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Production of hyper glycosylated darbepoetin alfa using *Leishmania tarentolae*

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Darbepoetin alfa known by the trade name of Aranesp is a hyper glycosylated and synthetic analog of erythropoietin (EPO) which is used as a drug in treating anemia in patients with chronic kidney failure and cancer. This study describes the expression of a codon-optimized recombinant darbepoetin alfa in *Leishmaina tarentolae* T7-TR host. The gene of darbepoetin alfa was optimized according to the codon usage of *L. tarentolae* and synthesized. The synthetic codon optimized gene was amplified by PCR and cloned into the pLEXSY vector. The resultant expression construct (pLEXSYDrabo) was purified, digested with SwaI enzyme and electroporated into *L. tarentolae*. The homologous recombination integration into the *L. tarentolae* genome was confirmed by different diagnostic PCRs. Expression of recombinant darbepoetin alfa was analyzed by ELISA, reverse-transcription PCR (RT-PCR) and Western blotting. The recombinant protein was purified and its biological activity was also measured.

After codon optimization, Codon Adaptation Index (CAI) was raised from 0.50 to 0.99 and GC% content was changed from 56% to 58%. Expression analysis showed and confirmed the presence of a protein band at about 40 kDa and its expression level was 11 mg/mL of culture medium. Reticulocyte experiment results revealed that the activity of expressed darbepoetin alfa was similar with that of Aranesp. In conclusion, we generated a recombinant *L. tarentolae* strain expressing the codon-optimized darbepoetin alfa gene.

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Imbalance between the apoptotic cell death (caspase-3 cleaved) and the cellular proliferation (PCNA) in lacrimal gland of female mice of hyperprolactinemic

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In this study the aim was to investigate the impact of the metoclopramide-induced hyperprolactinaemia on the apoptotic and proliferation in the lacrimal gland of female mice. Then, 20 female/groups: control group (non pregnant, Ctr1): 0.2 mL of saline (vehicle) and the experimental group (non pregnant, HPrl1): 200 μg/day of metoclopramide, dissolved in vehicle. After 50 days 10 females of each group were placed for mating with males and continued to receive treatment. The females non pregnant were euthanasia on 50th day (experimental I) and the females pregnant were euthanasia on 5.5th to 6.5th post-coital day (Ctr2: control group and HPrl2: experimental group). The blood samples were collected for hormone measurements. The lacrimal gland was processed for immunohistochemistry. The results were subjected to statistical test ($p < 0.05$). And results showed decreased immunoexpression of caspase 3 in the non pregnant controls compared to non-experimental group ($p < 0.05$). And increased cell proliferation (PCNA) in the pregnant control group compared to pregnant experimental group ($p < 0.05$). Serum prolactin levels were higher whereas the levels of estradiol and progesterone were lower in the animals that received metoclopramide compared to controls. These results suggest that the metoclopramide-induced hyperprolactinemia altered the cellular activity in lacrimal glands in non-pregnancy and pregnancy as a consequence of the imbalance between the apoptotic cell death (caspase-3 cleaved) and the cellular proliferation (PCNA). It is hypothesized that this effect might be related with decrease in the hormonal production of estrogen and progesterone.

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