

Dose-related Increased Binding of Nickel to Chromatin Proteins; and Changes to DNA Concentration in the Liver of Guinea Pigs Treated with Nigerian Light Crude Oil

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Abstract: The alteration in nuclear DNA concentration and the concomitant binding of xenobiotics (alkylating agents, heavy metals, etc.) to chromatin constituents may adversely affect gene structure and/or function, and thus initiate carcinogenesis. Binding of nickel to chromatin DNA has been reported to cause DNA damage (cross-links, single-strand breaks), and although many soluble nickel compounds and complexes have been shown to bind to chromatin, porphyrin-complexed nickel (PCN) in crude oils has not been studied. We have determined the dose-related increases in total and chromatin DNA concentrations, and the differential distribution (binding) of PCN (crude oil nickel-CON) to chromatin constituents in livers of adult male guinea pigs treated with 1.25, 2.50 and 5.0 ml/kg bw Nigerian Bonny light crude oil (BLCO) by intraperitoneal injection. The results showed large BLCO-induced increases in total DNA concentrations of 424%, 632% and 436% at 1.25, 2.50 and 5.0 ml/kg bw BLCO respectively over the untreated controls; while it induced equally large increases in chromatin DNA concentrations of 585% and 200% at 2.50 and 5.0 ml/kg bw respectively. In both cases, maximum increases occurred at 2.50 ml/kg bw BLCO. The distribution of PCN in BLCO between chromatin DNA and chromatin proteins (histones and non-histones) showed that at 2.50 and 5.0 ml/kg bw BLCO, nickel content in chromatin DNA reduced by 25% and 12.5% respectively over the controls; while its content in chromatin proteins also reduced by 26%; but increased by 166% at 2.50 and 5.0 ml/kg bw BLCO, respectively over the untreated controls. However, in intra-chromatin comparison, 38.8% more PCN bound to chromatin DNA than to chromatin proteins at 2.50 ml/kg bw; but at 5.0 ml/kg bw BLCO, 90.4% more PCN bound to chromatin proteins than to chromatin DNA. These results show a greater affinity of PCN in BLCO for chromatin proteins over chromatin DNA which may have played a role in the increased DNA concentrations. Also, the results may add critical information to understanding the reactions of porphyrin-complexed nickel in crude oils with chromatin since this has not been studied before. Furthermore, the probable carcinogenicity of BLCO may be implied.

Keywords: Porphyrin-complexed nickel; distribution; hepatic chromatin-DNA, proteins; BLCO.

Introduction

Crude oils are complex mixtures of a vast number of individual chemical compounds, the bulk of which are hydrocarbons [1]. However, all crude oils also contain traces of characteristic metallic compounds such as nickel and vanadium at levels ranging from a few parts per million (ppm) to 200 ppm nickel, and up to 1200 ppm vanadium which occur primarily as stable porphyrin

complexes [1]. The Nigerian light crude oil commonly referred to as 'Bonny Light Crude Oil' (BLCO) has been described to contain up to 4.0 ppm nickel [2].

The induction effects of other geological crude oils on the concentrations of cellular macromolecules such as nucleic acids, have been reported: oral administration of Prudhoe Bay crude oil at 5ml/kg bw daily for two days to male Charles River CD-1 mice, for instance, was reported to result in increases in hepatic proteins, RNA, glycogen

and total lipids [1]. However, no effect on total DNA concentration was mentioned and no such study on the effect of BLCO has been reported.

Similarly, the role of nickel contained in crude oils-porphyrin complexes[1] in potentiating crude oil carcinogenicity has not been explored; and this would be of valuable interest in the Niger-Delta region of Nigeria where all of Nigeria's light crude oil (BLCO) is produced, resulting in chronic oil spillages which threaten both human-health and the environment.

There is a wealth of data in the literature on the interaction of nickel in other nickel compounds (not porphyrin-complexed nickel – PCN) with the genetic substance (Chromatin DNA and associated proteins), and consequent DNA damage, that is single strand breaks, cross-linking, etc. events that potentiate carcinogenesis. No such data exist on BLCO-nickel.

For instance, in nickel-binding studies of various nickel compounds and complexes, nickel chloride was shown to bind, in a dose-related manner to chromatin, polynucleosomes and DNA [3]. However, in another study, nickel incorporated from nickel chloride was shown to have a higher affinity for cellular proteins than for DNA or RNA [4]. In the nuclear chromatin, nickel was found to associate with both the DNA and histone and non-histone chromosomal proteins; but following the intraperitoneal injection of nickel carbonate to male Sprague-Dawley rats, significant differences were found in the distribution of nickel between nucleic acids and associated proteins in DNA samples extracted from kidney and liver [3]; nickel was shown to associated with both kidney and liver nuclear DNA as early as three hours after injection and further increased by twenty hours [3]. In lung and liver of NMR1 mice, nickel was found to bind predominantly to a high-molecular weight protein [3], while in another study, a ternary nickel-protein-DNA complex more stable than binary nickel-DNA complex was identified [5]. DNA-protein cross-linking in the presence of nickel [II] - and nickel [III] – tetraglycine complexes and molecular oxygen was observed *in vitro* in calf-thymus nucleohistone; and the same complexes caused random polymerization of histones *in vivo* [6]. DNA-protein cross links and single strand breaks were found in rat kidney nuclei after intraperitoneal injection of nickel carbonate [5].

In this study, we aimed to determine whether; PCN in BLCO would interact/bind with chromatin DNA and/or associated proteins, on the one hand, and whether this interaction would be dose-related, on the other. Furthermore, we intended to determine whether the distribution of PCN in chromatin would be differential. We also aimed to measure the effect of varying doses of BLCO on total cellular DNA and chromatin DNA concentrations. Our results may be of interest to understanding the pattern of interaction of nickel when complexed in a porphyrin ring with DNA and proteins, specifically in chromatin, and may add critical information to the evaluation of the probable

carcinogenicity of light crude oils that contain low to moderate nickel as porphyrin complexes, such as BLCO.

Materials and Methods

All the experiments described in this article were carried out in compliance with the regulations of the Federal Republic of Nigeria guiding Animal Care and Use.

Bonny Light Crude Oil (BLCO)

Fresh BLCO was obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Rivers State, Nigeria in an amber container and brought to the laboratory. It was used two days later.

Treatment of Animals

A total of twenty adult male guinea-pigs weighing approximately 400 gm (0.4kg) each were housed in individual cages, and all were fed the same commercial rodent chow and given drinking water from the same source *ad libitum* throughout the duration of the experiment. Prior to treatment, all the animals were allowed a period of one week acclimatization in the Animal House of the laboratory. They were subsequently divided into four groups of five (5) animals per group. The first group received BLCO at a dose of 1.25 ml/kg bw each by intraperitoneal (i.p) injection; the second group received 2.5 ml/kg bw BLCO each also by i.p; the third group received BLCO at 5.0 ml/kg bw each also by i.p, while the fourth group of five animals served as the untreated controls.

All animals were sacrificed twenty-four hours after treatment; their livers were excised, pooled according to group and homogenized in either ice-cold 0.05M potassium phosphate buffer, pH 7.4 containing 0.2mM EGTA for total cellular DNA extraction or in 0.32M sucrose, 5mM MgCl₂ for nuclei isolation and subsequent chromatin preparation.

Isolation of Nuclei, Preparation and Fractionation of Chromatin

Nuclei were isolated from liver homogenates prepared in 0.32M sucrose, 5mM MgCl₂ as reported elsewhere [6]. Chromatin was immediately prepared from purified nuclei and characterized by protein/DNA and RNA/DNA ratios as similarly reported elsewhere [7]. DNA was isolated from chromatin, purified and the concentration was estimated by the diphenylamine method as described elsewhere [7]. Associated chromatin proteins were precipitated with excess methanol, centrifuged at 2000 rpm and washed once with distilled water-saturated ether and re-centrifuged. The protein pellet was collected and dissolved in 0.05M potassium phosphate buffer, pH 7.4.

Isolation of Total Cellular DNA

Total cellular DNA was isolated from the liver homogenate prepared in 0.05M potassium phosphate buffer pH 7.4 containing 0.2mM EGTA by a slight modification of the method of Maniatis, *et al* [8]. Briefly, the homogenate was first extracted once with buffer-equilibrated phenol and centrifuged at 2000 rpm for 5min. The aqueous phase (supernatant) was decanted to polyethylene tubes and the phenol and interface layers discarded. Next, the aqueous phase was extracted in a 1:1 (v/v) phenol:chloroform mixture for 10min. and centrifuged at 2000 rpm for 5 min; the aqueous phase was recovered as before and extracted once more with chloroform alone. Centrifugation was repeated and the aqueous phase re-collected and extracted with distilled water-saturated ether and centrifuged as before. The upper ether layer was discarded, and the bottom aqueous phase was collected and heated to 68°C for 5 min. to remove traces of ether. NaCl was added to the aqueous phase containing DNA to a final concentration of 0.1M and mixed well. Finally, two volumes of ice-cold absolute ethanol was added, mixed well and stored at 0°C for 18 hrs. DNA was subsequently recovered by high-speed centrifugation for 10 min. The pellet was dissolved in 1 ml 0.05M potassium phosphate buffer, pH 7.4; 0.2mM EGTA, heated to 37°C to aid dissolution of the DNA pellet and the concentration was estimated by the diphenylamine method [7].

Acid-digestion and Quantification of Ni²⁺

Chromatin DNA and associated proteins were separately acid-digested to free the total nickel bound to each macromolecule with a 3:1:1 (v/v/v) concentrated nitric: perchloric: concentrated sulfuric acids mixture, and quantified by the dimethylglyoxime reaction method as described elsewhere [2].

Statistical Analysis

Quantification assays for total cellular DNA, chromatin DNA and Ni²⁺ were carried out in two sets of triplicates each, and the arithmetic means with their standard deviations were calculated. Results are expressed as Means ± SDs.

The extent of change of each parameter over control is expressed as percentage increase or decrease.

Results

Table 1 shows dose-related large increases in total DNA and chromatin DNA concentrations at 1.25, 2.5 and 5.0ml/kg bw BLCO when compared to the untreated controls. At 1.25ml/kg bw, BLCO induced a significantly large (424%) increase in total DNA concentration, while at 2.5 and 5.0 ml/kg bw, it induced equally significantly large increases of 632% and 436%, respectively; and 585% and 200% increases in Chromatin DNA

concentrations, respectively. These across-board large increases would seem to agree with the findings of others on the induction effect of Prudhoe Bay crude oil on RNA concentration [1]. Furthermore, these increases are dose-related; the largest increases in both total and chromatin DNA concentrations occurred at 2.5 ml/kg bw BLCO, of 632% and 585% respectively.

Table 1: Total Cellular DNA and Chromatin DNA concentrations in Male Guinea Pigs Treated with 1.25 or 2.5 or 5.0 ml/kg bw Nigerian Bonny Light Crude Oil by Intra-peritoneal Injection.

Dose (ml/kg bw)	DNA Concentration (mg/ml)			
	Total Cellular DNA	Percent Increase	Chromatin DNA	Percent Increase
Nil (Control)	0.25 ± 0.11	-	0.21 ± 0.14	-
1.25	1.31 ± 0.56	424	Not Determined	-
2.50	1.83 ± 0.60	632	1.44 ± 0.78	585
5.0	1.34 ± 0.50	436	0.63 ± 0.12	200

In Table 2, a similar dose-related, albeit, zero distribution of PCN to chromatin DNA is shown. At the two doses tested (2.5 and 5.0 ml/kg bw BLCO) the nickel contents of chromatin DNA was lower than the untreated control. Indeed, at 2.5 ml/kg bw, there was a 25% decreased in Ni²⁺ content, while at 5.0 ml/kg bw, exactly half of that, a 12.5% decreased Ni²⁺ concentration was obtained in chromatin DNA. In essence, PCN in BLCO did not interact/bind with chromatin DNA at all under our experimental conditions.

Table 2: Nickel Concentration of Chromatin DNA in Male Guinea Pigs Treated with 2.5 ml/kg bw or 5.0 ml/kg bw Nigerian Bonny Light Crude Oil by Intraperitoneal Injection.

Dose (ml/kg bw)	Chromatin DNA nickel concentration (µg/ml)	Percent decrease
Control (Nil)	0.024 ± 0.007	-
2.5	0.018 ± 0.002	25
5.0	0.021 ± 0.008	12.5

Results in Table 3 however are different: PCN in BLCO seem to interact/bind substantially with proteins in chromatin and in a dose-related manner. Here, at the lower dose of 2.5 ml/kg bw BLCO, nickel concentration of chromatin proteins is actually 26% less than the untreated controls – a result near-identical with that of chromatin DNA. This suggests that PCN did not bind to

the proteins at all at this dose, when compared to the controls. However, at the higher dose of 5.0 ml/kg bw BLCO, nickel content of chromatin proteins increased markedly to 166% over the untreated controls, and 263% over the value obtained at 2.5 ml/kg bw. This pattern of the distribution of PCN to chromatin DNA and proteins in a dose-related manner seems to agree with the Ni²⁺ distribution pattern of soluble nickel compounds and complexes to chromatin, polynucleosomes and DNA as cited in introduction [3].

Table 3: Nickel Concentration of Chromatin Proteins in Male Guinea Pigs Treated with 2.5 ml/kg bw or 5.0 ml/kg bw Nigerian Bonny Light Crude Oil by Intraperitoneal Injection.

Dose (ml/kg bw)	Chromatin Proteins Nickel Concentration (µg/ml)	Percent change over control
Control (Nil)	0.015 ± 0.004	-
2.5	0.011 ± 0.005	- 26
5.0	0.040 ± 0.01	166

Table 4 shows the comparison of the dose-related differential interaction of PCN in BLCO with chromatin DNA and associated proteins, as measured by the Ni²⁺ content of each macromolecular type. As can be seen, compared to each other, at the lower dose of 2.5 ml/kg bw BLCO, the Ni²⁺ content of chromatin DNA is 38.8% higher than that of chromatin proteins; but at the higher dose of 5.0 ml/kg bw, the Ni²⁺ content of chromatin proteins is 90.4% higher than that of chromatin DNA. On balance therefore, PCN in BLCO seems to distribute predominantly more to chromatin proteins than to chromatin DNA.

Table 4: Comparison of the Dose-related Distribution of PCN between Chromatin DNA and Associated Proteins in the Livers of Male Guinea Pigs Treated with 2.5 ml/kg bw and 5.0 ml/kg bw BLCO by i.p.

Dose (ml/kg bw)	Ni ²⁺ Concentration (µg/ml)		Percent Difference
	Chromatin DNA	Chromatin Protein	
BLCO			
2.5	0.018 ± 0.002	0.011 ± 0.005	38.8% in favor of DNA
5.0	0.021 ± 0.008	0.040 ± 0.01	90.4% in favor of Proteins

Discussion

DNA concentrations, total and chromatin, in the livers of guinea-pigs treated by intraperitoneal injection with BLCO, increased in response to increasing doses of this crude oil, at 1.25, 2.50 and 5.0ml/kg bw, with peak increases occurring for both categories of DNA at the median dose of 2.5 ml/kg bw BLCO. This agrees in part with the findings of others [1], which showed that crude oil given orally to male Charles River CD-1 mice at 5.0 ml/kg bw induced increases in hepatic proteins and the nucleic acid, RNA among other indices. However, effect to DNA concentration was not reported. Both these findings suggest strongly that crude oils have the capacity to induce unscheduled DNA, RNA and protein syntheses, hallmarks of cell proliferation.

Our finding that porphyrin-complexed nickel (PCN) in BLCO interacted with chromatin DNA and associated proteins, albeit to differing extents, may be of interest. Other studies have reported varying degrees of binding of Ni²⁺ from soluble nickel compounds and complexes, notably nickel chloride, nickel carbonyl, nickel alloys and nickel carbamate to chromatin DNA and proteins [3,4,5,6], but none has reported the reactivity of PCN vis-à-vis binding of its Ni²⁺ to chromatin. Nickel is present in crude oils as a porphyrin complex [1]. The zero-binding of PCN to chromatin DNA at both 2.5 and 5.0 ml/kg bw when compared to the untreated controls, may suggest non-accessibility or affinity of PCN to the negatively-charged DNA phosphate moieties coupled with the structural nature of chromatin, in which DNA is known to be very tightly associated with histone proteins, which package and order DNA into nucleosomes [7].

Also of significance, is that histone cores in chromatin do not bind randomly to DNA, rather they are positioned such that they can play a role in the expression of some eukaryotic genes – more of a regulatory role. In this model therefore, the possible Ni²⁺ binding sites in DNA, the negatively charged phosphate groups, may not be easily accessible, where as nickel-complexing ligands, such as L-histidine in histones and, possibly also non-histone chromosomal proteins (NHCP) may be more accessible and available to Ni²⁺ from BLCO. In addition, we suspect that these protein ligands have higher affinities for Ni²⁺ and are more stable than the DNA phosphates, and therefore the enhanced binding of PCN to the chromatin associated proteins may be valid. One study however, reported enhanced binding of Ni²⁺ to nuclear DNA as early as three hours after injection to male Sprague-Dawley rats, with further increase by twenty hours; but the compound tested was said to be basic nickel carbonate [3]. Our findings were to the contrary in that PCN in BLCO did not distribute (bind) to chromatin DNA as Table 2 shows, when compared to the untreated controls.

PCN interaction with chromatin proteins on the other hand, was clearly different and dose-related. Maximal binding of Ni²⁺ to chromatin proteins, as measured by the total nickel content, was 166% higher than the controls at

5.0 ml/kg bw BLCO (Table 3), while Ni²⁺ content of chromatin DNA at this dose was essentially nil. However, when we compared the Ni²⁺ concentration of chromatin DNA with that of chromatin proteins after treatment of the guinea pigs with 2.5 and 5.0 ml/kg bw BLCO (Table 4), there was a moderately (38.8%) higher Ni²⁺ content in chromatin DNA than proteins at the lower dose of 2.5 ml/kg bw (0.018 and 0.011 µg/ml, respectively). On the contrary, at 5.0 ml/kg bw, Ni²⁺ content in chromatin proteins was a significant 90.4% higher than Ni²⁺ content in chromatin DNA (0.040 to 0.021 µg/ml, respectively). This may be due to increased amounts of PCN and increased availability of Ni²⁺ binding sites (ligands) in chromatin proteins.

In this study, in order to shed more light on the metabolism and probable toxicity and/or carcinogenicity of BLCO, we focused on two aspects: BLCO as a whole substance and its ability to alter nuclear (chromatin) DNA concentration and may be function; and PCN in BLCO as an individual entity capable of binding to chromatin proteins and possibly able to induce conformational changes which in turn may indirectly alter gene function; as cited earlier, Ni[II] and Ni[III] had been shown to induce DNA damage – cross-links and single strand breaks, when these bind directly to the nucleohistones. In both cases, we propose that these results in themselves may not be enough to evaluate the probable carcinogenicity of BLCO, but may add critical information to this effort.

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