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Original Article

Rapid hemodilution induced by desmopressin after erythropoietin administration in humans

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ABSTRACT

Sanchis-Gomar F, Martinez-Bello VE, Derbré F, García-López E, García-Valles R, Brioche T, Ferrando B, Ibañez-Sania S, Pareja-Galeano H, Gomez-Cabrera MC, Viña J. Rapid hemodilution induced by desmopressin after erythropoietin administration in humans. *J. Hum. Sport Exerc.* Vol. 6, No. 2, pp. 315-322, 2011. We have shown that treatment with desmopressin has a very effective hemodilution effect in healthy humans. These results led us to suggest the possible role of desmopressin to mask blood doping in sports. Based on our results, the World Anti-Doping Agency included the desmopressin in the 2011 List of Prohibited Substances and Methods. On this occasion, the aim of our study was to test the desmopressin-induced hemodilution after rHuEpo administration in humans. This was an intra-subject, crossover study in which five physically active males acted as their own controls. A basal blood sample was taken on their first visit to the laboratory. The next day, the subjects began the treatment. They received a subcutaneous rHuEpo injection three times/week for a two-week period. On the second visit to the laboratory, seventeen days later, a blood sample was taken. Thereafter, the subjects received an oral dose of 4.3 µg/kg of desmopressin and were instructed to ingest 1.5 liters of mineral water during the following fifteen minutes. Three hours after the water ingestion a second blood sample was obtained. The samples were analyzed for hematocrit (HCT), hemoglobin (Hb), reticulocytes (Ret%) and OFF Hr-Score. We found significantly higher HCT, Hb and Ret% levels after rHuEpo administration. Administration of desmopressin significantly decreased the HCT and Hb values but we did not find significant changes in Ret%. The values of the OFF Hr-Score also decreased after treatment with desmopressin. Desmopressin has a very effective hemodilution effect after rHuEpo administration and significantly modifies the hematological values measured by the anti-doping authorities to detect blood doping. We consider that these results reinforce the conclusions reported in our first study and confirm that desmopressin is a very effective masking agent for blood doping. **Key words:** HEMOGLOBIN, HEMATOCRIT, RETICULOCYTES, STIMULATION INDEX AND PLASMA VOLUME EXPANDERS

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INTRODUCTION

The World Anti-Doping Agency (WADA) implemented the Athlete Blood Passport (ABP) to detect athletes using performance-enhancing drugs such as rHuEpo (Ashenden et al., 2011). This strategy relies on detecting abnormal variations in hematological variables over time to facilitate indirect detection of doping on a longitudinal basis, rather than on the traditional direct detection of doping (Gilbert, 2010). The major physiological effect of the treatment with erythropoiesis stimulating agents, such as rHuEpo, is the increase in hematocrit/hemoglobin values and improvements in aerobic sport performance (Brien & Simon, 1987; Ekblom et al., 1976). Thus, athletes have adopted new strategies to manipulate their blood to adjust their values between the physiological limits (Thevis et al., 2000).

The prohibited list of substances in sports (WADA, 2011) includes a group of masking agents. Masking agents are drugs or compounds that are taken with the express purpose of hiding the presence of specific illegal drugs that are screened for athletic drug testing. Masking agents are prohibited at all times, in and out of competition, and they include diuretics, plasma volume expanders (PVEs) and probenecid (Ventura & Segura, 2010). When an athlete presents hematological values higher than those allowed, a fast way to decrease them is to use PVEs such as albumin, dextran, and/or hydroxyethyl starch (Guddat et al., 2005; Guddat et al., 2008).

Desmopressin, 1-desamino-8-D-arginine-vasopressin, is a synthetic analogue of vasopressin with increased antidiuretic activity and decreased pressor activity (Robinson, 1976). It works by limiting the amount of water that is eliminated in urine and binds to V2 receptors in renal collecting ducts, increasing water reabsorption (Levy, 2008). Desmopressin is used to prevent or control polyuria, polydipsia, and dehydration in patients with central diabetes insipidus (Kim et al., 2004) and in primary nocturnal enuresis (Dimson, 1977).

Our group recently characterized desmopressin-induced hemodilution in humans and its possible implications in blood doping (Sanchis-Gomar et al., 2010b). Based on our results, the World Anti-Doping Agency included desmopressin in the 2011 List of Prohibited Substances and Methods (WADA, 2011).

The main limitation of our first study was that we recruited subjects with physiological blood levels, i.e. subjects who were not previously treated with erythropoiesis stimulating agents such as rHuEpo, darbepoietin, CERA etc. The aim of the present study is to determine the effects of rHuEpo administration and the modifications achieved after desmopressin treatment on hematocrit and hemoglobin values, reticulocytes and the OFF-Hr Score.

MATERIAL AND METHODS

Five physically active male subjects volunteered for this study. All were non-smokers and free of any known illnesses as ascertained by a questionnaire.

This was an intra-subject, crossover study in which the subjects acted as their own controls. All the subjects were required to visit the laboratory on two occasions with an interval of seventeen days. All the subjects were instructed to follow the same dietary and training pattern the week before the analysis. Subjects were instructed to avoid any strenuous exercise for four days prior to the analysis. On their first visit to the laboratory, the subjects were seated for 30 minutes and a basal blood sample was taken in this position. The next day we began the rHuEpo treatments (Figure 1).

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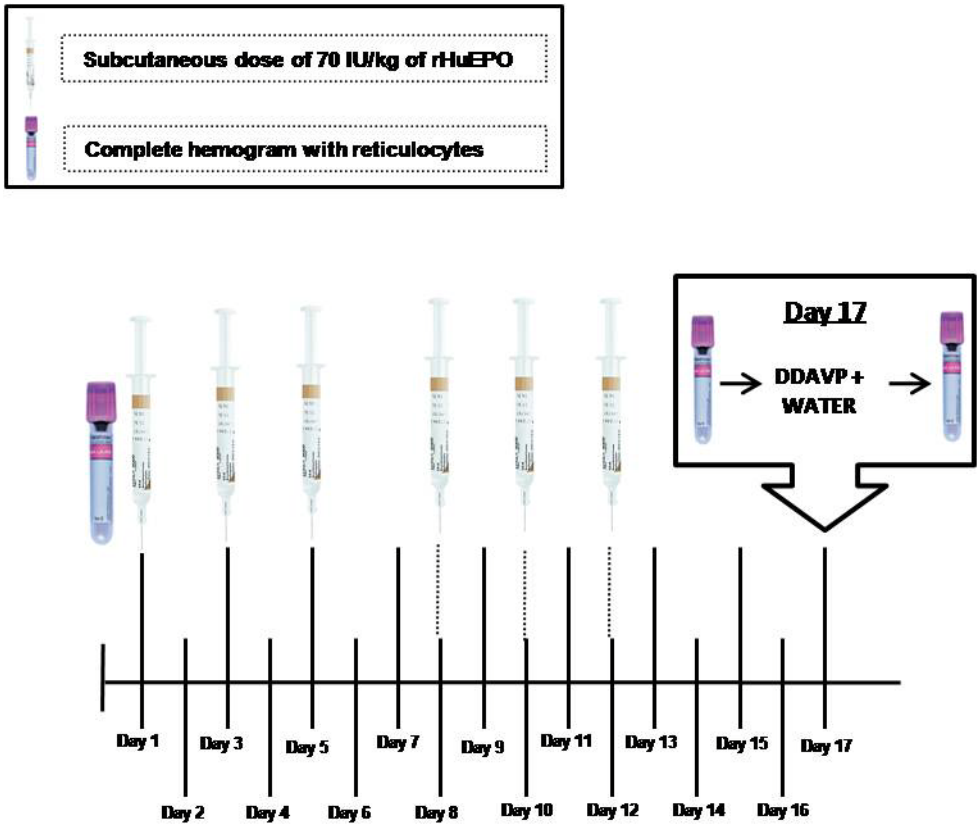

Figure 1. *Design of the study. On their first visit to the laboratory a basal blood sample was taken. The next day, subjects began the treatment. The received subcutaneous rHuEpo injections 3 times/week for a 2-week period. On the second visit to the laboratory, seventeen days after, a blood sample was taken. Thereafter, they received an oral dose of 4.3 μg/kg of desmopressin and were instructed to ingest 1.5 liters of mineral water during the following fifteen minutes. Three hours after the water ingestion, a second blood sample was obtained.*

Recombinant human erythropoietin treatment

The subjects received rHuEpo (Epoetin alpha; Eprex; Janssen-Cilag) injections at a dose of 70 IU/kg for a two-week period (three injections/week); the injections were given subcutaneously (see Figure 1).

The subjects were supplemented daily with iron tablets one week before the study commenced until one week after it terminated (Fero-gradumet; ~105 mg of elemental iron derived from 350 mg of dried ferrous sulphate).

Desmopressin treatment

On day seventeen, all the subjects visited the laboratory at 9 a.m. On arrival the subjects remained seated for 30 minutes and a basal blood sample was taken in this position. Immediately after the sample was taken, the subjects received an oral dose of 4.3 μg/kg of desmopressin and they were instructed to ingest 1.5 liters of mineral water (sodium content, 13.2 mg/L) during the following 15 minutes. Three hours after the water ingestion a second blood sample was obtained.

Therefore, three blood samples (Figure 1) were collected on days 0 and seventeen (on two occasions). All of them were collected at the same time and under the same conditions (temperature and humidity) from a superficial vein in the antecubital fossa while the subjects were seated. The blood samples were divided into three tubes: BD Vacutainer blood collection tubes containing EDTA.

All subjects were informed verbally and in writing about the nature of the study, including all potential risks. The Committee on Ethics in Research of the Faculty of Medicine, University of Valencia, granted ethical approval.

Determinations

Whole Blood analysis: Hematocrit, hemoglobin, and reticulocytes were analyzed within three hours of blood collection using a SYSMEX XT2000i hematology analyzer (Roche Diagnostics). The direct measurements performed by the SYSMEX XT2000i on erythrocytes included: count, size, and total hemoglobin. Direct reticulocyte measurements included the percentage of reticulocytes (R%). The stimulation index was calculated from hemoglobin (Hb in g/L) and reticulocytes (R in %), on the OFF-model score: Hb - 60√R.

Statistical analysis

For the statistical analysis of the results, the mean was taken as the measurement of the main tendency while the standard deviation was taken as the dispersion measurement. According to normality, continuous data were analyzed with the two-tailed paired samples *t*- test. The alpha level for statistical significance was set at $p<0.05$. We used SPSS software (version 17.0; SPSS Inc, Chicago, IL) for all the statistical analyses.

RESULTS

Effect of the administration of 4.3 µg/kg of desmopressin on hemoglobin, hematocrit, reticulocytes and on OFF-Hr Score, after rHuEpo administration.

In Figure 2 we show significantly higher Hb, HCT and Ret% levels after rHuEpo administration demonstrating that rHuEpo injections were effective in our study. The Hb values (g/dL) increased from 15.2 ± 0.6 before rHuEpo administration to 16.6 ± 0.5 ($p < 0.01$) after treatment. The HCT (%) increased from 45.7 ± 1.3 before rHuEpo administration to 49.4 ± 0.7 ($p < 0.01$) after treatment. The percentage of reticulocytes increased from 0.7 ± 0.1 before rHuEpo administration to 1.5 ± 0.4 ($p < 0.01$) after treatment. Finally, the OFF-Hr Score decreased from 101.7 ± 6.8, before rHuEpo administration, to 92.9 ± 10.6.

A statistically significant decrease in Hb and HCT concentration was observed three hours after treatment with desmopressin in all the subjects (Figure 2). The Hb concentration decreased from 16.6 ± 0.5 before the treatment to 15.5 ± 0.5 ($p < 0.01$) post treatment. The HCT percentage decreased from 49.4 ± 0.7 before the treatment to 46.6 ± 1.4 ($p < 0.01$) after the treatment. Although a decrease in Ret% was also found after treatment with desmopressin, this modification was not statistically significant (1.5 ± 0.4 to 1.3 ± 0.5). However, the OFF-Hr Score value, calculated using the Ret%, decreased significantly after treatment with desmopressin, from 101.7± 6.8 to 86.5 ± 10.4 ($p < 0.05$).

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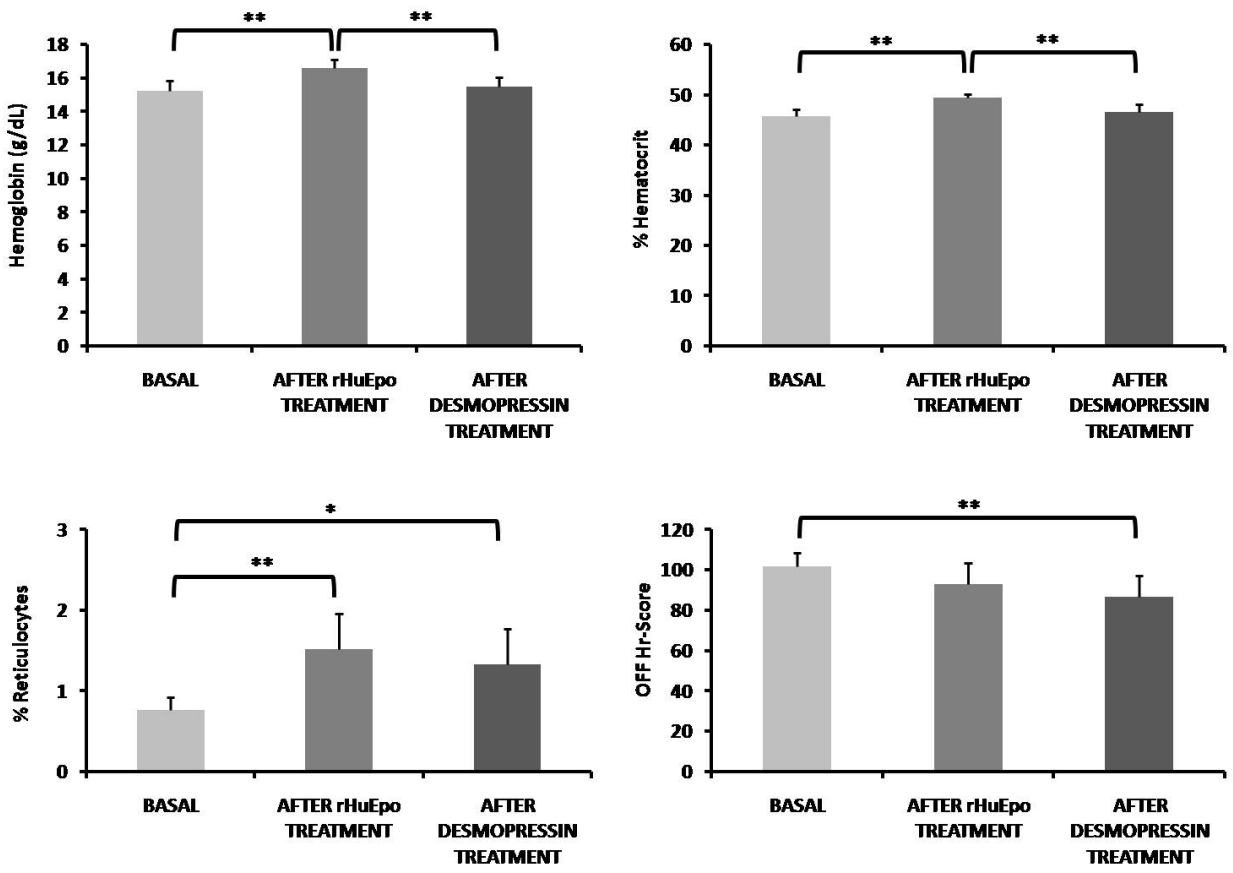

Figure 2. *Hemoglobin, hematocrit, reticulocytes concentration and OFF-Hr Score values after rHuEpo administration and after treatment with desmopressin. Data presented as mean (±SD). Hematological values were measured in basal and after treatment conditions. (*) indicates significant difference ($p < 0.05$, two-tailed paired samples t –test between two groups). (**) indicates significant difference ($p < 0.01$, two-tailed paired samples t –test between two groups).*

DISCUSSION AND CONCLUSIONS

Increasing the blood content by raising hemoglobin/hematocrit values is an easy method to improve oxygen delivery and improve sport performance (Ekblom et al., 1976). Illegal methods for raising hematocrit values include blood doping (infusion of packed autologous, homologous, heterologous, red blood cells) and administration of erythropoiesis stimulating agents such as rHuEpo, darbepoietin, CERA, etc. (Cooper & Beneke, 2008). In view of the long list of agents and techniques in blood doping practices, it is necessary to have a wide range of tests to detect them. The indirect method to detect blood doping is based on the determination of several blood parameters: Hb, HCT, Ret% and in the calculation of the stimulation index (OFF-Hr Score). This index consists of applying a statistical model using two blood parameters: percentage of reticulocytes and hemoglobin (Hb in g/L) on the OFF-model score: $Hb - 60\sqrt{R}$. Both parameters are substantially altered after a period of EPO administration (Gore et al., 2003; Parisotto et al., 2003; Parisotto et al., 2001; Sharpe et al., 2006). The reticulocytes are a crucial parameter in the ABP. Their values increase significantly after rHuEpo injection (Audran et al., 1999; Parisotto et al., 2001) or phlebotomy

(Damsgaard et al., 2006; Morkeberg et al., 2009a), and decrease when rHuEpo treatment ceases (Gore et al., 2003) or blood is re-infused (Morkeberg et al., 2009b). The accuracy of this method for the indirect detection of EPO abuse has been very well documented (Gore et al., 2003; Parisotto et al., 2001; Sharpe et al., 2006). This is why the OFF-Hr Score has been adopted by the anti-doping agencies (Gore et al., 2003).

It has been demonstrated that the combination of PVEs and rHuEpo can be advantageous for the cheaters. PVEs are banned because raising plasma volume can potentially be performance enhancing (preventing dehydration) (Guddat et al., 2008) and its use can mask a rise in hematological values caused by illegal methods. For instance, the members of the Finnish skiing team used hydroxyethyl starch to mask blood doping, prior to the world Nordic ski championship in 2001 (Cooper & Beneke, 2008).

Our results show a very significant increase in Hb, HCT, and Ret% after treating our subjects with rHuEpo at a dose of 70 IU/kg for a two-week period. This was accompanied by a significant decrease in the OFF-Hr Score after the treatment. Administration of 4.3 μg/kg of desmopressin + 1.5 liters of water was sufficient to restore the Hb and HCT values to almost the basal levels. Regarding reticulocytes, the administration of desmopressin+ water decreased its value (slightly from 1.5 ± 0.4, after rHuEpo treatment, to 1.3 ± 0.5 after desmopressin treatment) although the basal levels were not achieved (0.7 ± 0.1). Finally the OFF-Hr Score decreased significantly after treatment with desmopressin in combination with water. It has been reported that intravenous infusion of Gelofusine (a PVE), in recreationally active men, results in a significant reduction in Hb concentration (g/dL) (16.0 preinfusion vs. 14.7 postinfusion; $p < 0.001$) and in the HCT (%) (44.0 preinfusion vs. 41.0 postinfusion; $p < 0.01$) (Berger et al., 2006). Taking these values into account we consider that our results confirm that treatment with desmopressin is similar to the administration of a PVE (Sanchis-Gomar et al., 2010b).

In a recent paper we demonstrated the hemodilution effect of demospressin in a group of healthy subjects not being treated with erythropoiesis stimulating agents (Sanchis-Gomar et al., 2010b). The novelty of the present study lies in the demonstration that desmospressin has a very effective and fast hemodilution effect in a group of subjects treated with rHuEpo. Consequently, we consider that these results reinforce the main conclusion reported in our first study (Sanchis-Gomar et al., 2010b) and confirm that desmopressin is a masking agent for blood doping in sports.

In our study published in 2010, we concluded that the hemodilution effect of the treatment (desmopressin+1.5 liters of water) was due to the properties of the drug and not to the water. We administered 1.5 liters of mineral water to the subjects and no modifications in the hematological parameters tested were found (Sanchis-Gomar et al., 2010b). Therefore, our main conclusion is that treatment with 4.3 μg/kg of desmopressin and 1.5 liters of water, after rHuEpo administration, significantly decreases the hematological values measured by the anti-doping authorities to detect blood doping. This fact is extremely important. In 2009, the UCI's Biological Passport programme collected 6,165 blood samples but only 2,165 accompanying urine samples were tested for rHuEpo (Zorzoli & Rossi, 2010). Only if an athlete has an irregular blood value. The anti-doping authorities perform anti-EPO urine analyses (Lasne, 2001). Thus finding new hematological variables or methods resistant to plasma volume changes is crucial to detect blood doping (Sanchis-Gomar et al., 2010a).

Statement

None of the authors has any conflicts of interest with the funding agencies or professional relationships with companies or manufacturers who may benefit from the results of the present study.

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