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CO-INDUCTION OF REGENERATIVE DNA, PROTEINS AND GLUCOSE-6-PHOSPHATASE ACTIVITY AFTER TREATMENT OF PARTIALLY- HEPATECTOMIZED RATS WITH BONNY LIGHT CRUDE OIL

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ABSTRACT

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

Rats were treated by intraperitoneal injection six hours post partial-hepatectomy (pph), and sacrificed twenty-four hours pph. Control rats were partially hepatectomized but not treated; while reference rats (normal rat liver) were non-hepatectomized and not treated. Regenerative DNA (DNA synthesized de novo in partially-hepatectomized and regenerating rat liver) was partially purified from liver homogenates. Results show 59.3% and 9.8% increases in total homogenate protein concentration at 2.5 and 5.0ml/kg body weight (bw) BLCO respectively; and 68.2% and 46.0% increases in glucose-6-phosphatase activity at 2.5 and 5.0 ml/kg bw over the control, (4.1 μmol.Pi/ml/min.) respectively. Increases in partially-purified regenerative DNA concentrations also occurred of 13.7% and 20.5% over the controls at 2.5 and 5.0 ml/kg bw, respectively. Nigerian light crude oil (BLCO) induced increases in the concentrations of both regenerative DNA and total protein at the first wave of DNA synthesis (24hrs.pph) at the two dose levels tested, while it (BLCO) probably also increased microsomal (bio-transformation) activity or microsome synthesis as judged by the increased level of glucose-6-phosphatase activity.

These results may shed more light on the probable molecular mechanism(s) of BLCO's potential hepatotoxicity and/or geno-toxicity.

KEY WORDS: Partial-hepatectomy; regenerative-DNA; Bonny Light Crude Oil; Glucose-6-phosphatase.

INTRODUCTION

Bonny Light Crude Oil (BLCO) 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'; NNPC-personal communication).

The toxic effects of various geological crude oils have been reported widely in laboratory animals; for example, the oral administration of Prudhoe Bay 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 phospholipids (Khan, et al 1987); also Rahimtula et al (1987) reported 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 (Khan, et al 1987). Potential carcinogenic polyaromatic compounds, such as benzo(a)pyrene have been identified in crude oils (IARC, 1987) and these induce microsomal enzyme activities for detoxification (IARC, 1987). In spite of these, BLCO, 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 BLCO by skin application was shown to be time-dependent, as the highest urinary nickel level occurred at two days and returned to control level at 16 days (Oruambo, 2004).

Based therefore on the potential of BLCO to be hepatotoxic and/or genotoxic as a Nigerian geological crude, the study was designed to assess the effects of two varying doses of BLCO 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.

MATERIALS AND METHODS

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

Treatment of Animals: Fifteen (15) male adult albino rats weighing 200-250gm (0.2 –.25kg), were grouped into three of five (5) rats per group and kept in metabolic cages in the Animal Room of the laboratory. Group A received BLCO 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, two of the five lobes of liver were surgically excised (two-fifth partial hepatectomy), and the rats sutured and returned to the cage, as reported elsewhere (Oruambo, 1989).

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 BLCO 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%

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EDTA. Total protein contents and glucose-6-phosphatase activity were determined in all the homogenates (Groups A,B,C and Reference) by the Biuret method (Banerjee, et al. 1980) and by a slight modification of the Fiske-Subbarow method for the estimation of inorganic phosphate, respectively (Oruambo, 1989). Partial purification and quantification of DNA by the phenol: chloroform extraction: cold ethanol precipitation, and the diphenylamine reaction methods, respectively followed immediately as described elsewhere (Oruambo, et al 1989).

Preparation of Liver Homogenates: Homogenates were prepared from rat liver as described elsewhere (Banerjee, et al. 1980).

Enzyme Assay: Glucose-6-phosphatase (EC3.1.3.9) activity in rat liver homogenates was assayed colorimetrically by visible absorption spectrophotometry as also described in detail elsewhere (Oruambo, 1989). Enzyme activity is expressed as μmol inorganic phosphate (Pi) released x ml^{-1} x min^{-1} in the presence of a constant enzyme (protein) concentration of 10μg/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 as described in detail elsewhere (Oruambo, et al. 1989).

RESULTS AND DISCUSSION

Table 1: Effect of administration of 2.5 and 5.0 ml/kg bw BLCO on the induction of total regenerative proteins and glucose-6-phosphatase activity in regenerating rat liver homogenates.

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

ND- Not determined

Table 1 results show induced increases in total protein content and glucose-6-phosphatase activity which occurred at both dose levels, a dose-response, 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 response to partial-hepatectomy (ph) in rats not treated with BLCO over normal rat liver. This suggests a probable ph-induced activation of unscheduled biosynthesis of microsomal (endoplasmic recticulum) proteins or of bio-transformation activity associated with detoxification.

Similarly, the significant increases in the levels of this marker-enzyme activity in ph rats treated with BLCO, 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 BLCO because glucose-6-phosphatase is a endoplasmic recticulum (microsomal) membrane-bound marker-enzyme. This in turn may result in a concomitant increase in the biotransformation of BLCO components most likely the polyaromatic 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 (Khan, et al 1987, IARC, 1989).

Table 2: Effect of administration of 2.5 and 5.0 ml/kg bw BLCO on induction of total (partially-purified) regenerative DNA concentration in regenerating rat liver.

Dose (ml/kg bw)	Treatment (i.p.)	mg/ml	% change
0	Control (pph-not treated)	0.635	
2.5	BLCO	0.722	+13.7
5.0	BLCO	0.765	+20.5

In Table 2, a clearer pattern of the induction effect of BLCO 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 preclusive, because carcinogenic polyaromatic hydrocarbons contained in crude oil (including BLCO) such as benzo(a)pyrene, are known to bind covalently to DNA following in vitro microsomal activation (Banerjee, et al. 1980; Oruambo, et al. 1989).

Table 3: Comparative analysis of dose-related co-induction of total regenerative proteins, glucose-6-phosphatase activity and total regenerative (partially-purified) DNA concentration in regenerating rat liver.

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

ND = Not determined

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 Bonny 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 BLCO, 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 probable implications for geno-toxicity, such as carcinogenicity, or hepato-toxicity from these results are suggested.

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