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RESEARCH ARTICLE

Curcumin attenuates erythropoiesis in recombinant human erythropoietin-induced polycythemia in rats

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ABSTRACT

Background: Several studies documented the non-hematologic clinical therapeutic uses of recombinant human erythropoietin (EPO). On the other hand, hypertension, thromboembolism, and increased oxidative stress were toxic effects related to the increased hematocrit (Hct) with recombinant human EPO treatment. Accordingly, alternate strategies to reduce erythropoietic activity and other potential side effects of EPO will greatly improve its non-hematopoietic clinical applicability. **Aims and Objectives:** Our objective was to demonstrate whether curcumin treatment could attenuate the effect of recombinant human EPO on erythropoiesis in EPO-induced polycythemia, and if so, whether this effect is mediated by changing concentrations of iron and its key regulator hormone hepcidin in rats. **Materials and Methods:** Totally 24 male albino Sprague-Dawley rats were included in this study. Rats were equally divided into four groups: Control group, curcumin-treated group, EPO-induced polycythemia group, and curcumin + EPO-induced polycythemia group. Blood indices and serum concentrations of iron, ferritin, and hepcidin were measured. **Results:** EPO treatment caused significant increase in hemoglobin (Hb), red blood cells, and Hct versus other study groups ($P < 0.05$). Curcumin treatment significantly decreased Hct in curcumin-treated group versus control and EPO-induced polycythemia groups ($P = 0.021$ and 0.008, respectively). Serum iron concentrations were significantly decreased in curcumin + EPO-induced polycythemia group versus control group. Serum ferritin concentrations were significantly decreased in all treated groups versus the control group. Serum hepcidin concentrations were significantly decreased in EPO-induced polycythemia group and curcumin + EPO-induced polycythemia group versus control group. **Conclusion:** The presented data suggest a potentially attenuating effect of curcumin administration on recombinant human EPO-induced polycythemia. This effect may be mediated by promoting iron deficiency. However, further studies are required to address the safety of this combination treatment and interspecies differences in iron metabolism between rats and human in addition to have better understanding of the role of the hepcidin.

KEY WORDS: Curcumin; Erythropoietin; Erythropoiesis; Iron; Ferritin; Hepcidin

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INTRODUCTION

Erythropoietin (EPO) is a pleiotropic cytokine originally identified for its role in erythropoiesis and is used in the treatment of anemia, especially in renal failure. EPO has been suggested to have diverse biological functions, including neuroregenerative, angiogenic,

anti-inflammatory, and anti-apoptotic actions in the brain.[1] Shin and Cho suggested a novel strategy of EPO and granulocyte colony-stimulating factor combination therapy for stroke patients in their exploratory study.[2] In addition, EPO had a neuroprotective role in rotenone-induced parkinsonism in rats,[3] an anti-inflammatory effect in the development of experimental colitis,[4] and a role in the regulation of body weight, fat mass, and glucose metabolism.[5]

On the other hand, there is an increased mortality rate with recombinant human EPO treatment in stroke patients due to the toxicity of the high dose of EPO, this toxicity may partially result from increased hematocrit (Hct)-associated side effects, such as hypertension and thromboembolism.[1] Furthermore, systemic EPO treatment increased oxidative stress in rats.[3] Accordingly, alternate strategies to reduce erythropoietic activity and other potential side effects of EPO will greatly improve its non-hematopoietic clinical applicability.[6]

Curcumin is a phytochemical derived from the rhizome of *Curcuma longa*, present in the spice turmeric, and it gives Indian curry its yellow color. Curcumin has been used as a wound-healing agent and for treating a variety of diseases in traditional Indian and Chinese medicine. Curcumin has attracted the attention of researchers as an agent capable of inhibiting the proliferation of cancer cells. In addition, curcumin raises interest as an agent of potential use in therapy of many diseases including cardiovascular diseases, Alzheimer's disease, rheumatoid arthritis, and metabolic syndrome.[7]

Previous studies reported that curcumin treatment could decrease Hct in adult and aged rats[7] and in mice consuming a low-iron diet (5 mg iron/kg diet).[8] This finding was explained by the iron-chelating effect of curcumin.[8] In addition, it was found that the mice that were supplied with curcumin in their diet had a significant reduction in iron store in the liver and the spleen despite sufficient iron intake.[9]

Hepcidin, a recently discovered peptide hormone, is produced by hepatocytes and induces internalization and degradation of ferroportin. By inhibiting ferroportin, hepcidin prevents gut cells from allowing iron into the hepatic portal system, thereby reducing the absorption of dietary iron. Degradation of ferroportin also reduces iron release from macrophages.[10] At present, hepcidin is considered the key regulator hormone of systemic iron homeostasis.[11]

Hence, the aim of the current study was to demonstrate whether curcumin treatment could attenuate the effect of recombinant human EPO on erythropoiesis in EPO-induced polycythemia, and if so, whether this effect is mediated by changing iron and hepcidin concentrations in rats.

MATERIALS AND METHODS

Animals

Totally 24 male albino Sprague-Dawley rats, body weight 170-200 g, purchased from Center for Experimental Animals, Faculty of Veterinarian Medicine, Zagazig University, were used in this study. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals (1985) NIH, Bethesda. All rats were left to acclimatize for 1 week prior to the experiment and were housed in plastic cages maintained at controlled room temperature (22-24°C) with 12 h diurnal (day and night change) with free access to standard pellet animal diet and tap water. Then, the rats were equally divided into four groups: Control group, curcumin-treated group, EPO-induced polycythemia group, and curcumin + EPO-induced polycythemia group.

Methods

- Curcumin treatment in curcumin-treated and curcumin + EPO-induced polycythemia groups: Curcumin (HOC6H3 (OCH3) CH=CHCO] 2CH2, molecular weight 368.38 (Bio Basic Canada Inc.), was dissolved in tween 80 (Sigma) and it was administered intraperitoneally (IP) at a dose of 50 mg/kg. All injections were injected once daily for 21 consecutive days.[7]
- Recombinant human EPO treatment in EPO-induced polycythemia and curcumin + EPO-induced polycythemia groups: Animals received three subcutaneous injections weekly of EPO (300 IU/kg) (SEDICO, Egypt).[12]
- Measurement of blood indices: 1 ml of blood was withdrawn from retro-orbital venous plexus from each anesthetized rat into an ethylenediaminetetraacetic acid-containing Eppendorf tube to perform blood indices (red blood cell [RBC] count, hemoglobin concentration [Hb], Hct, mean cell volume, mean cell Hb [MCH], and MCH concentration). Blood testing was carried out using the fully automated hematology cell counter (Swelab, Sweden).[7]
- Serum iron and ferritin assay: The serum was obtained by centrifugation (2400 rpm, for 20 min at 4°C). The concentration of serum iron was determined by semi-automated chemistry analyzer (photometer 5010), and the concentrations of ferritin were determined by Vidas, BioMerieux.
- Serum hepcidin concentration assay: The concentration of hepcidin was measured by rat hepcidin ELISA kit (MyBiosource). Intra-assay precision: CV% is <8%; inter-assay precision: CV% is <10%.

Statistical Analysis

All data were expressed as mean ± standard deviation and were analyzed using Statistical Package for Social Sciences program version 20. All comparisons among groups were

carried out using one-way analysis of variance followed by Bonferroni *post-hoc* test. Data were considered statistically significant with p≤0.05.

RESULTS

Blood Indices' Results

EPO treatment caused significant increase in Hct, Hb, and RBCs in EPO-induced polycythemia group versus the control group, curcumin-treated group, and curcumin + EPO-induced polycythemia group. On the other hand, curcumin-treated group had significant decrease in Hct in curcumin-treated group versus control group and curcumin + EPO-induced polycythemia group (Table 1).

Serum Iron Concentration Results

All the treated groups had decreased serum iron concentrations versus the control group. However, the only statistically significant decrease occurred in curcumin + EPO-induced polycythemia group ($P = 0.036$) (Figure 1).

Serum Ferritin Concentration Results

All the treated groups had significant decrease in the concentrations of serum ferritin versus the control group ($P = 0.001$ in all comparisons) (Figure 2).

Serum Hepcidin Concentration Results

Serum hepcidin concentration was significantly decreased in EPO-induced polycythemia and curcumin + EPO-induced polycythemia groups versus the control group (P = 0.034 and 0.002, respectively) (Figure 3).

DISCUSSION

In the current study, we found that curcumin administration attenuated the erythropoietic activity of recombinant human EPO and resulted in normalization of the Hct in EPO-induced polycythemia in rats. Similar result concerning the curcumin effect on Hct in adult and aged rats was found.[7] This finding may represent a new strategy for increasing the non-hematopoietic clinical applicability of recombinant human EPO by controlling the increased Hct-associated side effects, such as hypertension and thromboembolism.[1]

To address the mechanism of the current finding, serum iron concentrations were assayed and a non-significant decrease was found with curcumin treatment and also with EPO treatment. However, with combined administration of curcumin and EPO, a significant reduction of serum iron concentrations was observed. Plasma iron levels are maintained in a relatively constant range by balancing the inflow of iron into plasma with the outflow. The inflow is determined by the release of iron from macrophages recycling old RBCs, release of iron from the liver stores, and absorption of iron from the diet.[13] Hence, the current finding may be explained

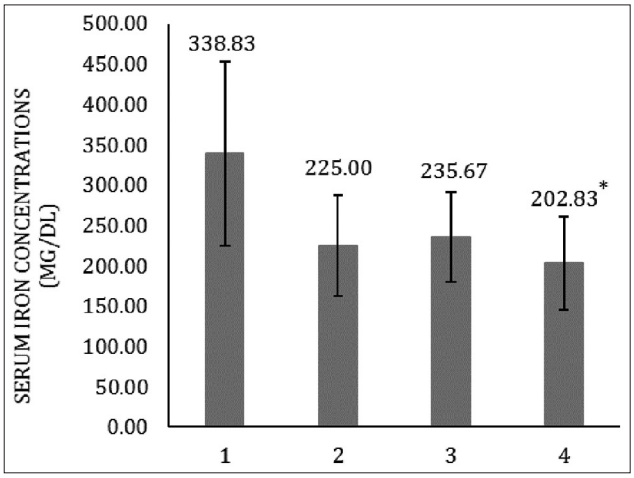

Figure 1: Serum iron concentrations among the studied groups. 1: Control group; 2: Curcumin-treated group; 3: Erythropoietin (EPO)-induced polycythemia group; 4: Curcumin + EPO-induced polycythemia group. *Significant decrease in serum iron concentrations (mean ± standard deviation) in curcumin + EPO-induced polycythemia group versus the control group ($P = 0.036$)

Table 1: Blood indices' results among the studied groups (mean±SD)

Blood indices	Control group	Curcumin-treated group	EPO-induced polycythemia group	Curcumin+EPO-induced polycythemia group
RBCs (millions of cells/μL)	7.99±0.7	7.24±0.7	10±1.3*	8.5±0.6
Hb (g/dL)	13.9±1.3	12.8±0.8	17.7±2*	15.1±1.2
Hct (%)	48.4±4.7	41.1±1.1*[a]	59.1±5.5*	49.3±1.9
MCV (fL)	61.1±7.7	57.3±6.3	59.4±3.2	58.6±4.1
MCH (pg)	17.5±1	16.8±1.1	17.8±1	17.4±1.1
MCHC (g/dL)	29±3.9	31.6±2	29.9±1.1	29.6±1.2

*Significant increase in RBCs, Hb, and Hct in EPO-induced polycythemia group versus the control group, curcumin-treated group, and curcumin+EPO-induced polycythemia group (RBCs: P=0.004, 0.000, and 0.038, respectively; Hb: P=0.001, 0.000, and 0.022, respectively; Hct: P=0.001, 0.000, and 0.001, respectively). *[a]Significant decrease in Hct in curcumin-treated group versus control group and curcumin+EPO-induced polycythemia group (P=0.021 and 0.008, respectively). RBC: Red blood cells, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean cell volume, MCH: Mean cell hemoglobin, MCHC: Mean cell hemoglobin concentration

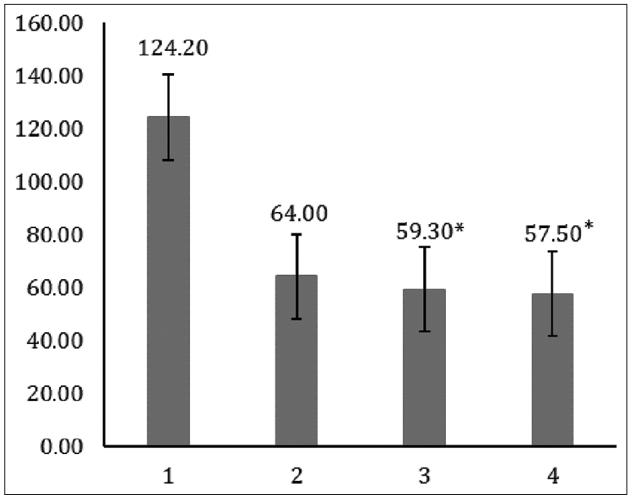

Figure 2: Serum ferritin concentrations among the studied groups. 1: Control group; 2: Curcumin-treated group; 3: Erythropoietin (EPO)-induced polycythemia group; 4: Curcumin + EPO-induced polycythemia group. *Significant decrease in the serum ferritin concentrations (mean ± standard deviation) in curcumin-treated, EPO-induced polycythemia, and curcumin + EPO-induced polycythemia groups versus the control group (P = 0.001 in all comparisons)

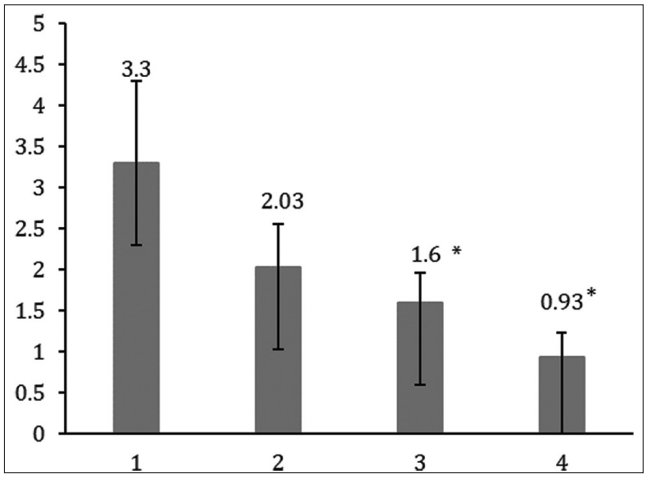

Figure 3: Serum hepcidin concentrations among the studied groups. 1: Control group; 2: Curcumin-treated group; 3: Erythropoietin (EPO)-induced polycythemia group; 4: Curcumin + EPO-induced polycythemia group. Significant decrease in the serum hepcidin concentrations (mean ± standard deviation) in EPO-induced polycythemia and curcumin + EPO-induced polycythemia groups versus the control group (P = 0.034 and 0.002, respectively)

by the fact that the increased erythropoietic activity induced by EPO required more than the available plasma iron and functional iron deficiency developed.[14] In addition, true iron deficiency may develop during chronic EPO administration due to a progressive shift of iron from body stores to the bone marrow.[15] On the other hand, in agreement with the current study, Jiao et al. found that curcumin significantly decreased the plasma iron concentrations in mice fed with low iron diet and supplemented with curcumin and suggested that curcumin had an iron-chelating effect and impaired iron absorption.[8] Hence, in the current study, administration of EPO may result in functional iron deficiency and administration of curcumin may decrease the absorption of iron, and the sum of these two effects was the observed significant decrease in serum iron concentrations.

In the current study, serum ferritin concentrations significantly decreased with curcumin administration, EPO administration, and combined curcumin and EPO administration. In agreement with these results, other studies reported similar results concerning the administration of curcumin[8,9] and administration of EPO.[16] However, to our knowledge, no study reported the effect of combined administration of curcumin and EPO on serum ferritin.

In the current study, serum hepcidin concentrations were significantly decreased with recombinant human EPO treatment alone or in combination with curcumin. In agreement with this result, several studies reported that recombinant human EPO treatment reduced hepcidin secretion.[17,18]

The current finding is explained by understanding the methods of hepcidin regulation including plasma iron concentrations, body iron stores, infection and inflammation, and erythropoiesis.[19] The observed decrease in serum iron and ferritin concentrations with combined treatment of EPO and curcumin may suppress hepcidin in a classical endocrine feedback system in which hepcidin production is stimulated by plasma iron and iron stores.[20] Furthermore, erythropoietic precursors in the bone marrow are the main consumers of iron, so expansion of a precursor population in response to the administration of EPO suppresses hepcidin to increase iron absorption and release of iron from stores to match iron supply to the increasing demand. Increased erythropoietic activity suppresses hepcidin possibly through a mediator released by the bone marrow which exerts its effect on hepatocytes. This mediator is erythroferrone, a hormone produced by EPO-stimulated erythroblasts.[20,21]

In contrast to the current finding of absence of a significant effect of curcumin treatment on serum hepcidin concentrations, Chin et al. found that the expression of hepcidin was significantly reduced in the liver of curcumin-fed mice along with depleted iron stores in the liver and the spleen.[9] The difference in the current study may be attributed to the change of administration method of curcumin (I/P daily injection for 21 days in the current study versus oral administration for 6 months in the mentioned study). In addition, in the current study, rats were fed standard pellet animal diet, whereas in the mentioned study, mice were fed a Western-type diet (20% fat and 10% sugar), and a high-fat diet has been suggested to promote iron deficiency by reducing duodenal iron absorption and by inducing inflammation.[9]

CONCLUSION

The presented data suggest a potentially attenuating effect of curcumin administration on recombinant human EPO-induced polycythemia. This effect may be mediated by promoting iron deficiency. Therefore, combined administration of recombinant human EPO and curcumin may represent a new strategy to improve recombinant human EPO non-hematopoietic clinical applicability. However, further studies are required to address the safety of this combination treatment and interspecies differences in iron metabolism between rats and human in addition to have better understanding of the role of hepcidin.

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