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ERYTHROPOIETIN INCREASES Epo AND EpoR EXPRESSION IN DLD-1 CELLS

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ABSTRACT

Introduction. Supplementation of recombinant human erythropoietin (rHuEpo) is one of the methods for the treatment of anemia for patients with colon cancer. However, the results of *in vitro* studies investigating the influence of rHuEpo on cancer cells are contradictory.

Aim. The aim of the present study was an assessment of the effect of rHuEpo on proliferation, as well as Epo and EpoR protein expressions in normoxia and hypoxia conditions on human colon adenocarcinoma cells (DLD-1).

Materials and methods. The cells were cultured in medium with rHuEpo in concentrations of 1 and 3 IU without (normoxia) or with (hypoxia) cadmium chloride for 48 hours. Cell viability was counted using a haematocytometer and trypan blue 0.4% (w/v) dye. Expression of Epo and EpoR protein was assessed by western blot.

Results and Discussion. We observed a decrease in the number of colon cancer cells in hypoxia. Addition of rHuEpo did not modify cell numbers in normoxia and hypoxia. We found a significant increase of EpoR expression in all cells growing in medium with cobalt chloride in comparison with respective normoxic cells. We also noted that rHuEpo in concentration of 3 IU significantly increased expression of Epo and EpoR protein in colon cancer cells in normoxia and hypoxia conditions.

Conclusions. We concluded that Epo and EpoR are constitutively expressed in DLD-1 cells. In hypoxia as well as in the presence of rHuEpo the increase of Epo and EpoR protein was found. However, the expression of Epo and EpoR protein in these cells does not seem essential to their growth.

Key words: erythropoietin (Epo), erythropoietin receptor (EpoR), recombinant human erythropoietin (rHuEpo), colon cancer cells.

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INTRODUCTION

Erythropoietin (Epo) is a 30.4-kDa glycoprotein hormone produced and secreted in the kidneys of adults and in fetal liver in response to hypoxia as a 166-amino acid peptide [13]. During maturation a carboxy-terminal arginine in position 166 is removed resulting in a circulatory mature 165-amino acid protein. Endogenous as well as exogenous peptide (recombinant human Epo, rHuEpo) has the same composition [15]. The main physiological role of Epo is the stimulation of erythropoiesis. However, the results of numerous *in vivo* studies have shown that Epo protects against ischemia and trauma of the brain, retina, and spinal cord in animals [7, 8, 10]. Moreover, in *in vitro* studies it has been shown to stimulate angiogenesis, cell proliferations and vessel formation [15].

The effect of Epo is mediated by binding to the erythropoietin receptor (EpoR), the expression of which has been shown in nonhematopoietic cells and tissues such as endothelial cells, brain, female genital tract, placenta, myoblasts, kidney, intestine and various cancers [13]. EpoR is a transmembrane protein. The presence of EpoR in these cells may suggest the participation of Epo in autocrine or paracrine mechanisms. The binding of Epo to EpoR activates also others cascades that lead to the enhancement of proliferation, differentiation and survival [13, 16]. Thus, an endogenous Epo/EpoR system plays a prominent role in developing many tissues, including cancers.

In solid cancers, including colon cancer, Epo and EpoR expressions are mainly regulated by hypoxia via hypoxia inducible factors-1 (HIF-1) [1]. HIF-1 is composed of HIF-1A and HIF-1B and under normoxic as well as hypoxic conditions mRNA in both of them are constantly expressed in a number of mammalian cell lines. However, HIF-1A protein is markedly increased by hypoxia, whereas HIF-1B protein is constantly present regardless of oxygen tension [9]. Then, HIF-1A is the main regulator of Epo and EpoR expressions in hypoxic cancer cells.

In vitro studies investigating the role of Epo and Epo–EpoR signaling in tumor growth and angiogenesis have yielded contradictory results. Yasuda et al. observed the inhibition of angiogenesis and tumor cell survival in stomach and melanoma xenografts following the blockade of Epo–EpoR signaling [25]. These results are in opposition to the findings of Hardee et al. who did not observe any effects on angiogenesis and tumor growth in colon and head and neck xenografts after Epo administration [12].

AIM

At present in literature there have been no reports describing the influence of rHuEpo on human colon adenocarcinoma cells (DLD-1). Thus, the aim of our study was to estimate whether rHuEpo might directly affect human colon cells in normoxia and hypoxia. We also assayed the influence of rHuEpo on Epo and EpoR protein expressions in DLD-1 cells in normoxia and hypoxia conditions.