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Aspirin Protective Effect on Cyclophosphamide Induced Hematological Toxicity

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Abstract

BACKGROUND: Bone marrow toxicity is the essential factor limiting use of cytotoxic drugs in cancer treatment. It has recently been demonstrated that enzyme inhibitors of prostaglandin (PG) synthase enzymes can enhance cytotoxic effect of chemotherapeutic agents on cancer cells. However, it is not clear whether these inhibitors can also influence the toxicity of cytotoxic drugs to bone marrow or other tissues.

MATERIALS AND METHODS: The effects of aspirin (as a PG synthase enzyme inhibitor) on cyclophosphamide bone marrow toxicity were investigated in this study, and peripheral blood counts were used as a substitute for bone marrow damage.

RESULTS: 50 mg/kg iv dose of cyclophosphamide results in reduction in total white blood cell count, non-granulocyte count, granulocyte count, hemoglobin percent, and platelets count. 75, 150, and 300 mg/kg doses of aspirin were protected from a decrease in white blood cell count measured 5 days after cyclophosphamide injection (50 mg/kg) into mice. Moreover, this protection was dose-independent. The best results were obtained at the dose of 300 mg/kg. Aspirin was unable to prevent changes in hemoglobin %.

CONCLUSION: Aspirin has been discovered to improve in the prevention of bone marrow toxicity-induced through cytotoxic medications for instance the alkylating agent's cyclophosphamide, for which there is now no antidote. According to some researchers, aspirin partially expresses this activity by reducing the negative inhibitory effect of leukotrienes, or excess PG, which are formed during bone marrow damage, on growth factors produced by hematopoietic progenitor cells.

Introduction

Cyclooxygenase is inhibited in the metabolism of amino acids (AA) by aspirin and comparable anti-inflammatory medications [1]. As a result, they increased the quantity of AA available to lipoxygenase, hence enhancing leukotriene production [2]. Glucocorticoids reduce the action of phospholipase A3, hence inhibiting the leukotrienes and prostaglandin (PGs). Numerous triggers produce these mediators, which contribute to the development of inflammation-related signs and symptoms [3].

WBC

The presence of colony-stimulating factors is required for proper granulocyte–monocyte progenitor proliferation. Both granulocyte-monocyte progenitor cells and colony-stimulating factor are inhibited by PGE1 [4]. PGE1 was said to exert a selective effect on B-lymphocyte activities, hence suppressing the humeral antibody response. In addition, PGs influence T-lymphocytes, which play a role in tumor growth inhibition and cell death [5]. PGE prevents sensitized T-lymphocytes from producing and releasing lymphokines [6]. Although leukotriene B4 is a strong chemotactic agent for polymorph leucocytes, other leukotrienes lack this feature [7]. The primary effect of PGs has been to suppress immunologic response [8]. Certain cancers' immunosuppressive action may be associated with their capacity to synthesize PGs [9].

Platelet

PGD2 and PGE1 are platelet aggregation inhibitors. At low concentrations, PGE2 is a stimulant, whereas at large concentrations, it is an inhibitor. Thromboxane (TX) A2 is an extremely potent inducer of platelet aggregation. There is little evidence to suggest that PGs have a direct role in the generation of megakaryocyte-derived platelets [10]. The influence of PGs on platelet aggregation has been linked to tumor metastasis through hematogenous dissemination. PGI2 administration before or during tumor cell injection significantly inhibits tumor colonization; this is related to platelet aggregation suppression instead of vasodilation [11].

RBC

PGE2 and PGE1 reduce the fragility of RBC at low concentrations, while increasing it at

high concentrations [12]. PGE2 may impact red cell maturation and result in a decrease in the amount of hemoglobin produced [13].

Cyclophosphamide

Cyclophosphamide is the most often utilized alkylating agent in the treatment of cancer in both experimental and humans. The use of cytotoxic chemicals in cancer chemotherapy has yielded positive outcomes in the treatment of hematological and lymphoid cancers. However, the toxicity of these agents on normal tissues of the body, particularly bone marrow, is the primary impediment to their use. Moreover, it is used an immune suppressant in many autoimmune diseases [14]. Numerous approaches have been developed to minimize the toxicity of cytotoxic drugs on normal tissues of the body, allowing for the safe use of greater doses while also enhancing tumor cell death. Mesna and N-acetyl cysteine [15] are two medications that have been utilized to reduce cyclophosphamide toxicity [16]. We used cyclophosphamide in our study, since it is a common alkylating drug used in cancer treatment and immune suppression.

Aim of the study

The aim of this study was to assess whether aspirin has any effect in mitigating cyclophosphamide toxicity, many doses of the drug will be administered to test a number of hematological factors.

Materials and Methods

Animals

During the test, adult male rabbits weighing 1–2 kg were free to consume commercially available food pellets and water.

Drugs

All the drugs used in the experiments were freshly produced. Medications such as the ones listed below were used:

- Cyclophosphamide (Endoxan-Baxter; Baxter Oncology GmbH Kantstrasse2 D-33790 Halle, Germany, 200 mg/10 cc vials)
- Aspirin (Aspegic: From the French laboratories synthelabo; 500 mg/5 cc vials).
- Heparin (Leo Heparin: From Leo pharmaceutical products, Denmark, vials containing 25,000 units/5 cc). Before blood sampling, it was given to rabbits at a dose of 5000 units/kg.
- Hematological indices used: A comprehensive blood count (in form of WBCs, platelets, RBSs, and hemoglobin) was determined using an automatic electronic counter after blood samples were collected from the animals (Beckman Coulter, ACT. 5 diff. USA).

Procedure

The doses implemented, here, are predetermined by a previous pilot study (unpublished).

Interactions between different aspirin doses and cyclophosphamide (50 mg/kg): Four groups, each has six rabbits included, that received aspirin i.m. in doses of 75, 150, and 300 mg/kg, respectively, daily for 3 days. The second injection of aspirin was given simultaneously with cyclophosphamide 50 mg/kg i.v. Blood samples were drawn from the marginal ear vein before administration of the first aspirin dose and on a daily basis for 5 days following the administration of the cyclophosphamide injection to determine hemoglobin percent, white blood cell counts (differential and total), and platelet counts (Figure 1).

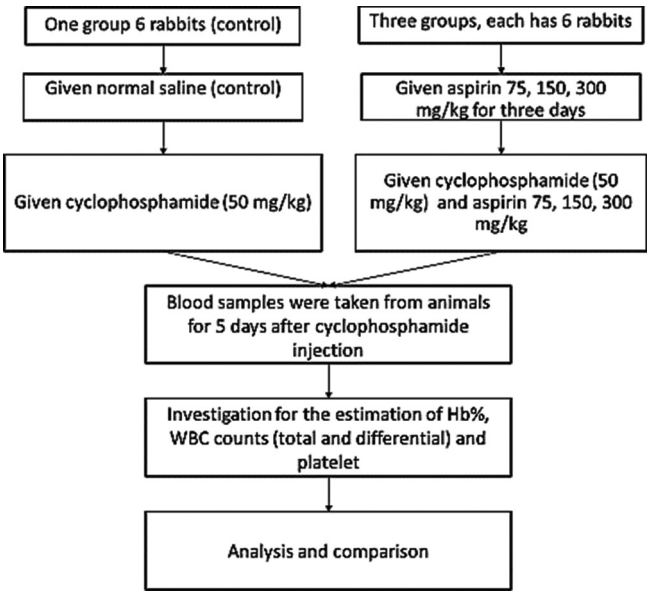

Figure 1: Flowchart showing the study research protocol

To account for the substantial variation in absolute platelet or WBC counts between animals, % were calculated by taking each animal's pre-treatment value as 100% and dividing it by the total number of animals. As previously stated, we counted total granulocytes, because heparin interferes with accurately differentiating them into neutrophils, eosinophils, or basophils. However, because neutrophils account for most granulocytes, differences in this count are likely to be mostly attributable to variations in the neutrophil count. Similarly, heparin impairs the ability to discriminate between granulocytes and non-granulocytes in a reliable manner (lymphocytes and monocytes). In contrast, because lymphocytes account for most non-granulocytes, it is expected that changes in total non-granulocyte counts will be driven mostly by changes in lymphocyte counts.

Statistical analysis

ANOVA was used to compare treatment groups to their respective controls. When $p < 0.05$, significance was used to reject the null hypothesis. The Dunnett's multiple comparisons test was used to detect the dose-dependent effect of medicines.

Results

The parameters, we examined, showed high changes throughout the study period, that is, 5 days when the experimental animal was exposed to the effects of cyclophosphamide. Therefore, it turns out that the most practical and decisive strategy are to rely on the fifth. Another important issue is that the significance level shown in the results is one way, that is, an increase in the parameters studied. For example, in Figure 2a, a significant reduction in WBC numbers ($p < 0.01$) was observed when 75 mg/kg of aspirin was used compared to the control. Nevertheless, this big impact is in the negative direction, that is, the number of WBCs is reduced, which is a detrimental effect.

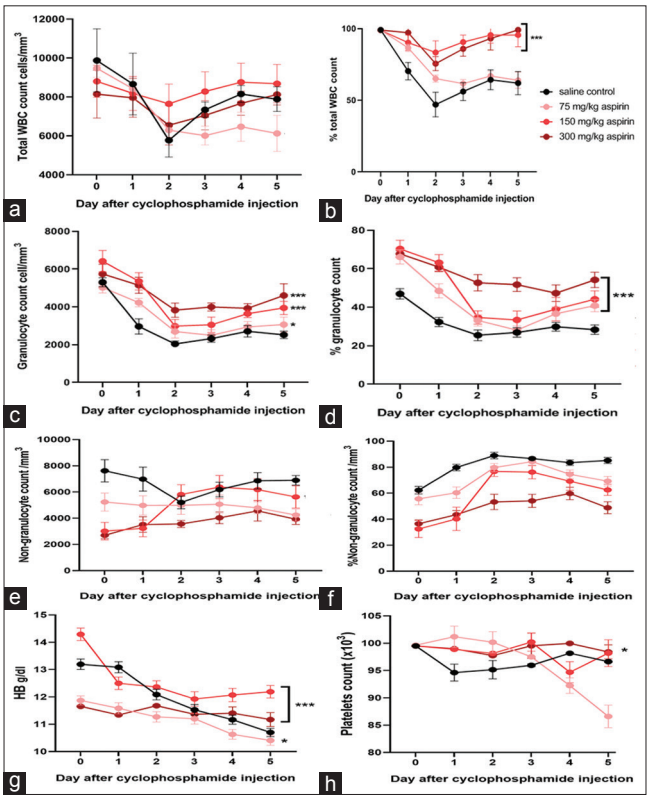

*Figure 2: Effect of aspirin (75, 150, and 300 mg/kg intramuscularly) on the blood picture of rabbits treated with cyclophosphamide (50 mg/kg intravenously). On the overall WBC count: (a) refers to the absolute value, while (b) refers to the percentage Effect on the number of granulocytes, (c) stands for absolute and d for percentage. Effect on the count of non-granulocytes, (e) stands for absolute, (f) for percentage. (g): Hemoglobin percentage; (h): platelet count percentage. *p < 0.05, **p < 0.01, and ***p < 0.001, respectively, as determined by Dunnett's multiple comparisons and ANOVA test*

Thus, we searched for beneficial changes in the 150 and 300 mg/kg dose ranges. The findings for these two doses are not statistically significant. They do, however, represent a protective effect against the harmful effects of cyclophosphamide.

Aspirin significantly increased the relative count of WBC in cyclophosphamide-treated laboratory animals at all dosages except 300 and 150 mg/kg, which is shown in Figure 2b.

At all doses, aspirin was protective against cyclophosphamide-induced granulocyte depletion (absolute numbers and percentages), with high statistical significance, especially at the dose of 300 mg/kg (dose of 300 mg/kg) in Figure 2c and d.

However, as illustrated in Figure 2e and f, aspirin had no effect on the relative and absolute non-granulocyte counts.

As shown in Figure 2g, aspirin significantly increased hemoglobin levels at 150 and 300 mg/kg; however, 75mg/kg had a negative effect on hemoglobin levels.

In Figure 2h, the platelet count increased significantly at the 300mg/kg dose, but not at the 150mg/kg dose, while the dose of 75 mg/kg had a negative effect on the number of platelets.

Discussion

The hematopoietic system is particularly vulnerable to the effects of cyclophosphamide [17]. The primary impediment to employing these medicines in cancer treatment is bone marrow depression. Cyclophosphamide, as a chemical damage to the bone marrow, is related with the de novo secretion and synthesis of several PGs, which may have a further detrimental effect on hemopoiesis. Colony-stimulating factors (CSFs) are produced in situ by T macrophages and lymphocytes and are required for the maturation and proliferation of both non-granulocytes and granulocytes [18].

Interleukin-3isexamplesofCSF(IL3),Granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and granulocyte colony-stimulating factor (G-CSF).

After intravenous infusion, G-CSF and GM-CSF elicit a significant, dose-dependent increase in neutrophil counts; in addition, GM-CSF increases monocytes, the absolute counts of lymphocytes, and eosinophils [19]. CSFs should also definitely be used in conjunction with other medications to address mucositis and bone marrow injury caused by chemotherapy [20]. Concurrent application of these parameters is expected to enable the use of

high dosages of cytotoxic drugs in cancer treatment, resulting in the death of bigger cells with minimal toxicity, and may also assist to avoid the establishment of drug resistance among vulnerable cells. Alternatively, agents that enhance the endogenous level of these growth factors by decreasing their catabolism and/or increasing their production, or that potentiate their activity by removing any endogenous inhibitors, may attain the same consequences.

Given that, PGs are known to suppress that these growth factors, using PGS inhibitors for instance aspirin, may be one method to accomplish this goal of conserving the bone marrow while allowing for greater selectivity in the use of cytotoxic drugs.

We selected cyclophosphamide in our study that it is one of extensively used cytotoxic drugs. In addition, its bone suppressive action may be applicable to trauma of bone marrow caused by other physical, chemical, or cytotoxic agents. Moreover, it is a widely used immune suppressant for many autoimmune diseases.

In our study, different doses of cyclophosphamide resulted in a dose-dependent decrease in total leucocyte count, which peaked on the 2nd day after injection. Recovery of blood indices on the 5th day after injection is most likely due to the elimination of the drug, whose half-life is 4-6 h, and the reactivation of progenitor cells and resistant stem.

This decrease is mostly attributable to a large decline in granulocytes relative to non-granulocytes. Given that, neutrophils are the major granulocytes; this likely implies a greater degree of harm to neutrophil precursors in bone marrow caused by cyclophosphamide metabolites that cause dose-dependent alkylation of myelocyte and myeloblast.

Aspirin has a mild ameliorative impact on total WBC count at 300 and 150 mg/kg doses, but it is not significant. As there is a considerable ameliorating effect on WBC % at these levels, it is maximum at 300 mg/kg.

PGE2 has been found to be a dose-dependent inhibitor of the normal growth of colony-forming cells [21] which have a sustained ability to increase intracellular cAMP levels [22], resulting in a decrease in the number of granulocyte progenitor cells.

In this study, we found that inhibiting the formation and release of PGs with the PGs inhibitor aspirin may mitigate their detrimental influence on bone marrow hemopoietic progenitor cells. This describes its protective effect against the cyclophosphamide-induced decrease of granulocytes, particularly neutrophils.

In fact, aspirin has been demonstrated to exacerbate percentages and non-granulocyte counts. The granulocytes exhibit mirror-image alterations, as well as differential counts of non-granulocytes, corroborating, and validating our findings. Cyclophosphamide also caused a dose-dependent drop in Hb levels. Alkylation may impact red cell precursors in the same way as it affects granulopoiesis precursors in bone marrow [23].

There is a direct correlation between erythropoietin and the levels of renal prostaglandin (PGE2). PG synthesis inhibition caused by aspirin's action on cyclooxygenase can result in decreased erythropoietin levels [24]. Nonetheless, inhibiting cyclooxygenase might transfer the scales toward the generation of leukotrienes. The leukotrienes (LT) C4 and B4 induced a dose-dependent decrease in erythroid colony counts and granulocyte-macrophage [25]. Thus, it appears that leukotrienes had a stronger effect than PGs in our experimental conditions.

Cyclophosphamide, like most cytotoxic agents, is widely documented to cause thrombocytopenia. However, aspirin has been found to have a strong and considerable protective impact on platelets at a dose of 300 mg/kg.

It appears that a similar mechanism protects non-granulocytes, such as lymphocytes and monocytes, and that our PGSIs function primarily by rectifying this detrimental influence of PGs on leucopoiesis.

Furthermore, aspirin's inhibitory action on PG production is insufficient to protect bone marrow erythrocyte precursors from alkylation and damage caused by cyclophosphamide metabolites, resulting in a decrease in hematocrit or hemoglobin percent. Although they virtually entirely inhibit cyclooxygenase-mediated PG formation, their use is related with increased accessibility of AA for lipoxygenase, resulting in increased generation of leukotrienes [2]. It is hypothesized that elevated levels of leukotrienes have the same detrimental effect on bone marrow erythropoiesis like PGs.

In addition, aspirin protein binding may be implicated in the protective influence of PGSIs on lowering cyclophosphamide toxicity and leucopoiesis. Aspirin is highly covalently linked to plasma proteins [26]. Cyclophosphamide binds less strongly to plasma proteins [27], and hence, coadministration would increase the cyclophosphamide-free percentage.

Finally, it appears that aspirin may assist to mitigate promote recovery and injury from bone toxicity-induced through cytotoxic medicines for instance that there is no specific antidote of the cyclophosphamide agent of alkylating.

Conclusion

Aspirin impact may be achieved by removing the detrimental inhibitory impact of leukotrienes (produced through bone marrow trauma) or excess

PGs on hemopoietic progenitor cell growth factors. It is hypothesized that, in the future, treatment with these drugs would improve the safety and selection of these cytotoxic agents, allowing for the use of higher doses to boost cancer cell death and likely preventing the development of drug resistance.

Limitation of the study

1. As it is conducted on animals, the application of findings on human requires further investigation and research.
2. The study findings does not show the impact difference of using aspirin on normal versus malignant blood cells. Such difference requires a further study.

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