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RESEARCH ARTICLE

Oral Administration of Ginger Rhizome Extract Protects against Side Effects of Azathioprine on Erythropoiesis

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ABSTRACT

Ginger has many therapeutic properties, being antioxidant and able to inhibit the formation of inflammatory mediators that are also important in driving erythropoiesis. The aim of this study is to detect the role of ginger rhizome in protecting and regulating erythropoiesis while using Azathioprine (AZA) as an immunosuppressive drug. Three groups of guinea pigs were allocated randomly, four in each group, twelve in total. Group I was treated orally with 50 mg/kg AZA daily for five days. Group II received 50 mg/kg body weight of AZA in combination with 50 mg/ml of ginger rhizome extract in the same protocol, while the guinea pigs in group III were left untreated as a control group. Erythrocyte count and RBC indices were measured at the end of the study period in all animals, using a standard haematology analyzer (Sysmex). AZA was found to affect erythropoiesis, causing significant elevation ($p < 0.05$) of erythrocyte count, Hb and PCV, but not, in group I (AZA) compared to the control group. MCV was found also to be significantly higher ($p < 0.05$) in groups I and 11 compared to the control group, causing macrocytic anaemia. The effect of AZA was neutralized by ginger rhizome, as the results for erythrocyte count, Hb and PCV in group II was found to be the same as in the control group without significant variation ($p > 0.05$). In conclusion the results of the present study reveal that the ginger rhizome extract might have a role in counteracting the stimulatory effect of AZA on erythropoiesis.

KEYWORDS

Macrocytic anaemia, erythropoiesis, ginger rhizome, azathioprine and RBC aplasia

INTRODUCTION

Azathioprine (AZA) is widely used as an immunosuppressive drug in organ transplantation and autoimmune diseases. This medication is found to be toxic to some vital organs such as liver and kidney that are important in metabolism of drugs[1]. These organs have also a significant role in synthesis of some erythropoietic growth factors that are regulated in vivo erythropoiesis, for instance erythropoietin (EPO)[2].

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Therefore the current study aimed to evaluate the effects of AZA on these organs and subsequently erythrocyte and red blood cell (RBC) indices.

Mustafa et al. found that ginger is able to inhibit the activity of cyclooxygenase and lipoxygenase and therefore minimize pain in headaches and rheumatism[3]. The rhizome of *Zingiber officinale* (ginger) is known for its protective effect on health in traditional medicine. It is utilized to treat rheumatism, peptic ulcer, headaches, burns, dyspepsia, impotence and depression[4]. The oleoresin from the rhizome has been shown to have anti-inflammatory, antipyretic, antihepatotoxic, analgesic and cardiotonic properties[5].

Immunosuppressive agents such as AZA will cause a reduction in RBC aplasia, which is characterised by the presence of less than 0.5% mature erythroblasts in the bone marrow (BM), a reticulocyte count of less than 1% and a severe anaemia[6]. This model can be exploited generating immune-compromised hosts to study a variety of viral and bacterial infections and features related to immune suppression.

The changes that occur in RBC indices as a result of Azathioprine medication causemacrocytosis[7]. Particularly, the alterations in erythrocytic parameters reflect the anaemia and parameters relating to erythropoietin and growth factors of erythropoiesis. The main growth factors involved are colony-stimulating factor (G-CSF), insulin growth factor-1 (IGF-1), Flt-3, IL-3, SCF and interleukins- (IL-3, IL-1, IL-6, IL-4, IL-9, IL-11)[2].

Azathioprine is used to prevent rejection following kidney or liver transplantation, usually in conjunction with, other immunosuppressive therapy, corticosteroids, and local radiation therapy[8,9]. This study was carried out on a guinea pig model, to observe drug interactions between azathioprine and Ginger through the alteration in erythrocyte count and RBC indices as a result of the combined treatment.

MATERIAL AND METHODS

This study was carried out at the animal research facility and laboratories of the College of Applied Medical Sciences (CAMS) at Aljouf University during the period from April to May 2014.

Ginger Extraction

Ginger was prepared and extracted as reported by Almarshad (2014) on the effects of ginger rhizome extract on lymphocytopenia[10]. Firstly, 0.5 kg fresh ginger rhizomes were obtained and washed with water. Secondly, an electric press was used to squeeze the fresh ginger rhizomes after slicing them into small pieces.

Thirdly, 1ml of the liquid obtained was evaporated under reduced pressure at 70° C. The weight of the dried powder was found to be 50 mg/ml and it was used as the concentration of the ginger extract.

Experimental Animals

Twelve 2-3 yrs old male guinea pigs, each weighting 800-1200 g were used in the current study. All procedures were performed in compliance with relevant laws and National Committee of Bioethics (NCBE) guidelines and with approval from Aljouf University Institutional Bioethics Local Committee. The experimental animals were randomly divided into three groups, four guinea pigs in each as follows;

Group I- received daily oral administration of 50 mg/kg body weight of AZA in combination with 50 mg/ml of ginger rhizome extract for five days.

Group II – was treated with 50 mg/kg body weight of AZA daily for five days.

Group III- was a control group which received neither azathioprine nor ginger.

The animals were kept individually and all of them were examined on a daily basis for signs of morbidity.

Sample Analysis

All guinea pigs were anesthetized with chloroform and then sacrificed by decapitation and blood allowed dripping into an EDTA tube. Blood samples of about four ml were collected for the Erythrocyte count and RBC indices.

These haematological parameters were analyzed, using a haematology analyzer, SYSMEX SE-9500[11] at the haematology laboratory at the College of Applied Medical Sciences (CAMS). The haematological parameters were compared statistically (two-tailed t test) between different treatment groups using GRAPHPAD software. A p value < 0.05 indicated statistically significant differences.

RESULTS

The findings for erythrocytic parameters in the azathioprine (AZA) and AZA plus ginger groups versus control samples are set out in Table 1. The salient features are represented graphically in Figures 1 and 2. The MCV in groups 1 and II

was significantly higher ($p< 0.05$) than in the control samples (Figure 1).

MCH, MCHC and RDW-CD were slightly higher but without significant variation in the AZA plus ginger group compare to the control group (Table 1). This table also shows that Hb and PCV were significantly raised in AZA group I but not group II, compared to controls.

Table 1: Erythrocyte counts and related parameters

Parameter	Azathioprine Mean (± SD)	AZA + ginger Mean (± SD)	Control Mean (± SD)
Hb g/dl	13.8 (0.9) [a]	13.1 (0.3)	12.2 (0.2)
PCV /l	40.8 (2.2) [a]	38.7 (1.2)	36.8 (0.7)
MCH/pg	26.7 (0.5) [b]	26.4 (0.5) [b]	25.3 (0.1)
MCHC mg/dl	33.8 (0.5) [b]	33.9 (0.5) [b]	32.9 (0. 5)
RDW-CV	11.6 (0.2)[b]	11.7 (0.4) [b]	11.8 (0.4)
RDW-SD	31.45 (0.9) [b]	34.1 (0.9) [b]	33.5 (1.8)

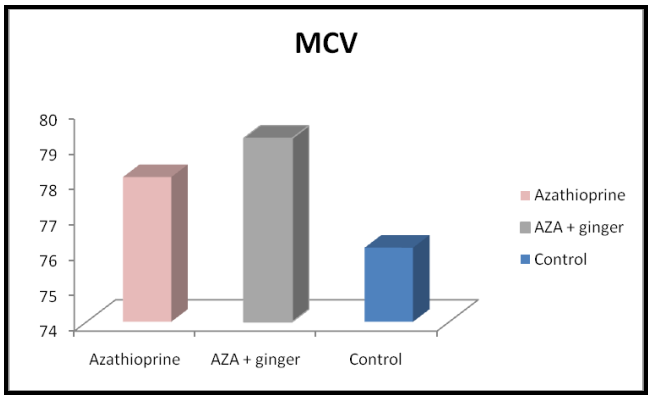

Figure 1: Mean cell volume in the experimental groups

Mean vales plotted, four animals per group

The erythrocyte count was significantly higher ($p<0.05$) in group I (AZA) than III (control) samples, but it was not significantly higher in group II (AZA plus ginger) (Figure 2).

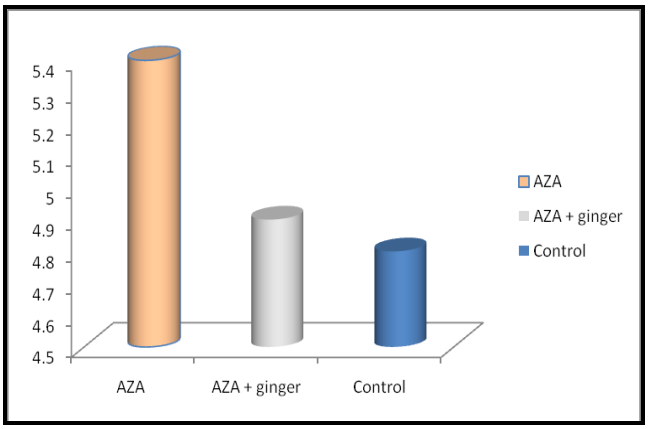

Figure 2: Erythrocyte counts in the experimental groups

Mean vales plotted, four animals per group

DISCUSSION

In this study, the erythrocyte count and RBC indices were determined in groups of guinea-pigs given: I - azathioprine (AZA), II - AZA plus ginger and III – control. Azathioprine readily affects proliferating cells including *erythrocyte precursors, and populations of* lymphocytes, because it acts as a pro-drug for mercaptopurine, inhibiting an enzyme that is essential for DNA production[12]. Another study also reported that AZA is toxic to some vital organs such as liver and kidney, which are essential in synthesis of erythropoietic growth factors such as EPO[2]. The objective of the current study was to detect any role for ginger rhizome extract in protecting and regulating erythropoiesis while using AZA as an immunosuppressive drug.

The results showed that MCV in the animals treated with AZA were significantly higher than in the control samples. This is similar to results from a study of 91 patients receiving azathioprine by DeClerk et al[7]. It is also similar to another finding that azathioprine activates suicidal erythrocyte death, an effect most probably contributing to azathioprine-induced anaemia[13]. This means that, AZA might affect erythropoiesis processes, causing clear elevation in MCV so that megaloblastic anaemia emerges.

Macrocytosis developed in the group I because AZA might cause ineffective or dys-erythropoiesis, causing megaloblatic anaemia to arise. This is consistent with previous studies among human beings receiving long-term azathioprine therapy[6,7].

Ginger (*Zingiber officinale*) was used in this study in an attempt to inhibit or alleviate the complications of AZA. It potentially inhibits lipid peroxidation by maintaining levels of antioxidants[14]. Several studies have also found antiemetic, anticholesterolaemic and antiplatelet effects of ginger[15,16]. In the current study, 50 mg/kg body weight of AZA in combination with 50 mg/ml of ginger rhizome extract was given to the AZA + ginger group II, as daily oral administration for five days.

The MCV was elevated in the combined treatment group compared to the group II (AZA only). This is in agreement with a report that ginger may assist iron absorption and was found to be useful as a supplement in treating patients with iron deficiency anaemia, as it contains ascorbic acid, reducing sugar[17].

The erythrocyte count and its parameters, Hb and PCV in the AZA group were significantly higher ($p< 0.05$) than in the control samples. This means that AZA might lead to a high initial *erythropoietic* rate, causing erythroid hyperplasia and reticulocytosis. The elevation was not unexpected as it had been found in the first three weeks after using AZA previously[18].

However, the levels of these haematological parameters might be decreased dramatically among individuals on AZA, because of RBC aplasia[13] due to disturbances in the erythropoiesis stimulating agents by the action of AZA. RBC aplasia is characterized by a severe normocytic anaemia, absence of erythroblasts and reticulocytopenia from an otherwise normal bone marrow (BM)[13].

Interestingly, the effect of AZA was inhibited clearly by ginger rhizome, as the erythrocyte count, Hb and PCV in group II was found to be indistinguishable from the control group (Figure 2 and Table 1). The main adverse effect of azathioprine is BM suppression, which can be life-threatening, especially in people with a genetic deficiency of the enzyme thiopurine S-methyltransferase[19]. It is listed as a carcinogenic to humans by the International Agency for Research on Cancer[20].

CONCLUSION

Ginger has emerged as an alternative medication to alleviate some complications of AZA administration. Selective erythroid toxic reaction is a potential problem that must be considered when anaemia develops in patients receiving long-term azathioprine therapy.

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