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Biochemical changes in male albino rats following single exposure to crude oil and ciprofloxacin

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Abstract. Biochemical techniques were used to investigate the development of hepatotoxic effects caused by single exposure to crude oil (CO, 3.0 ml/kg) and ciprofloxacin (CFX, 200 mg/kg) in male rats. The results showed that hepatic P-450 content in crude oil exposed groups induced after 1 day to 4 fold and reached maximum 7.6 fold after 2 day compared with control and then fell to first day degree. CFX exposed group significantly inhibit the hepatic P-450 while in crude oil and CFX co-exposed group it induced but in lesser degree than that of crude oil alone. Inhibition of cytochrome P-450 has no effect on the activity of lipid peroxidation (LPO) while its activation latter was caused by elimination of the inhibitor from the body and delayed activation of cytochrome P-450. Single exposure of both crude oil and CFX lead to destructive changes in liver cells and inhibition of CYP-450 by ciprofloxacin could not protect against lipid peroxidation induced by crude oil.

Key words: Crude oil, Ciprofloxacin, CYP 450, Lipid peroxidation

Introduction

Kazakhstan has the second largest oil reserves among the former Soviet republics after Russia and therefore the problem of pollution of the territory by crude oil is one of the main ecological problems of Kazakhstan [1-3]. Crude oil derived from crude petroleum contains complex mixture of chemicals, varying widely in composition of hydrocarbons and hydrocarbon like chemicals [4, 5]. Crude oil also contains some trace elements like vanadium, nickel, iron, aluminium, copper, and some heavy metals like lead and cadmium [6].

Unfortunately many of compound of crude oil are highly toxic and cancer causing. The most hazardous components of crude oil are aromatic compounds as like benzene [7]. One of cause of toxicity of crude oil is the process of metabolism in which the metabolite of a compound is more toxic than the parent chemical. The first step in the metabolism of petroleum by vertebrates is oxidation, catalyzed by the cytochrome P-450 monooxygenase system. There are numerous isoforms of cytochrome P-450 which is induced by crude oil. Some isoforms of cytochrome P-450 binds and activates oxygen and the generated reactive oxygen species is inserted

into the petroleum hydrocarbon [8]. A number of studies found that aromatic hydrocarbons trigger the induction of cytochrome P-450 1A proteins via an intracellular aryl hydrocarbon receptor (Ah-R), predominantly found in liver but also in extra-hepatic tissues [9]. However, the role of this isoform of cytochrome P-450 in the development of toxic effects of the influence of oil is not sufficiently investigated and requires further research. So, the purpose of present study is to examine the hepatoxic effects caused by single exposure to crude oil and/or ciprofloxacin (CFX) in male albino rats and if the toxic effects of crude oil could be changes by ciprofloxacin, specific inhibitor of CYP-450.

Materials and methods

Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Total protein kits were obtained from Vital Diagnostics (Saint-Petersburg, Russia). Other chemicals were obtained from high commercial company (Almaty, Kazakhstan). Fresh crude oil was obtained from the oilfield Biikzhal (western Kazakhstan).

Adult male albino rats in the weight range

of 250–300 g used in this study were obtained from the Animal House, Faculty of Biology and Biotechnology – Almaty – Kazakhstan, and were acclimatized for 3 weeks before putting them into different treatments. Animals were randomly assigned into four groups of 20 animals each. Animals of group I served as control while animals of groups II, III, IV injected intraperitoneal once with (crude oil at a dose of 3.0 ml/kg bw, ciprofloxacin (CFX) at a dose of 200 mg/kg bw and crude oil + CFX at the same doses as in groups II and III for crude oil and CFX) respectively. CFX administration on the forth group was one hour after crude oil injection. Throughout the experiment, animals were housed in plastic cages placed in a well-ventilated rat house, provided with rat pellets (protein 21%, fat 6.78%, fiber 3.26%, salts and vitamins) and water *ad libitum*, and subjected to natural photoperiod of 12/12 h light–dark, constant temperature: 19–20 ℃. Four animals from each group after giving light ether anesthesia were sacrificed after 1, 2, 3, 5 and 8 days of treatments.

Blood samples were taken by puncturing the abdominal aorta of the animals after giving light ether anesthesia. The collected blood samples were kept at room temperature for 30 min and then were centrifuged at 2000 rpm for 10–15 min to separate the serum. Serum was used for the estimation of the liver marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Then the animals were sacrificed by exsanguination under light anesthesia. Liver was removed immediately for the estimations of enzymes activities and perfused with normal saline (0.9%, w/v) in order to take care of red blood cell contamination.

Biochemical analysis

Microsomes were isolated by differential centrifugation according to Schenkman and Cinti [10] with minor modifications. In brief; 1 g of frozen tissue was homogenized in 4 ml of ice-cold Tris-sucrose buffer (10 mM Tris-HCl, 0.25 M sucrose, pH 7.4). The homogenate was centrifuged at 13,000 × g for 10 min at 4°c and the precipitate was discarded. To the supernatant, calcium chloride was added to yield a final concentration of 10 mM. The solution was stirred for 15-20 min, and then centrifuged at 25,000 × g for 10 min at 4°c. The firmly packed pellets of microsomes were re-suspended by homogenization in 100 mM Tris-HCl buffer containing 20% w/v glycerol and 10 mM EDTA, pH 7.4. The microsomes were stored at -70 ℃ until use.

CYP-450 content was determined by the method of Omura and Sato [11]. Briefly, the microsomes were diluted in the ratio 1:9 with 0.1 M potassium phosphate buffer, pH 7.4, containing 0.5% Triton X-100, and 1 mM EDTA. The solution was stirred thoroughly, and divided into 2 tubes each containing 5 ml. The sample and reference cuvettes containing the microsomal preparations were saturated with 30 to 40 bubbles of carbon monoxide, at a rate of about 1 bubble/sec. Sodium dithionite was added only to the sample cuvette, to obtain a reduced carbon monoxide Vs oxidized carbon monoxide difference spectrum. An extinction coefficient of 106.1/mmol/cm was used for the determination of CYP-450 content nmol/mg [12].

Lipid peroxidation was determined as malondialdehyde (MDA) content in liver by the method of Burlakova *et al.* [13]. Briefly, portions of liver (250 mg) was homogenized in 2 ml of 0.1 M ice-cold potassium phosphate buffer (pH 7.4), then centrifuged at 6000 rpm for 40 min at 4 ℃. To 2 ml of the obtained supernatant 0.5 ml of 0.1M potassium phosphate buffer was added. Then the tube vigorously shakes immediately after adding 1 ml TCA (Trichloroacetic acid). All samples were centrifuged (15 min, 4000 rpm, 4 ℃) then the supernatant was separated and 1 ml TBA [(Thiobarbituric acid) (0.75 – 0.80 %)] was added to 2 ml of it and placed for 10-12 min in a boiling water bath. The content of MDA (nmol/g tissue) measured as the increase in absorbance at 532 nm.

Conjugated diene (CD) content in the rat liver was determined by the method of Burlakova *et al.* [13]. Briefly, portions of liver (250 mg) was homogenized in 2 ml of 0.1 M ice-cold potassium phosphate buffer (pH 7.4), then centrifuged at 6000 rpm for 40 min at 4 ℃. To 1 ml of the obtained supernatant 0.1 ml of 1.15% potassium chloride and 4.5 ml of isopropyl alcohol were added. Then the tube vigorously shakes for 3-4 min and centrifuged at 3000-4000 rpm for 15 min at 4 ℃. To obtained supernatant was added 4.5 ml heptane and shaken for 3-4 min after that the tubes left in dark place until a clear phase separation (at least 5 h). The content of conjugated diene (CD) nmol/mg tissues was measured in the heptanoic phase as the increase in absorbance at 233 nm.

Statistical analysis

All data expressed as mean ± SE and statistical analysis was made using the Statistical Package for Social Sciences (SPSS 18.0 software and Microsoft

Excel 2010). For tests, analysis of differences between groups consisted on a one-way analysis of variance (ANOVA) with repeated measures, followed by post-hoc comparisons (LSD test). Differences were considered statistically significant at $p < 0.05$ [14].

Results

The effect of single exposure to crude oil and/or CFX on CYP-450 in rats' liver is depicted in Fig. 1. Induction of hepatic P-450 content in crude oil exposed group was found to be significant ($p < 0.001$) from the first day of exposure by (298%) and reached maximal value by the second day by (658.9%) compared with the control group and then fell to the first day degree after 3, 5 and 8 days. Similarly hepatic P450 of the forth group animals (crude oil and CFX) was significantly ($p < 0.001$) induced after the first and second day by (109.8 and 222%) with the maximal increase after the second day compared with control but in lesser degree than that induced by crude oil alone while at the period between 3 and 8 days, the level of P-450 was sharply decreased to the control level. On the other hand the content of cytochrome in liver of the third group animals (CFX alone) significantly ($p < 0.05$) decreased after 1 and 2 days by (36 and 34%) respectively and then decreased insignificantly ($p > 0.05$) after 3 days by (15.4%) and finally recovered to the control level.
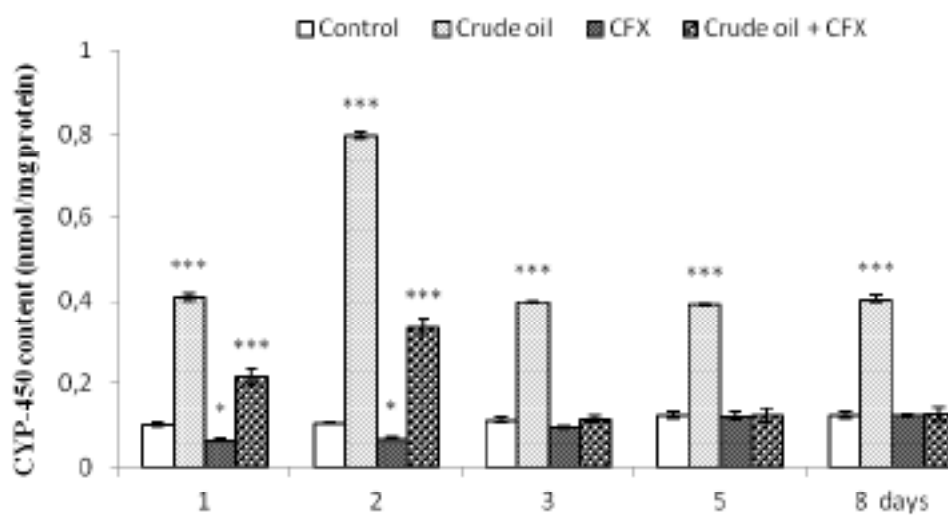

Figure 1. The changes of liver CYP-450 content in male albino rats at 1, 2, 3, 5 and 8 days. Data are represented as mean ± standard error (M ± S.E) of 4 rats. The mean difference is significant at the 0.05 level, * P < 0.05, *** P < 0.001.

Table 1

The changes in the activities of aminotransferases (ALT and AST) in the serum of rats exposed to crude oil and cipro-floxacin

Para-meter	ALT (Unit/l)					AST (Unit/l)				
	Exposure period (days)					*Exposure period (days)*				
Treatment	1	2	3	5	8	1	2	3	5	8
Control	0.726± 0.01	0.739± 0.02	0.727± 0.02	0.725± 0.01	0.717± 0.02	0.645± 0.04	0.652± 0.02	0.653± 0.02	0.641± 0.03	0.649± 0.02
Crude oil	1.111± 0.05***	0.831± 0.01*	0.733± 0.02	0.726± 0.03	0.716± 0.03	1.138± 0.05***	0.940± 0.02***	0.679± 0.01	0.645± 0.04	0.650± 0.03

| CFX | 0.954± 0.04*** | 0.829± 0.03* | 0.729± 0.01 | 0.729± 0.04 | 0.711± 0.02 | 1.045± 0.04*** | 0.880± 0.03*** | 0.665± 0.03 | 0.645± 0.03 | 0.640± 0.03 |
| CO + CFX | 1.181± 0.03*** | 0.795± 0.01 | 0.731± 0.04 | 0.722± 0.03 | 0.711± 0.03 | 1.204± 0.03*** | 0.893± 0.01*** | 0.679± 0.01 | 0.642± 0.03 | 0.645± 0.03 |

Data are represented as mean ± standard error (M ± S.E) of 4 rats. The mean difference is significant at the 0.05 level, * P < 0.05, *** P ≤ 0.001.

Table 2.

The changes in malondialdehyde (MDA) and conjugated diene (CD) contents in the liver of rats exposed to crude oil and ciprofloxacin

Para-meter / Treat-ment	MDA (nmol/g tissue)					CD (nmol/g tissue)				
	Exposure period (days)					Exposure period (days)				
	1	2	3	5	8	1	2	3	5	8
Control	1.361± 0.02	1.302± 0.03	1.306± 0.01	1.322± 0.01	1.322± 0.01	1.293± 0.04	1.219± 0.02	1.201± 0.02	1.194± 0.01	1.228± 0.01
Crude oil	1.413± 0.02	1.425± 0.02*	1.956± 0.05***	1.326± 0.02	1.495± 0.05**	1.817± 0.03***	1.963± 0.05**	2.225± 0.02***	2.340± 0.04***	2.338± 0.01***
CFX	1.365± 0.02	1.308± 0.03	1.310± 0.03	1.316± 0.02	1.308± 0.03	1.316± 0.01	1.223± 0.01	1.208± 0.02	1.191± 0.02	1.223± 0.02
CO + CFX	1.365± 0.02	1.860± 0.05***	1.302± 0.01	1.334± 0.02	1.653± 0.03***	1.693± 0.03***	2.297± 0.03**	2.375± 0.01***	1.209± 0.02	1.233± 0.03[†]

Data are represented as mean ± standard error (M ± S.E) of 4 rats. The mean difference is significant at the 0.05 level, * P ≤ 0.05, ** P ≤ 0.01, ***P ≤ 0.001.

The effect of single exposure to crude oil and/ or CFX on serum levels of ALT and AST in rats is depicted in Table 1. ALT and AST values were significantly increased in animals plasma of the second group after 1 and 2 days by (53.1, 12.5%, p<0.001 and 0.01) and (76.5, 44.2%, p<0.001) respectively while after 3, 5 and 8 days they fell to almost the control degree and the same changes were found in the third and fourth groups.

The effect of single exposure to crude oil and/ or CFX on conjugated diene content is depicted in Table 2. The content of lipid peroxidation products dienes and malondialdehyde in animals liver of the third group were insignificantly (p>0.05) different from the control. In animals liver of the second group, the content of dienes were increased significantly (p<0.001) at the all experimental days by (40.6, 61, 85.3, 96 and 90.4%) respectively. The increase in conjugated diene was time dependent.

Animals from fourth group had more unstable values as the contents of dienes were significantly (p<0.001) more than control after 1, 2 and 3 days by (30.9, 88.4 and 97.8%) respectively and then fell to almost the control level.

The effect of single exposure to crude oil and/ or CFX on malondialdehyde content is depicted in Table 2. In comparison with the control, the content of malondialdehyde (MDA) in animals liver from second group was significantly increased after 2 and 3 days by (9.5, 49.7%, p<0.05, 0.001) respectively then recovered to control level after 5 days and finally after 8 days, MDA significantly (p<0.05) increased again by (13.1%). Similarly in the liver of animals from the fourth group, the content of MDA increased significantly (p<0.001) for the first time after 2 days by (42.9%) then fell to control level after 3 and 5 days and finally, MDA content significantly (p<0.001) increased again after 8 day by (25%).

Discussion

The relationship between CYP isozymes and chemical carcinogenesis by polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene, has extensively been studied [15]. Cytochrome P450, a heme protein, is a heterogeneous system of microsomal enzymes responsible for the oxidative biotransformation of many chemicals (including drugs) to polar metabolites, thereby facilitating the pharmacological inactivation of these chemicals and their elimination from the body [16-18]. Cytochrome P450 exists in multiple forms, and the composition of these isoenzymes, as well as their relative concentrations in tissues, are influenced by treatment with different chemicals [17]. The duration and intensity of action of xenobiotics within a biological system are determined by the rate of their biotransformation to pharmacologically active or inactive metabolites. The compositional change in CYP isoforms has been observed in liver of rats exposed to high concentrations of hydrocarbon solvents (e.g. toluene, xylene, benzene) [19, 20]. Our results have shown that following intraperitoneal injection of crude oil (3 ml/kg bw) there was significant induction of hepatic P450 level from the first day and reached maximal value by second day compared with control and then fell to the first day degree. Induction of the hepatic P-450 was decreased when CFX co-administrated with crude oil. These results indicate that CFX probably acts as an inhibitor of CYP-450.

Exposure to petroleum-contaminated environment and the ingestion of petroleum-contaminated diet have been reported to stimulate the formation of lipid peroxidation products in animals [21, 22]. Lipid peroxidation that is a consequence of the activity of oxygen free radicals (e.g. superoxide anion, hydroxyl radical and alkyl-peroxyl radical) has been implicated as a mediator in oxidative stress in animals [23]. Oxidative stress is the term used to describe the condition of oxidative damage that results when the critical balance between free radical generation and antioxidant defenses are unfavorable [24, 25]. In the present study the contents of products of lipid peroxidation dienes and malondialdehyde in liver of animals treated with crude oil were increased. Dienes content was significantly increased from the first day and stayed stable till the end of experiment. Malondialdehyde content was significantly increased after 2 and 3 days then recovered to control level after 5 days and finally it increased again after 8 days. dienes and malondialdehyde production in the present study, suggested participation of free radical induced oxidative cell injury in mediating the toxicity of crude oil.

Ciprofloxacin (CFX) is an effective and relatively safe antimicrobial used in a variety of human infections. However, adverse drug reactions and positive results in genotoxic tests are reported, in the present study the contents of products of lipid peroxidation dienes and malondialdehyde in liver of animals treated with CFX were similar to that of the control. It is known that ciprofloxacin induces lipid peroxidation but only short time in condition and probably we did not register valid changes [26]. Oxidative stress in the kidney was observed by Weyers *et al.,* [27] after 15 min. of CFX administration at the dose of 10 mg/kg.

Conclusion

Thus, the results of biochemical studies revealed that single exposure of both crude oil and ciprofloxacin lead to destructive changes in liver cells. The inhibition of CYP1A by ciprofloxacin could not protect against lipid peroxidation induced by crude oil. Moreover we observed biphasic process of activation of lipid peroxidation with two peaks (2-3 days and 8 days). Probably second peak may be connecting with late activation of CYP-450 which was inhibited by ciprofloxacin and first peak likely induced another isoform of cytochrome.

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Шалахметова Т.М., Махмуд К.Е., Умбаев Б.А.
Биохимические изменения у белых крыс самцов после однократного воздействия сырой нефти и ципрофлоксацина

Биохимиялық әдістердің көмегімен еркек егеуқұйрықтарда шикі мұнайдың (CO 3,0 мл / кг) және ципрофлоксациннің (CFX, 200 мг / кг) бір рет әсер еткендегі гепатотоксикалық әсері зерттелді. Шикі мұнаймен әсер еткенде жануарлардың бауырындағы Р-450 цитохромы мөлшерінің, бақылаумен салыстырғанда, 1 күннен кейін 4,0 есе, ал 2 күннен кейін 7,6 есе жоғарылайтындығы, ал содан кейін алғашқы күнгі деңгейге дейін түсетіндігі анықталды. CFX Р-450 белсенділігін едәуір тежейді, ал шикі мұнай мен CFX әсері фермент белсенділігін индукциялайды, бірақ ол екеуінің біріккен әсері, шикі мұнайдың жеке әсерімен салыстырғанда, төмендеу болады. Р-450 цитохромы белсенділігінің тежелуі липидтердің (ПОЛ) тотығу процестеріне әсер етпейді, ал организмнен тежегіштерді алып тастаған кезде олардың белсенділігінің жоғарылауына және Р-450 цитохромы белсенділігінің кідіруіне алып келеді. Шикі мұнай және CFX-тің бір қайтара әсері бауыр клеткаларында деструктивті өзгерістерге әкеледі, нәтижесінде CYP-450-дің ципрофлоксацин көмегімен тежелуі шикі мұнай әсерінен болған липидтердің асқын тотығуынан қорғай алмайды.

Шалахметова Т.М., Махмуд К.Е., Умбаев Б.А.
Мұнай мен ципрофлоксацинмен бір рет уландырылған ақ егеуқұйрықтардағы биохимиялық өзгерістер

Гепатотоксические эффекты, вызванные однократным воздействием сырой нефти (CO, 3,0 мл / кг) и ципрофлоксацина (CFX, 200 мг / кг), были изучены у крыс-самцов с помощью биохимических методов. Результаты показали, что содержание печеночного цитохрома Р-450 индуцируется у животных, подвергнутых воздействию сырой нефти через 1 день в 4,0 раза, а через 2 дня – в 7,6 раза (максимум) по сравнению с контролем, а затем падает до уровня первых суток. CFX значительно ингибирует Р-450 в печени, тогда как воздействие сырой нефти и CFX индуцирует фермент, но в меньшей степени, чем только сырая нефть. Ингибирование цитохрома Р-450 не влияло на активность процессов перекисного окисления липидов (ПОЛ), а его активация позже была вызвана элиминацией ингибитора в организме и задержкой активации цитохрома Р-450. Однократное воздействие сырой нефти и CFX приводит к деструктивным изменениям в клетках печени, и ингибирование CYP-450 ципрофлоксацином не может защитить от перекисного окисления липидов, вызванного воздействием сырой нефтью.