aquatic life, and human-health.[1] The toxic effects of various geological crude oils have been reported widely in laboratory animals; for example, the oral administration of a crude oil at 5.0ml/kg body weight daily for two days to male Charles River CD-1 mice resulted in increases in liver weight, hepatic proteins, RNA, glycogen, total lipids, cholesterol, triglycerides and phospho-

Dept. of Chemistry, Rivers State University of Science and Technology, Port Harcourt, Nigeria

lipids; also a 15-20 fold increase in hepatic ornithine decarboxylase activity at 12hrs, and a 34-fold increase in hepatic putrescine levels over those in controls following the intraperitoneal administration of 4ml/kg bw crude oil.[2] Potential carcinogenic polyaromatic compounds, such as benzo(a)pyrene have been identified in crude oils, and these induce microsomal enzyme activities for detoxification.[3] In spite of these, LCO, as a geological crude, has not been adequately studied. Recently, the pattern of the urinary excretion of nickel in guinea pigs treated with a single dose (5.0ml/kg bw) of LCO by skin application was shown to be duration-dependent, as the highest urinary nickel level occurred at two days and returned to control level at 16 days.[4]

Based therefore on the potential of LCO to be carcinogenic and/or genotoxic as a Nigerian geological crude, we carried-out this pioneer study. The study describes the effects of two varying doses of LCO on total concentrations of regenerative DNA and proteins; and on glucose-6-phosphatase (microsomal marker-enzyme) activity in rats treated six hours post partial hepatectomy (pre-replicative phase), and sacrificed twenty-four hours post-partial hepatectomy (pph), the maximum synthetic phase. The potential implication as bio-markers of probable genotoxicity and/or carcinogenicity is discussed.

Materials and Methods

Fresh light crude oil (LCO) was obtained from the Nigerian National Petroleum Company (NNPC) in Port Harcourt, Rivers State, Nigeria, and brought to the laboratory in an amber container.

Treatment of Animals: Fifteen (15) male adult albino rats were grouped into three of five (5) rats per group: Group A received LCO at a dose of 2.5 ml/kg body weight (bw); Group B, 5.0ml/kg bw; and Group C were the untreated controls. Treatment was by intraperitoneal (i.p) injection. All fifteen rats were individually anaesthetized with diethyl ether and partially-hepatectomized.[5]

A separate group of three rats served as the non-partially hepatectomized and untreated reference, i.e., normal rat liver. Groups A and B rats were treated with LCO at the appropriate doses 6 hours post-partial hepatectomy (pph), while Group C control rats were partially-hepatectomized, but not treated.

Twenty-four (24) hrs pph, all fifteen rats were sacrificed, their livers excised, pooled by Group, and 10% homogenates prepared in 0.05M potassium phosphate buffer, pH7.4; 0.01% EDTA. Total protein contents and glucose-6-phosphatase activity were determined in all the

Table 1
Dose-related induction of total regenerative proteins and glucose-6-phosphatase activity in regenerating rat liver homogenates by administration of Light Crude oil (LCO) at 2.5 and 5.0ml/kg b/w

Dose *(ml/kg bw)*	*Treatment *(i.p.)*	Total Protein	% change *(mg/ml)*	Enzyme Activity *(mmol Pi x ml^{-1} x min^{-1})(10^{-12})*	% change
Nil	(Normal) Reference	N.D.	–	1.3	
Nil	Control (pph-not treated)	115.2	–	4.1	+215
2.5	LCO		183.6	+59.3	6.9
+68.2					
5.0	LCO		126.6	+9.8	6.0
+46.0					

N.D. - Not determined

** Control (Group C) consisted of five (5) untreated adult male albino rats whose livers were pooled following sacrifice at 24 hrs, pph. Liver homogenates were prepared and total protein concentration and glucose-6-phosphatase activity were measured by the Biuret and Fiske-Subbarow methods, respectively, as described under Materials and Methods. Normal or Reference consisted of three untreated and non-partially hepatectomized rats whose livers were similarly pooled, homogenates prepared and enzyme activity determined as described. Treated rats of Groups A and B consisted of 5 male rats per group; Group A received 2.5 and Group B 5.0ml/kg bw LCO six hours pph, and sacrificed 24hrs pph. Their livers were similarly pooled by Group and homogenates prepared as already described. In the homogenates of all rats (Groups A, B and C) regenerative DNA was extracted, partially purified and quantified by the diphenylamine reaction, as described elsewhere.*

homogenates (Groups A, B, C and Reference) by the Biuret method,[6] and by a slight modification of the Fiske-Subbarow method for the estimation of inorganic phosphate, respectively.[5] Partial purification and quantification of DNA by the phenol: chlorofom extraction: cold ethanol precipitation, and the diphenylamine reaction methods, respectively, followed immediately.[7]

Preparation of Liver Homogenates: Homogenates were prepared from rat liver as per standard procedure.[6]
Enzyme Assay: Glucose-6-phosphatase (EC3.1.3.9) activity in rat liver homogenates was assayed colourimetrically by visible absorption spectrophotometry.[5] Enzyme activity was expressed as mmol inorganic phosphate (Pi) released x ml^{-1} x min^{-1} in the presence of a constant enzyme (protein) concentration of 10mg/ml. Protein content in liver homogenate was determined by the Biuret method.

Extraction and Partial Purification of Total Regenerative DNA: Total regenerative DNA was extracted from the rat liver homogenates and partially-purified by the phenol: chloroform: cold ethanol precipitation method, and quantified by the diphenylamine reaction method.[7]

Results

The results of the study are displayed in **Tables 1** and **2**.

Table 2
Dose-related induction of total (partially-purified) regenerative DNA concentration in regenerating rat liver by administration of Light Crude Oil (LCO) at 2.5 and 5.0 m1/kg bw

Dose *(ml/kg bw)*	Treatment *(i.p.)*	Total Protein *(mg/ml)*	% change
Nil	Control (pph-not treated)	0.635	
2.5	LCO	0.722	+13.7
5.0	LCO	0.765	+20.5

Discussion

As Table 1 shows, induced increases in total protein content and glucose-6-phosphatase activity occurred at both dose levels, a dose-response of 59.3% and 68.2%, respectively over untreated controls at 2.5ml/kg bw; and 9.8% and 46.0% respectively over untreated at 5.0ml/kg bw.

Significantly, liver glucose-6-phosphatase activity increased markedly at 215% in partially-hepatectomized (ph) rats not treated with LCO over normal rat liver, suggesting the accelerated ph-induced activation of unscheduled biosynthesis of microsomal proteins, or of bio-transformation activity associated with detoxification.

Table 3
Comparative analysis of dose-related co-induction of total regenerative homogenate proteins, homogenate glucose-6-phosphatase activity and total regenerative (partially-purified) DNA concentration in regenerating rat liver by administration of Light Crude Oil (LCO) at 2.5 and 5.0 ml/kg bw

Dose *(ml/kg bw)*	Treatment *(i.p.)*	Total Protein *(mg/ml)*	% change	Enzyme Activity *(mmol Pi x ml^{-1}x min^{-1}) ($x10^{-12}$)*	% change	Total DNA *(mg/ml)*	% change
Nil	Normal Rat Liver	N.D.	–	1.3		N.D.	–
Nil	Control (pph-not treated-)	115.2	–	4.1	+215	0.635	–
2.5	LCO	183.6	+59.3	6.9	+68.2	0.722	+13.7
5.0	LCO	126.6	+9.8	6.0	+46.0	0.765	+20.5

N.D. - Not determined

Similarly, the significant increases in the levels of this marker-enzyme activity in ph rats treated with LCO, at both dose levels suggest the probable induction of the synthesis of microsomal proteins which contain the cytochrome P-450 and 448-dependent mixed-function oxidases by LCO because glucose-6-phosphatase is a microsomal marker-enzyme. This in turn may result in a concomitant increase in the biotransformation of LCO components, most likely the polyaromtic hydrocarbons, to reactive metabolites. This agrees with the findings by others that other geological crude oil increased several-fold the levels of various enzyme activities, most notably detoxification enzymes and hepatic proteins and RNA.[2,3] In Table 2, a clearer pattern of the induction effect of LCO is shown. Here the two doses (2.5 and 5.0m1/kg bw) induce moderate increases in total regenerative DNA contents in a classic dose-response manner; increases occurred at 2.5m1/kg bw of 13.7% over the partially-hepatectomized but untreated controls, and increased further (20.5%) at the higher dose of 5.0ml/kg bw, over the controls, although the increase is not proportional. This suggests strongly that the reactive metabolites from induced microsomal enzyme activities may preferentially interact at control sites thereby modulating regulatory sequences of DNA replication, inducing the accelerated synthesis of DNA. These events may have resulted in the significantly increased concentration of total regenerative DNA we obtained. This interpretation is plausible, though not conclusive, because carcinogenic polyaromatic hydrocarbons contained in crude oil such as benzo(a)pyrene (including LCO) are known to bind covalently to DNA following in vitro microsomal activation.[6,7]

Table 3 shows the comparative analysis of the dose-related increases (induction) in total regenerative homogenate proteins, homogenate glucose-6-phosphatase activity and partially-purified regenerative DNA concentrations by the intraperitoneal administration of Light Crude Oil to adult male albino rats at 2.5 and 5.0 ml/kg bw.

In essence, all three parameters were co-induced to differing extents in rats treated 6hrs pph (pre-replicative phase) with 2.5 ml/kg bw and 5.0 ml/kg bw of LCO, and sacrificed 24 hours post-partial hepatectomy (maximum synthetic phase). The differences in extent of induction of proteins, glucose-6-phosphatase activity and regenerative DNA may suggest differences in the mechanism or pathway of induction/activation of DNA and microsome syntheses. The implications for geno-toxicity, such as carcinogenicity, or hepato-toxicity from these results are not known.

Acknowledgement

The author wishes to acknowledge the technical contributions of Mr. Amadi, Ikenna and Mr. Oguara, Jeptha of the Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt, Rivers State, Nigeria.

These experiments comply with the laws and regulations of the Federal Republic of Nigeria regarding Animal Care, Use and Handling.

REFERENCES

1. Akpofure EA, Efere ML, Ayawei P. Oil spillage in Nigerian Niger Delta: Integrated Grassroots Post Impact Assessment of Acute Damaging Effects of Continuous Oil Spills in Niger Delta. Psycho-morphological and Empirical overview. (1998-2000).
2. Khan S, Irfan M, Rahimtula AD. The hepatotoxic potential of a Prudhoe Bay Crude Oil: effect on mouse liver weight and composition. Toxicology. 1987; 46: 95-105.
3. International Agency for Research on Cancer (IARC). Occupational exposures in petroleum refining: Crude oil and major petroleum fuels. 1987; 45: 119-126.
4. Oruambo IF. Urinary excretion of nickel after dermal application of Nigerian Light Crude Oil. J Environ Pathol Toxicol Oncol. 2004; 23(4): 303-306.
5. Oruambo IF. Alteration of glucose-6-phosphatase activity, and regenerative, and total DNA concentrations in regenerating and normal rat liver of aqueous extracts of two Nigerian Plants. J Environ Pathol Toxicol Oncol. 1989; 9(2): 191-199.
6. Banerjee S, Van Duuren BL, Oruambo, IF. Microsome-mediated covalent binding of 1,2 dichloroethane to lung microsomal protein and Salmon sperm DNA. Cancer Res. 1980; 40: 2170-2173.
7. Oruambo IF, Van Duuren BL. Evidence for the covalent interaction of carbon tetrachloride with mouse liver chromatin DNA in vitro. J Environ Pathol Toxicol Oncol. 1989; 9: 13-19.