Blood Doping

The effects of microdose recombinant human erythropoietin regimens in athletes

This study appraised the veracity of claims that athletes can evade doping controls by injecting *microdoses* of recombinant human erythropoietin (rHuEPO), which rapidly disappear from the circulation. We confirmed that microdosing can reduce the window of detection to as little as 12-18 hours post-injection, suggesting that authorities must adopt appropriate counter measures.

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Athletes can illicitly use rHuEPO to boost red cell mass and thereby oxygen carrying capacity and endurance performance.[1] There are persistent suggestions that athletes have learnt to use rHuEPO, but test negative, by titrating rHuEPO dosage regimens in order to minimize the appearance of basic isoforms in urine samples (rHuEPO can be detected via electrophoresis because rHuEPO isoforms are more basic than endogenous erythropoietin isoforms).[2]

It is vital for antidoping agencies to determine whether existing deterrent strategies have been circumvented. To establish whether it is possible to confound detection strategies by titrating rHuEPO dosages, our study simulated a so-called 'microdose' rHuEPO regimen and measured the level of basic isoforms in urine collected during and after the administration protocol.

Two well-trained male subjects (28 years old, 74 kg, 176.5 cm, regional level triathlete; 31 years old, 62 kg, 170 cm, national level long distance runner) gave informed consent to participate in this study which was reviewed and approved by the Regional Ethics Committee. Initially red cell production was rapidly accelerated in both subjects using high doses of rHuEPO (~260 IU/kg injections on days 0, 2, 4, 7, 9 and 11) in conjunction with a single intravenous iron treatment (100 mg), with the goal to elevate hemoglobin (Hb) concentration to approximately 170 g/L.

Over the next 3 weeks, injections were given every 2-3 days (injections on days 15, 17, 19, 22, 24, 26, 29, 31 and 33) and dosages were adjusted by a pharmacologist guided only by basic hematologic information (blood and reticulocyte counts, no urine profiles were provided as feedback). Microdosages were less than 10% of the initial dose (exact dosage undisclosed to prevent replication by athletes). Urine samples were collected three times per day during the microdose phase (7-9h, 11-13h, 19-21h), and analyzed for the presence of rHuEPO at the French national antidoping laboratory (Laboratoire National de Dépistage du Dopage, Paris).

As expected high dose rHuEPO treatment rapidly elevated Hb concentrations within ~2 weeks (140 to 166 g/L; 148 to 174 g/L; subjects 1 and 2, respectively). We found that it was possible to maintain elevated Hb values using microdoses of rHuEPO. After 3 weeks of the microdose regimen Hb concentrations were still 164 g/L and 170 g/L respectively (and 164 g/L and 162 g/L 1 week after all injections ceased). During the microdose phase reticulocyte percentages ranged in value from 0.8-1.2% and 0.4-1.1% for the two subjects. Urine samples collected more than 24 hours after a microdose injection typically had less than 80% basic isoforms, which until recently was the criterion used to declare a sample positive (Figure 1).

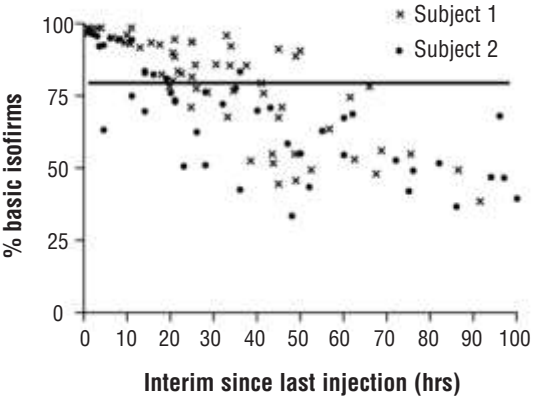

Figure 1. The percentage of basic isoforms in urine samples collected at various intervals after each of the nine *microdose* injections of rHuEPO. Injections were given over a 3-week period during which time both subjects maintained an elevated haemoglobin concentration of approximately 170 g/L. The horizontal line depicts an 80% threshold level that has been used previously to declare a sample positive for the presence of rHuEPO.

In some instances samples collected just 12-18 hours after the last injection fell below the 80% threshold. It is noteworthy that our pharmacologist was able to quickly devise an effective microdose regimen utilizing limited feedback and with few prior attempts.

Interestingly, isoelectric profiles showed the reappearance of endogenous erythropoietin bands during the microdose phase (*results not shown*). This is in contrast to the existing paradigm which holds that endogenous erythropoietin production is suppressed when the red cell mass has been increased beyond the homeostatic set point.

The implications of this remain unclear, however it can be speculated that were an athlete to receive microdoses of rHuEPO for an extended period (>2-4 weeks), it is conceivable that reappearance of endogenous bands of erythropoietin would be of sufficient magnitude to further reduce the effective window of detection of the test for rHuEPO.

Our results show that it is conceivable for athletes to maintain illicit rHuEPO doping even during multiday endurance events when competitors may be tested at the end of each day (ie at 24 hour intervals). The electrophoretic test has proven legally defensible and remarkably robust.

The recent adoption of improved detection criteria further enhances the discriminatory capacity of the urine test,[3] although whether this carries over to microdose samples awaits further research. The fact that microdoses of rHuEPO disappear rapidly from the circulation could be exploited by athletes to evade detection. This implies that authorities should supplement the urine test with an approach providing greater

reach-back. This research also sharpens awareness that, to be efficient, urine tests should be based on out of competition testing.

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