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**Detection window of CERA abuse through haematology profiles post single injection**

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**Abstract**

BACKGROUND: CERA (Continuous erythropoietin receptor activator) is a PEG (polyethylene glycol) linked erythropoietin preparation, which was designed for treating anemia. With the biological passport program pursued by the World Anti-Doping Agency recent years, an integrated evaluation of all the parameters and variables in both blood and urine specimen post CERA injection could be helpful.

OBJECTIVE: Our work presents a comparison of changes in haematology parameters between single injections of CERA and a Chinese produced recombinant erythropoietin.

METHOD: 4 volunteers were subcutaneously injected a single dose of Mircera\textsuperscript{®} (150 µg). Another 4 volunteers were treated with Yibiao\textsuperscript{®} at a comparable dosis (10,000 IU). Urine and blood samples were collected pre and post administration. Biological Passport parameters were determined immediately after blood samples were collected. Additionally, soluble transferrin receptor, CERA and total erythropoietin were measured in sera.

RESULTS: The concentration of both pharmaceuticals peaked and declined as expected, and a significant individual difference was observed for one of the volunteers. Obvious alterations were observed in some of the monitored haematology parameters.

DISCUSSION: Differences between the two preparations were observed. By combining with our previous work on urine IEF profiles post CERA injection, the detecting window of CERA could be could be more specifically described.

**Introduction**

Continuous erythropoietin receptor activator (CERA) is a polyethylene glycol (PEG) linked erythropoietin preparation, which was designed for treating anemia but has been abused in endurance sports sometimes. The classic IEF plus western blot method for detecting recombinant EPO and its analogues could be used as screening and confirmation procedures, while other specifically designed approaches such as Sarcosyl-PAGE and the made-for-purpose ELISA kits also proved effective \cite{1,2}. In the meantime, the Athlete Biological Passport (ABP) was pursued by WADA. Hence, an integrated evaluation of ABP haematology parameters post CERA injection could be helpful. Our work presents a comparison of haematology parameter profiles between single injections of CERA and a Chinese produced recombinant erythropoietin.

**Experimental**

4 volunteers were subcutaneously injected a single dose of Mircera\textsuperscript{®} syringe, while other 4 were injected Yibiao\textsuperscript{®} with at a comparable dosis. All volunteers were between 20 and 41 years of age and with a BMI between 20-28. The volunteers’ gender, dosage, and sample collection information is listed in Table 1.

Biological Passport parameters were determined immediately after blood samples were collected. Soluble transferrin receptor, CERA, and total erythropoietin concentrations were measured by ELISA kit (R&D, DTFR1; MicroCoat Biotechnologie GmbH, 609008) and DPC Immulite system (Siemens, Immulite 1000, LKP1), respectively. All blood parameters were calculated using the ABP software.
Results and Discussion

The results calculated with second and third generation [3,4] haematology formulas are presented in Figure 1 and results from ABP software are presented in Figure 2. With single injection of CERA, few samples could give a positive result with the on-model of the second generation formula; and only one sample from CERA injection group has a z-score of 3.09 which meet the third generation formula positive criteria. The off-modules use RET% as a marker are more sensitive. According to the ABP software [5], all volunteers from CERA group showed a positive result with both RET% and off-HR scores. But in the EPO group, male volunteers showed higher positive possibility than females, and none of them reach the recommended criteria of 99.9% possibility. On the contrary, HGB levels of most of the volunteers could be considered steady during the study; only volunteer 4 got a result of 87% possibility with it. This might due to the fact that none of the volunteers were given any supplements regularly after the injection.
Figure 1: Score results of second and third generation haematology parameters assays.

\begin{align*}
\text{ON-he} &= \text{Hb} + 9.74 \ln(\text{EPO}) \\
\text{ON-hes} &= \text{Hb} + 6.62 \ln(\text{EPO}) + 19.4 \ln(\text{sTfr}) \\
\text{OFF-he} &= \text{Hb} - 50 \sqrt{\text{Ret}} - 7 \ln(\text{EPO}) \\
\text{OFF-hr} &= \text{Hb} - 60 \sqrt{\text{Ret}}
\end{align*}

- \text{z-score} = \frac{\text{Current mean}/\text{Control mean}-\text{subject variance}^*}{1+\ln(1)}
- \text{Criteria of } z \text{ score exceeding 99\% threshold was 3.05}

Figure 2: HGB, RET\% and Off-HR scores from ABP software.
To find a proper test frequency, some of the data were deleted to simulate a testing period. With a 5 days gap between tests, starting from 3 days after injection, the module could easily find the profile suspicious after 3 tests and gave a positive result after 6 tests. And when the second time gap prolonged to 20 days, or the first test start from 9 to 14 days after the injection, RET% and OFF-HR score could still report the profile suspicious. These results are presented in Figure 3.

Figure 3: RET% scores with selective data from ABP software.
Conclusions

Compared with the classic urine IEF test detection window of our previous study [6], calculating blood parameters with the ABP software resulted in a longer period of CERA detection. Also the ABP method was more sensitive detecting CERA abuse than Chinese produced recombinant erythropoietin. However, as none of the volunteers involved in this study were elite athletes, their haematology parameters could be different. Additionally, most of the formulas were based on a data pool where the majority was from non-Asian individuals, which might explain that some of the modules were less sensitive than expected.

References


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