

“Endogenous erythropoietin stimulation by CO-breathing and by stabilizing HIF-1 by oral Cobalt application”

Pr. W. Schmidt, Dr. D. Schwenke, (University of Bayreuth, Germany)

PROJECT REVIEW

Among the misuse of erythropoietic stimulating agents, the application of substances that induce EPO gene expression by stabilizing HIF 1 as cobalt (II) ions or carbon monoxide inhalation seems to be widely distributed. For athletes, the advantages of the misuse of both substances are low costs, high erythropoietic effectiveness, and no risk of detection, because they are not yet included into the prohibited list of WADA.

The aim of this project is to demonstrate significant effects of cobalt and CO-inhalation on the erythropoietic system which can be detected by the Athlete Biological Bloodpass and direct methods. Special aims are: 1. to monitor the physiological effects of different doses of cobalt and carbon monoxide on plasma-EPO, Hb-mass, and aerobic performance, 2. to prove if the effects of cobalt and CO can be detected by the recently developed Bayesian probabilistic inference techniques, which are the basis for the athletes biological passport, 3. to develop and evaluate direct detection methods for cobalt-misuse, and 4. to evaluate whether the endogenously produced EPO after stimulation by cobalt and CO differs from normal native EPO.

In a first study the optimal dosage of cobalt and carbon monoxide on endogenous EPO production will be determined. In a second step, application of either cobalt or CO for 3 weeks will provide data on (1.) performance effects of both drugs, (2.) stimulation of endogenous EPO, (3.) changes of hemoglobin mass, and (4) changes of hematological parameters which will be analyzed by the statistics of the athlete biological passport. Cobalt concentration and EPO isoforms will be analyzed in urine and/or blood samples. We expect to develop methods which can be used for the detection of endogenous stimulation of erythropoietin by cobalt and carbon monoxide.

Results and Conclusions:

Introduction: From the mid 40ies until the late 70ies of the last century cobaltous ions (Co⁺⁺) were used as an effective drug to treat anemia of different origins. Due to severe side effects this kind of treatment was replaced by other erythropoietic stimulating agents like recombinant human erythropoietin. Co⁺⁺ acts similarly as a hypoxic stimulus; it stabilizes HIF-1 α and HIF-2 α and thereby increases the endogenous erythropoietin production. Because of its possible performance enhancing effect Co⁺⁺ has been added to WADA's list of prohibited substances and methods. Co⁺⁺ can be easily purchased and it is recommended from suppliers of nutritional supplements to athletes to boost their performance. The aim of this project was to investigate the effects of low dose Co⁺⁺ ingestion and to determine the

minimum oral dosage which is necessary to increase erythropoietic processes.

Methods: Three sub-studies were carried out: I. Single Co⁺⁺ dosages between 1mg and 10mg; II. Co⁺⁺ administration for 5 days with the lowest dosage found to be effective in study I; III. 3-week Co⁺⁺ administration with the lowest effective dosage (5 mg/day). We determined plasma [EPO], all parameters used for the athlete's biological passport (ABP) as well as in study III hemoglobin mass and performance. In total, 63 male recreational athletes participated at the studies which were conducted in a double blind design.

Results and discussion: Study I: Single dosages until 2mg Co⁺⁺ had no erythropoietic effect. Plasma [EPO] increased 5h after the 5mg administration by $18 \pm 14\%$ ($p < 0.05$) and until 7h following the 10mg Co⁺⁺ administration by $41 \pm 15\%$ ($p < 0.001$). Study II: Following the 5-day Co⁺⁺ application 5mg showed no significant effects on any parameter while 10mg increased [EPO] by $28 \pm 26\%$ ($p < 0.05$) and the immature reticulocyte fraction by $50 \pm 21\%$ ($p < 0.001$). [Ferritin] decreased in the 10mg group by $22 \pm 17\text{ng/ml}$, $p < 0.01$). Study III: During the 3-week application period plasma [EPO] increased by $30 \pm 39\%$ ($p < 0.001$) and total hemoglobin mass by $17.1 \pm 16.8\text{g}$ ($p < 0.001$) while Ferritin decreased by $17 \pm 18\text{ ng/ml}$ ($p < 0.01$). Although VO₂max was not affected, submaximal performance ($+11 \pm xy$ Watts at 2 mmol/l lactate, $p < 0.05$) and time until exhaustion ($35 \pm xy$ sec., $p < 0.05$) was slightly improved after the 3-week administration period.

Discussion: Co⁺⁺ administration using dosages 2-5-times above the recommendations of suppliers of nutritional supplements and ~ 2-times above the amount which is considered to be safe and without any side effects in case of life-long daily administration show measurable erythropoietic and performance-enhancing effects. We therefore recommend to install threshold limits for Co⁺⁺ concentrations in urine and in blood to deter and detect blood manipulation by Co⁺⁺ administration.