Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (10)

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Sport und Buch Strauß, Köln, 2002

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Urinary Charge Patterns of Recombinant Human Erythropoietin during Controlled Administration in Athletes
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (10). Sport und Buch Strauß, Köln, (2002) 245-247

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Urinary charge pattern of recombinant human erythropoietin during controlled administration in athletes

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Background

Well-trained male athletes received 5000 U rhEPO three times weekly in one month (epoetin beta,181-232 U/Kg wk) in a double blind, placebo controlled study. During EPO administration the hemoglobin concentration increased from 15,5 to 17,5 g/dl (figure 1) and maximal oxygen uptake increased 7 % (2).

Summary

Isoelectric focusing (IEF) with immunoblotting (1) was used to screen urinary samples from well-trained male athletes during a month administration of rhEPO (epoetin beta). Urine samples obtained from three athletes in the EPO-receiving group (N=10) and one from the control group (N=10) were analysed with respect to the urinary charge pattern of EPO on IEF. In the treated subjects, a significant change in acidity of the urinary EPO glycoforms was observed during the four weeks of rhEPO administration.

Methods

Urine samples from athletes both in the EPO-receiving group and the control group were collected during the treatment (days 1-30) and post-treatment period (days P1-P28). The urine samples were concentrated 500 times and analysed with respect to the EPO charge pattern on IEF (pH 2-6) and detected by immuno-blotting (1) and a CCD (Charge coupled device) camera (LAS-1000 Plus). A cut off-value was defined by means of the most acidic form of rhEPO. % basic glycoforms were calculated as a measure of the relative amount of rhEPO in the urine samples, above the cut off-value.

Results

One week after start of the subcutaneous rhEPO injections, the IEF pattern of EPO had changed to a more basic one (figure 2). The urine samples obtained after two weeks revealed charge patterns that were compatible with rhEPO. Until day 5 after the last injection, the IEF patterns were similar to rhEPO. Then the EPO glycoforms gradually became more acidic. Analysis of the urine samples from day 14 after the treatment was stopped showed an acidic IEF-pattern of hEPO corresponding to the charge profile of normal urine.

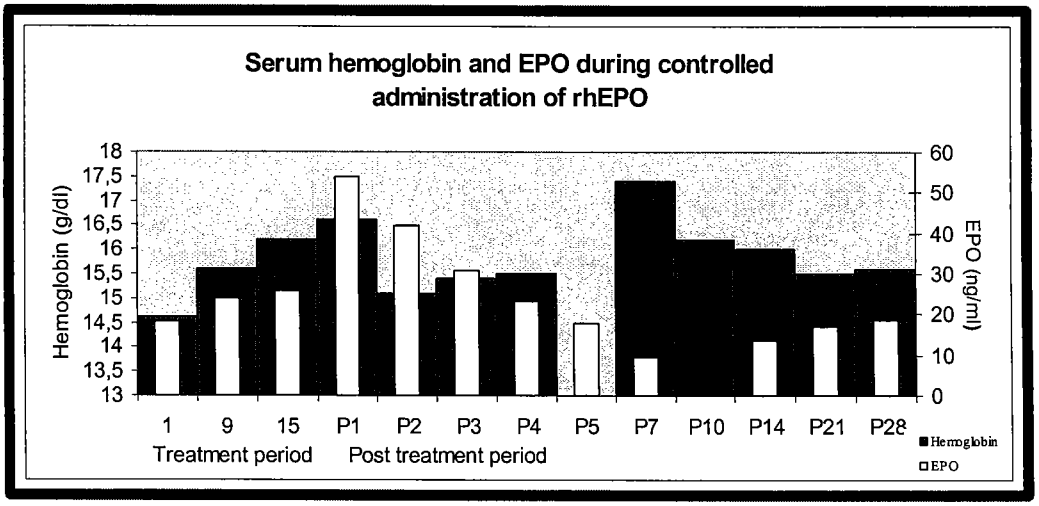

Figure 1. Serum hemoglobin and EPO during controlled administration of rhEPO

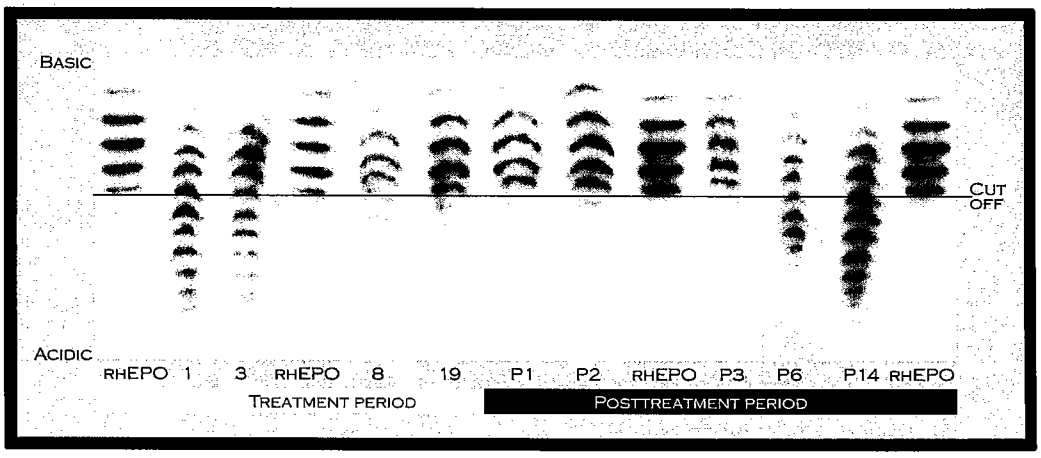

Figure 2. IEF patterns of urinary EPO from treated athletes.

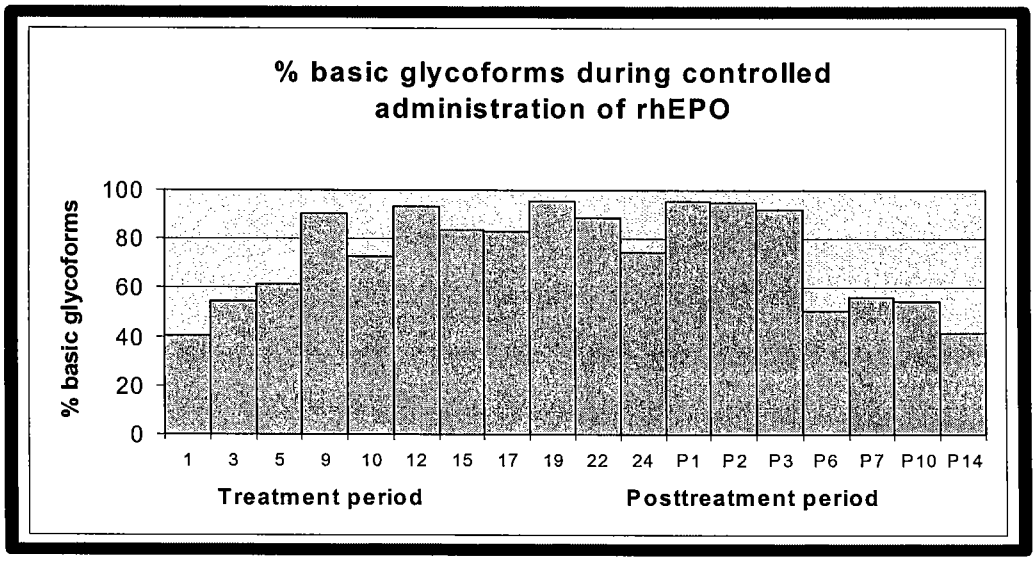

Figure 3. The % basic glycoforms calculated are related to a standard preparation of rhEPO

Conclusion

The urinary charge patterns of EPO from treated athletes compared to the control group were significantly different. For the treated subjects the time-course for the increase in % basic glycoforms, correlated with the increase of hemoglobin (figure 1 and 3). However, the hemoglobin levels stayed elevated at least until 3 weeks post administration. This was in contrast to the serum concentration of EPO that decreased rapidly, consistent with the decrease in the % basic hEPO glycoforms in the urine (figure 1 and 3). The study showed that detection of rhEPO in urine is possible at least 3 days after the end of treatment.

References

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2. Birkeland, K. I. et al. Effect of rhEPO administration on serum levels of sTfR and cycling performance. Med. Sci Sports Exerc. 1238-1243, 2000.