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Impact of Omega 3 on the Genotoxicity of Irinotecan on Bone Marrow and Spleen of Rats: *in-vivo* Study

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Abstract

The present study is designed to explore the genotoxicity of irinotecan through measurement of mitotic index in the bone marrow and the spleen cells and to describe the protective actions of omega 3 against irinotecan induced genotoxicity in the bone marrow and the spleen of rats.

Twenty four (24) rats (Sprague-Dawley) of both sexes were randomly-divided into four groups: Group I, rats received a single oral daily dose of distilled water (2 ml/kg) for 25 days (negative control); Group II/Induced Group (by irinotecan), received a single daily oral dose of (2 ml/kg) distilled water for 25 days by the oral gavage and subsequently-received irinotecan (50mg/kg) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection; Group III, received an oral dose of Omega-3 fish oil (600mg/kg/day) daily for 25 successive days by an oral gavage (Omega-3 fish oil-treated); Group IV (Omega-3 fish oil + irinotecan), received oral dose of Omega-3 fish oil (600mg/ kg/ day) given daily for 25 successive days by oral gavage and received subsequently irinotecan (50mg / kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection.

Mitotic index in the bone marrow and the spleen cells were shown to be significantly-decreased (P<0.05) in rats intraperitoneally-injected with irinotecan (Group II) compared to corresponding levels in the negative control (Group I) rats; Orally-administered Omega-3 fish oil with a total cumulative dose of irinotecan (Group IV), resulted in significant elevation (P<0.05) of the mitotic index in the bone marrow and the spleen cells compared to corresponding levels in rats treated with irinotecan (Group II).

Results of the current study suggested that the administration of omega-3 fish oil could be useful supplement that may alleviate irinotecan induced genotoxicity through the elevation of mitotic indices in the bone marrow and the spleen cells of the rats; but, at a mild level.

Keywords: Omega-3 fish oil, Irinotecan, Bone Marrow, Spleen, Mitotic Index.

تأثير أوميغا ٣ على السمية الجينية للإرينوتيكان على نخاع العظم وطحال الجرذان: دراسة في الجسم الحي

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الخلاصة

صممت الدراسة الحالية لوصف السمية الجينية للإرينوتيكان من خلال قياس مؤشر الانقسام في نخاع العظم وخلايا الطحال. ولاستكشاف التأثيرات الوقائية المحتملة للأوميغا ٣ ضد السمية الجينية التي يسببها الإرينوتيكان في نخاع العظم والطحال في الجرذان.

أربعة وعشرون (٢٤) جرذ Sprague-Dawley من كلا الجنسين قسمت عشوائيا إلى أربع مجموعات: المجموعة I ، تلقت الجرذان جرعة يومية واحدة من الماء المقطر عن طريق الفم (٢ مل / كغم) لمدة ٢٥ يوما (مجموعة المراقبة السلبية) ؛ المجموعةII (المعالجة بالإرينوتيكان) تلقت جرعة يومية واحدة من الماء المقطر عن طريق الفم (٢ مل / كغم) لمدة ٢٥ يومًا وتلقت الإرينوتيكان (٥٠ ملغم / كغم) في الأيام: ٥ ، ١٠ ، ١٥ (الجرعة الإجمالية = ١٥٠ ملغم / كغم) عن طريق الحقن داخل الصفاق ؛ المجموعة III، تلقت الجرذان جرعة فموية من زيت السمك أوميغا ٣ (٦٠٠ ملغم / كغم / يوم) يومياً عن طريق الفم لمدة ٢٥ يومًا متتاليًا (المعالجة بزيت السمك بأوميغا ٣) ؛ المجموعة VI (ألاوميغا ٣ + إرينوتيكان) ، تلقت الجرذان جرعة فموية من زيت السمك أوميغا ٣ (٦٠٠ ملغم / كغم / يوم) يوميًا عن طريق الفم لمدة ٢٥ يومًا متتاليًا ، وتلقت الإرينوتيكان (٥٠ ملغم / كغم من وزن الجسم) في أيام : ٥ ، ١٠ ، ١٥ (الجرعة الإجمالية = ١٥٠ ملغم / كغم) عن طريق الحقن داخل الصفاق.

أظهر مؤشر الانقسامية في نخاع العظم وخلايا الطحال انخفاضًا معنويا (P <0.05) في الجرذان المعالجة بالإرينوتيكان (المجموعة الثانية) مقارنة بالمستويات المقابلة في المجموعة السلبية (المجموعة الأولى) من الجرذان؛ أدى تناول زيت السمك أوميغا ٣ عن طريق الفم مع إجمالي الجرعة التراكمية من إرينوتيكان (المجموعة الرابعة) إلى ارتفاع معنوي (P <0.05) في مؤشر الانقسام في نخاع العظم وخلايا الطحال مقارنة بالمستويات المقابلة في الفئران المعالجة بالإرينوتيكان (المجموعة الثانية).

تشير نتائج الدراسة الحالية إلى أن تناول مركب زيت السمك أوميغا ٣ قد يكون مفيدًا ليخفف من السمية الجينية التي يسببها الإرينوتيكان من خلال ارتفاع المؤشرات الانقسامية في نخاع العظم وخلايا الطحال في الجرذان ؛ لكن بمستوى متوسط .

الكلمات الرئيسية: زيت السمك أوميغا ٣ ، إرينوتيكان ، نخاع العظم ، الطحال ، ومؤشر الانقسام.

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Introduction

Irinotecan, which is a semisynthetic anticancer drug derived from the natural alkaloid Chinese plant camptothecin, was approved in 1996, by the Food and Drug Administration, for colorectal cancer treatment [(1).]

Such a drug which is a prodrug for SN-38 the active metabolite is widely used for pancreatic, lung, and colorectal cancer treatment [(2)]. Researchers reported that irinotecan showed considerable effects against the refractory-5-fluorouracil colorectal cancer; and this lead to a comprehensive program to evaluate the use of irinotecan as a single agent and also as a part of the combination therapies [(3)].

Irinotecan is an anticancer agent which belongs to the topoisomerase I enzyme interactive class, targets the complex of DNA-topoisomerase I, lead to prevent the reannealing of nicked DNA strands resulting in the arrest of the DNA replication [(1)] and consequently might lead to fast-proliferating cell death as in the intestinal basal cells and the bone marrow cells; and the main dose-limiting toxicities associated with irinotecan treatment were the gastrointestinal (GI) and the hematologic toxicities; severe delayed diarrhea and sever neutropenia [(4)].

The spleen acts as a phagocytic filter through the removal of the senescent and the damaged cells, and it also produces antibodies by initiating an immune response versus the encapsulated bacteria so the removal of the spleen is associated with a large reduction of the IgM memory B cells [(5)].

Dietary fish oil shows to have a beneficial effect on some chronic degenerative diseases as rheumatoid arthritis [(6)], cardiovascular disease [(7,8)], other autoimmune diseases [(9,10)], diabetes [(11)], and cancer [(12,13)]. These beneficial effects of the fish oil are due to the high level of ω-3 polyunsaturated fatty acids (PUFAs) as the docosahexaenoic acid (DHA) and the eicosapentaenoic acid (EPA) [(14)].

All three omega 3 fatty acids; docosahexaenoic acid (22:6), eicosapentaenoic acid (20:5), and α linolenic acid [(18:3)]; are directly-inhibit the arachidonic acid production from the linoleic acid [(15)].

Omega-3 PUFA dietary supplements have many beneficial effects if giving prior to or during the cancer therapy including reversing the drug resistance of the tumor cell, reducing haematological, cardiac or GI side effects of different chemotherapeutic agents, protection from alopecia, and reduce the cancer cachexia [(16)].

Objectives

The present study was designed to explore the genotoxicity of irinotecan through measurement of mitotic index in the bone marrow and the spleen cells and to describe the protective actions of omega 3 against irinotecan induced genotoxicity in the bone marrow and the spleen of rats.

Materials and Methods

Reagents: Absolute Methanol, BDH (England); Giemsa stain, Fluka (Switzerland); Glacial acetic acid, Fluka (Switzerland); Potassium chloride powder Fluka, Switzerland; and Glycerin Fluka (Switzerland).

Drugs***:*** Irinotecan 100mg vials obtained from Fresenius Kabi, India; Colchicine tablet, Aventis (France); and Omega-3 fish oil capsule 1000mg/ml, NATROL (USA).

Animals and experimental design

Twenty-four (24) adult Sprague-Dawley rats of both sexes, each weighing 150-200gm taken from the Animal House of College of Pharmacy/ University of Baghdad. Duration of the study from April to October 2021. The institutional animal care and the use Committee of the College of Pharmacy/ University of Baghdad approved the protocol of this study and the conduction of the work was performed under the controlled-conditions of the conventional laboratory ethics. Experimental rats are kept in stainless steel cages, in temperature (25°C), natural light/dark cycle and a relative humidity. Standard laboratory rodent tap water and chow supplied *ad libitum*, and animals adapted for one week period before the experiment. The animals were divided into four groups of six rats each as follows:

•**Group I**: Rats administered a single oral daily dose of distilled water (2ml/kg) for 25 successive days by oral gavage. This group served as **control**.

•**Group II** (**irinotecan/induction group**): Rats received a single oral daily dose of (2 ml/ kg) distilled water for 25 successive days by oral gavage, and subsequently IP injected with irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) [(17)].

•**Group III** (**Omega-3 fish oil-treated):** Rats orally-administered omega-3 fish oil at a dose of (600mg/kg/day) [(18)] daily for 25 successive days by oral gavage.

•**Group IV** (**Omega-3 fish oil-irinotecan**): Rats received oral dose of omega-3 fish oil (600mg/kg/day) daily by oral gavage for 25 successive days, and subsequently-injected with irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection.

Twenty-four (24) hours after the end of treatment that is to say at day 26, all the rats used in the experiment were euthanized by diethyl ether anesthesia and then samples of BM and spleen cells were taken for cytogenetic analyses.

Preparation of solutions

Colchicine solution: the colchicine solution was prepared by dissolving 2 tablets of (1mg) colchicine in 4ml distilled water to prepare (0.5mg/ml) solution [19].

Hypotonic solution, potassium chloride (KCl), for the chromosomal aberration: potassium chloride in a concentration of 0.075M prepared through adding KCl salt (5.75gm) to be dissolved in 1000 ml distilled water; then sterilized by the autoclaving and then stored in the refrigerator (at 4ºC) [20].

Fixative Solution: the fixative solution is freshly prepared by adding three parts of the absolute methanol to one part of the glacial acetic acid [21].

Preparation of the Chromosome using the Somatic Cells of the BM and the spleen of Rats

This procedure is done as follows [21]:

a. First 1ml of colchicine are injected into the rats intraperitoneally, the concentration of colchicine is (1mg/2ml), two hours before their scarification.
b. On day 26 the animals were sacrificed.
c. By using a plate of anatomy each experimental rat was fixed so that the animal abdomen swabbed with its thigh region with ethanol 70%.
d. The animal femur bone was taken and then cleaned from tissues and other muscles, by using forceps the bone gapped vertically from the middle over a test tube edge, using a sterile 5ml syringe of PBS that was injected in the test tube to drop the BM in it.
e. Then in Petri dish extract the spleen of the rat and inject it by PBS in various spots.
f. By centrifuging the test tubes that spanned at 2000 rpm for about 10min.
g. Removing of the supernatant layer then adding 5ml of KCl (0.075M) as a hypotonic solution, then put the tubes in the water bath for 30 min. at 37ºC and every 10 min. shaking the tubes.
h. After that the tubes were put in the centrifuging for 10min (at 2000 rpm).
i. Remove the supernatant layer with the addition of 5ml fixative solution drops by drops on the test tube in siding walls with shaking continuously. Then well shaking the test tube.
j. Cells are fixed by keeping tubes at 4ºC for about 30min.
k. These tubes were put for centrifuging for 10min (at 2000 rpm). This was repeated three times then in 2ml fixative solution the cells are suspended.
l. From three feet height by using a Pasteur pipette few drops dropped vertically on the chilled slide at 4-5 drops rate for giving chance for well spreading of the chromosomes. Then dried the slides at room temperature.
m. Stained the slides by the Giemsa stain, 15min left the slides and washed by D.W.
n. Two slides were prepared for the cytogenetic studies.

Assaying of the Mitotic index

The following slides (by using the light microscope) were examined through the 40X power, count (1000) cells of both divided and non-divided. The only dividing cells are calculated by the percentage according to the following equation [22]:

$$\textbf{Mitotic index}\ (\%) = \frac{\textbf{Number of divided cells}}{\textbf{Total count}\ (\textbf{1000})} \times \textbf{100}$$

Statistical Analysis

The data were expressed as values of the mean ± the standard deviation (SD); and analyzing was performed utilizing the computerized program of Statistical Package for Social Sciences (SPSS) (version 23). Statistical significance of differences among various groups was determined by the one way-analysis of variance (one way-ANOVA). The statistically-significant differences between the groups were considered when the P value was less than 0.05 (P<0.05).

Results

Irinotecan (**Group II** rats), caused a significant-reduction (P<0.05) in the mitotic index in bone marrow cells (Figure 1), and in the spleen cells (Figure 2) as compared to corresponding levels in the control (**Group I**) rats.

Furthermore, in **Groups III** rats/orally received Omega-3 fish oil (600mg/kg/day) produce non-significant differences (P>0.05) in the mitotic index in the bone marrow cells (Figure 1), and spleen cells (Figure 2) respecting to the corresponding levels in **Group I/Control** rats.

Administration of omega-3 fish oil (600mg/kg/day) in association with irinotecan (**Groups IV**) rats, there was a significant-elevation (P<0.05) of the mitotic index in bone marrow cells (Figure 1), and in the spleen cells (Figure 2) compared to corresponding index in rats' tissues of **Group II**.

Rats pretreated with 600mg/kg of omega-3 fish oil for 25 successive days with the induction of genotoxicity by irinotecan administration (**Group IV**), there was a significant decreased (P<0.05) in the mitotic index in the bone marrow cells (Figure 1), and in the spleen cells (Figure 2) compared to corresponding index in rats' tissues of **Group I**.

Additionally, rats pretreated with 600mg/kg of omega-3 fish oil for 25 successive days with the induction of Genotoxicity by irinotecan administration (**Group IV**), there was a significant decrease (P<0.05) in the mitotic index in the Bone marrow cells (Figure 1), and the spleen cells (Figure 2) compared to such index in rats' tissues pretreated with 600mg/kg of omega-3 fish oil for 25 successive days (**Group III**).

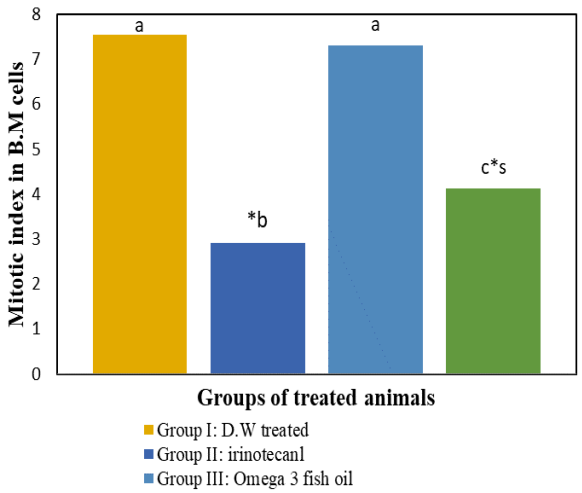

Figure 1. The effects of omega 3 fish oil and irinotecan intoxication on the mitotic index in bone marrow cells.
Data expressed as Mean±SD, n =6.
(*): Significantly different with respect to **Group I** (P<0.05). Values with non-identical superscripts (a, b and c) were significantly different (P<0.05).
s superscript: significant difference with respect to the irinotecan treated group.

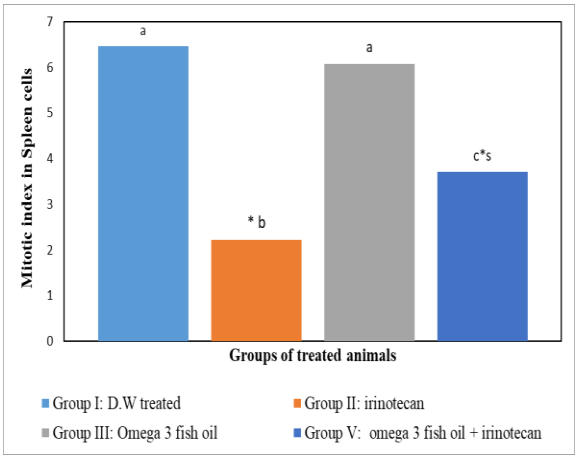

Figure 2. The effects of omega 3 fish oil and irinotecan intoxication on the mitotic index in spleen cells.
Data expressed as Mean±SD, n =6.
(*): Significantly different with respect to **Group I** (P< 0.05).
Values with non-identical superscripts (a, b and c) were significantly different (P<0.05).
s superscript: significant difference with respect to the irinotecan treated group.

Discussion

It has been reported that, myelosuppression caused by irinotecan treatment resulted in neutropenia, which can be considered as the predominant hematological adverse effect of such chemotherapeutic drug [23]. In animals treated with irinotecan showed a significant reduction in the WBC count and in the RBC count [24].

Moreover, a link between irinotecan treatment and steatohepatitis development was mentioned by authors [4]. A recent study confirmed that irinotecan results in liver toxicities, as was evidenced through the elevations in MDA content; in addition, there were reduction in serum total antioxidant capacity (TAOC) and elevation in serum AST, and ALT [17].

Researchers reported that treatment with irinotecan can cause activation of the cascade of many apoptotic-related signaling pathways via breakages of the double strands DNA; thus, ultimately resulting in apoptotic-cell death [17; 25].

Previous studies indicated that omega 3 fatty acids decrease the total myeloid progenitor cell frequencies and also promoted the differentiation of the specific progenitor cell types in mice bone marrow [15].

Omega 3 fatty acids resulted in the inhibition of proliferation, induction of apoptosis, and promotion of differentiation in many cancer types by mechanisms including the regulation of the signaling pathways and the gene expression through the peroxisome proliferator receptor activator γ (PPARγ), that omega 3 fatty acids are one of its natural ligands [26].

Other mechanisms of action of the omega 3 fatty acids include the inhibition of cyclooxygenase 2 (COX2) unregulated in different cancer types and it is known to have antiapoptotic and proliferative effects [27]. The phospholipase A2 (PLA2) enzyme liberate the arachidonic acid (AA) from phospholipids of the membrane, then the COX2 transfer the AA to the pro-inflammatory mediators, so through the inhibition of PLA2 and the COX2 enzymes, omega-3 fatty acid decreased the pro-inflammatory mediators [28].

Moreover, both the activation of the PPARγ and the inhibiting of COX2 expression were shown to inhibit the proliferation and also induce apoptosis in pancreatic cancer [29].

Also, researchers reported that omega-3 fatty acid possesses a beneficial therapeutic action in patients who were suffering from neurological disorders as epilepsy [28]. Furthermore, omega 3 fatty acids have inhibitory effects on the immune system that led to the usage of fish oils for the management of different autoimmune and inflammatory diseases [30].

The current study showed that omega 3 fatty acids (600mg/kg/day) attenuate irinotecan-induced reduction in Mitotic index in Bone Marrow Cells (Figure 1), and the spleen cells (Figure 2).

Conclusion

According to the results of this study it can be concluded that omega 3 fatty acids have protective effects on the genotoxicity induced by irinotecan on bone marrow and spleens of rats.

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