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Genotoxic and Cytotoxic Effects of Aspirin on the Mitotic Index in Bone Marrow Stem Cells of Albino Swiss Mice

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Abstract

Aspirin (acetylsalicylic acid) is among the most used over-the-counter drugs worldwide for the treatment of various conditions including analgesic, antipyretic and anti-inflammatory. Effects on its large therapeutic spectrum allows the use of Eleuphyllin, its use in clinical practice is limited by the cytogenetic and genotoxic effects that this drug may promote with prolonged or uncontrolled use. The mitotic index (MI) serves as a key cytological marker that reflects the proliferative activity of cells and identifies experimental disturbances associated with cell division. The goal of this study is to evaluate the genotoxic effect of aspirin in laboratory mice, utilizing the mitotic index of bone marrow stem cells. The dividing cells mitotic index (MI) was used as a measure of cytogenetic damage and mitotic inhibition. Significant dose-dependent decreases in mitotic index were seen in the aspirin-treated mice compared to the controls. The results indicate that aspirin may affect normal cell division and has genotoxic effects on bone marrow cells.

Keywords: Aspirin, mitotic index, genotoxicity, bone marrow stem cells, and cytogenetic effects.

1. Introduction

Aspirin (acetylsalicylic acid) is one of the most widely used and extensively studied pharmacological agents in clinical practice. Aspirin belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs). Exhibits multiple therapeutic effects, including analgesic, antipyretic, anti-inflammatory, and antithrombotic actions through the inhibition of cyclooxygenase (COX) enzymes [1, 2]. In oncology, a high mitotic index is often associated with aggressive tumors behavior and poor, whereas a reduction in MI may indicate cytotoxic effects or inhibition of cell division [3]. A major milestone in aspirin research was achieved with the identification of its antiplatelet mechanism, which involves

the irreversible acetylation of a serine residue on the COX-1 enzyme in platelets.

This results in irreversible inhibition of thromboxane A_2 production and blocks platelet aggregation, making aspirin a mainstay for the prevention of cardiovascular diseases including myocardial infarction and ischemic stroke [1]. Aside from its cardiovascular function, a growing body of evidence holds that aspirin is chemically preventive, particularly colorectal cancer, through inhibition of COX-2 and decrease of prostaglandin mediated tumor progression [2, 3]. Although the therapeutic properties of aspirin are well documented, there are also many concerns on cytotoxic and genotoxic activities after prolonged or uncontrolled use of aspirin.

Certain pharmacological agents such as NSAIDs theoretically disrupt DNA synthesis [2], perturbing presumptively normal mitotic development, which can reflect MI. Prognosis, as a decrease in MI may suggest cytotoxicity of cell division inhibition [3]. processes, resulting in genomic rearrangements include chromosomal instability and aberrant cellular proliferation [4]. Mitosis, or mitotic cell cycle, is the process of eukaryote cell division and its regulation governs the proper replication and segregation of genetic material. S phase, the stage in the cell cycle between the G1 and G2 stages, is when the DNA is replicated to give identical sister chromatids that are segregated at mitosis (to achieve stable genomic information and a functionally non-overlapping cell cycle) [4]. Disruption of this pathway can result in genomic instability and cell death. A measure of cell proliferative activity and cytogenetic stability, the mitotic index (MI), which corresponds to the number of cells in mitosis at the chosen time in relation to total cells [5]. This has far-reaching implications in fields such as toxicology, developmental biology, and cancer research.

As increased MI may mirror cytotoxic or cell division inhibiting effects, in oncology a large mitotic index (MI) is generally associated with malignant Tumor behavior and prognosis usually is determined by MI [5, 6]. Aspirin is the most widely used drug worldwide, and this is even more essential because compared with data from October 2023, it will be linked to higher use [2]. The objective of this study is to monitor the effect of aspirin on the mitotic index of bone marrow stem cells of Albino Swiss male mice in a dose- dependent manner to check its cytotoxic and genotoxic effect.

2. Materials and Methods

The present experimental work included twenty Albino Swiss mice aged from 8 to 12 weeks with an average body weight of (25 ± 2 g). Animals were obtained from the National Center for Drug Control and Research Ministry of Health Baghdad, Iraq. Mice were kept in a temperature-controlled room from 23 to 25 °C throughout the experimental period, with ad libitum access to standard food pellets and water to minimize any additional environmental distress. Aspirin tablets (100 mg; Julphar, U.A.E.) were dissolved in distilled water as previously described [7-10].

Cytogenetic protocols, different dosage concentrations were prepared from this solution. Animals were randomized into one of four study groups (n = 5/ group). Aspirin was given by intraperitoneal (IP) injection to 3 groups consisting of an injection of 1, 10, or 30 mg/kg body weight once daily for 3 days. A fourth group acted as the control and was injected with 0.25 ml of distilled water by IP under same conditions. The bone marrow from all the animals was collected for cytogenetic analysis 24 hours after the last dose.

The primary cytogenetic parameter to assess the cytotoxic effect of aspirin was the mitotic index (MI). Bone marrow preparations were done according to routine air-drying methods previously described by Evans et al. [7] and Allen et al. [8]. The mitotic index (MI) which is defined as the number of metaphase cells compared with the total number of examined cells was calculated based on methods referred to previously [9, 10].

Mitotic Index (%) = (Number of metaphase cells / Total number of examined cells) × 100

The proportion of cell dividing per sample is calculated by determining the number of dividing cells in a fixed number of cells observed by light microscope.

2. 1 Statistical Analysis

Data was provided as mean ± SE. Statistical analysis was performed using a two-sample t-test through the Minitab software package and the differences between groups were considered statistically significant at $P \leq 0.05$.

2. 2 Mitotic Index (MI) Assay

MI was used to examine the impact of aspirin on cell growth. Bone marrow smears were prepared by standard cytogenetic techniques and examined microscopically using a light microscope with a 40 ×

objective. Samples included five slides/samples, each slide in each sample was screened systematically. All bone marrow cells were counted in slides, including both diving and not-diving (1000 cells each slide). Cytogenetic analysis focused on cells in metaphase as they are the clearest cells and most appropriate for this type of analysis. The mitotic index only computed for metaphase cells. The formula given below was used to determine the mitotic index.

Mitotic Index (MI) (%) = (Number of dividing cells / Total number of examined cells) × 100.

The procedure and calculation method were performed according to previously established cytogenetic protocols [9, 10].

3. Results

Mitotic index (MI) decreased significantly ($P < 0.05$) in all aspirin-treated groups compared to the control group. Bone marrow cells from treated mice showed clear cytological alterations, such as more pronounced morphological signs of apoptosis while observed microscopically (suggesting the cytotoxic potential of aspirin).

As indicated in table 1 these results revealed a significant dose-dependent decrease in the mitotic index. The rate of cell division, defined as the mitotic index, was normal (6.94%) in the control group. As a comparison, mice treated with 1 mg/kg of aspirin had a MI of 3.1% and those treated with 10 mg/kg had an even lower MI of 2.6%. The highest level of decrease was observed in the highest dose (30 mg/kg), where the mitotic index was determined as 1.96%.

These findings show that progressive inhibition of cell division in bone marrow cells is associated with increasingly higher doses of aspirin. This observed trend establishes a significant dose response relationship between aspirin exposure and suppression of mitoses. Mitotic reduction and cytological abnormalities as shown in figure one, where table 1 lists the comprehensive characterization results revealing a significant dose-dependent decrease in the mitotic index.

Table1: Mitotic index for aspirin comparison with control group in mice bone marrow stem cell.

Groups of injected mice	Animal	Examined Cells	Mitotic index	
			Samples	%
control group	5	5000	347	6.94
1 mg/kg of aspirin	5	5000	155	*3.1
10 mg/kg of aspirin	5	5000	130	*2.6
30 mg/kg of aspirin	5	5000	98	*1.96

*Significant at ($p \leq 0.05$).

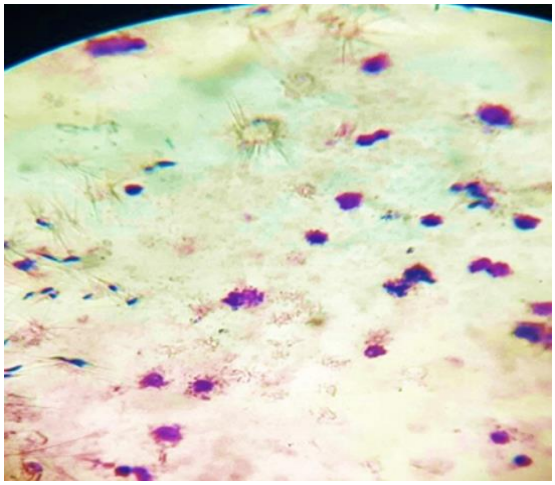

Figure 1: Mitotic index.

4. Discussion

Aspirin treatment was able to significantly reduce the MI of mouse bone marrow cells compared to controls ($P < 0.001$) indicating an obvious effect on cell proliferation inhibition in the present study. However, the decreases in SA probably are attributable to the cytotoxic effects of aspirin on rapidly dividing cells, particularly by disrupting the normal cell division cycle.

This may be due to inhibition of DNA synthesis (S-phase blockade) or other mechanisms, for example G2-phase cell cycle arrest that prevent cell passage to mitosis such as for aspirin [11]. Third, aspirin has the potential to change mitotic spindle structure, leading to chromosome misalignment and mis-segregation in mitosis. This cytotoxic effect is dose dependent which is further confirmed due to gradual decrease in MI with increase in doses. Effect of aspirin and other NSAIDs in decreasing mitosis at prophase, metaphase anaphase and telophase have been previously observed, and results partially substantiate and confirm these observations [12].

This staged disruption conceivably corresponds to this remarkable inhibition of mitosis observed here, in addition to the reduced mitotic frequencies, certain cytogenetic aberrations like micronuclei, chromosome-break and chromosome stickiness were also observed. These features are associated with clastogenic activity and are indicative of structural chromosome damage [14, 15].

The micronucleus assay is also a strong indicator of chromosomal breakage and confirms the clastogenic potential of the aspirin in the marrow [14]. Among the most notable findings within the analysed blood were the cyclic down regulation of genes recognized as leading to immune system suppression and chromosomal damage, similar findings have been found in experimental models when exposing cytotoxic and the function of anti-cancer agents by means of gene expression profile [16].

The ionic cellular damage that takes place may also be partially attributed to mechanisms functioning via oxidative stress.

Reactive oxygen species (ROS) generated by up-regulated non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin induce DNA damage, lipid peroxidation and compromise of cellular function [19].

The cytotoxic and genotoxic effects observed in the present study can, explained by this oxidative pathway. Aspirin has obvious clinical benefits, particularly in cardio protection. Further study of risks of excess or uncontrolled application is warranted. Biological effects, particularly at the level of dependent tissues, such as bone marrow, of chromosomal damage and its possible inhibition are characterized by high proliferative activity.

Aspirin for traditionally negative components of platelet action and situations such as uncommon situations giving rise to the Reye's syndrome [17, 18]. On the other hand, aspirin, having an anticancer effect, was considered to act through several COX dependent pathways as well as through COX-independent pathways such as regulation of MMR proteins, triggering of apoptosis, and inhibition of metastasis-related gene expression [20].

In addition, aspirin may reduce oxidative stress by activating AMPK, a critical regulator of cellular energy regulation and antioxidant responses [21]. Regardless, it is a beautiful demonstration that the protective effects are local and there is little chance for systemic cytotoxicity [22]. In conclusion, the data from this work imply that aspirin has Mitoclastic (via disrupting mitosis) and clastogenic (via damaging chromosomes) effects in bone marrow cells. Although the therapeutic efficacy of CBD is well documented.

These findings highlight the significance of regulated use and tailored research toward the dose-dependent and context-dependent nature of CBD cellular effects. There are some limitations to the current study that should be mentioned, despite its important findings. Two limitations to these findings, using sending and receiving machines was short, it may be short of the chronic effects of aspirin on bone marrow cells.

Moreover, the study was related to the features of cytogenetic, such as the index of mithosis, and chromosomal anomalies, without exploring the molecular mechanisms of aspirin-induced cytotoxicity. Additional research to investigate the molecular pathways involved in aspirin-induced genotoxicity, especially oxidative stress, DNA damage response, and cell cycle pathways, would therefore be beneficial. Increasing the sample size and having longer

exposure duration in the future investigation is needed. In addition, comparisons to other NSAIDs may elucidate whether these effects are unique to aspirin or represent a wider pharmacological class effect.

5. Conclusion

Aspirin can increase mitotic index, chromosome aberration and micronucleus in mice bone marrow stem cells, The combination effects of these drugs demonstrated very aggressive defects in mice bone marrow stem cells, which may belong to the interaction of both chemicals' combination of drugs. Further studies are required to find a less harmful dose of aspirin on stem cells to investigate the interaction effects between aspirin and other drugs.

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