Effects of low-dose recombinant human erythropoietin treatment on cognitive performance

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ABSTRACT

INTRODUCTION: High-dose recombinant human erythropoietin (rhEpo) has been shown to improve cognitive performance in both healthy volunteers and in patients suffering from diseases affecting the brain. The aim of this study was to examine whether administration of low-dose and even micro-dose rhEpo improves cognitive performance in healthy volunteers.

METHODS: We enrolled 25 healthy volunteers in a double-blind, randomised, placebo-controlled study to receive either low-dose rhEpo (n = 8, 60 IU/kg/week), micro-dose rhEpo (n = 9, 20 IU/kg/week), or saline (n = 8) for 4 weeks. Two cognitive performance-tests, the Raven Standard Progressive Matrices (Raven) and the Number Finder (NUFI), were performed during the first and last day of the study period. Semi-structured interviews were conducted weekly and were coded according to a scale.

RESULTS: Subjects receiving micro-dose rhEpo improved significantly measured by the Raven score (p = 0.04), and subjects receiving low-dose rhEpo treatment improved significantly measured by the NUFI score (p = 0.047), whereas no improvement was found in experienced cognitive performance in any of the groups. We found no significant difference in either Raven, NUFI or self-reported results between the groups.

CONCLUSIONS: In this small study, we found no significant effect of low-dose or micro-dose rhEpo on visual attention, cognitive performance in complex cognitive tasks or self-experienced cognitive performance compared with placebo.

FUNDING: The Aase and Ejnar Danielsen's Foundation. Danish Ministry of Science, Innovation and Higher Education.

TRIAL REGISTRATION: ClinicalTrials.gov identifier: NCT03093506

Recombinant human erythropoietin (rhEpo) has a well-known stimulating effect on the erythropoiesis [1], but it also has effects on neuroprotection, brain development and cognitive performance [2, 3].

In studies in mice, Epo and Epo receptors have been found to be expressed in the brain [4], and when these receptors are activated, cognitive performance improves [5]. It has been shown that rhEpo improves the cognitive performance in healthy human volunteers [2]. Furthermore, it has been shown that high-dose rhEPO has a positive effect on cognitive dysfunction in patients suffering from depression and bipolar disorder with effects on sustained attention, speed of complex information processing and working memory accuracy [6-8]. In patients with schizophrenia, long-term high-dose rhEpo administration also seems to improve cognitive performance [9, 10], whereas no improvement has been found in patients suffering from multiple sclerosis [11, 12].

RhEpo crosses an intact blood-brain barrier in a dose-dependent manner [13]. Most studies investigating the relationship between rhEpo treatment and cognitive performance have used high doses of rhEpo (30,000-40,000 IU). It is not clear whether rhEpo in lower doses also improves the cognitive performance. One study using low-dose rhEpo (5,000 IU/week) failed to show any cognitive effect [14]. In a recent study from our unit, low-dose rhEpo (5,000 IU/week) was administered to healthy subjects who in general experienced an improved cognitive performance [15]. The possible changes in cognitive performance were, however, not measured directly.

We hypothesised that 4-week administration of low-dose rhEpo (60 IU/kg/week) and even micro-dose rhEpo (20 IU/kg/week) improves the cognitive performance of complex cognitive tasks, tasks involving visual attention and self-experienced cognitive performance in healthy volunteers.

METHODS

Subjects

A total of 25 men volunteered to participate. The inclusion criteria were: healthy males between 18-45 years of age, a normal medical examination and weekly exercise for 0-5 hours. The exclusion criteria were: daily smoking, previous use of performance-enhancing drugs, elite athletes and presence of cardiovascular or metabolic disease. All subjects agreed to maintain their level of physical activity unchanged throughout the trial period. The primary endpoint was improvement in mitochondrial function, whereas the cognitive improvement presented in this article was a secondary endpoint.

Subjects were informed about the possible risks and discomfort involved before giving their written consent to participate. The study was conducted according to

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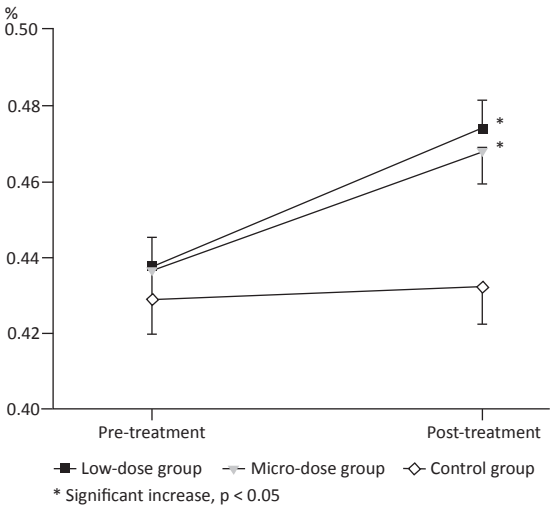
Dan Med J 2017;64(9):A5403

FIGURE 1

Haematocrit values of the groups before and after treatment.



the Declaration of Helsinki and was approved by the Ethical Committee of Copenhagen (H-1-2011-098 and H-1-2011-099).

Experimental design

The subjects were randomly allocated for either low-dose rhEpo (NeoRecormon Roche, Schweiz, 60 IU/kg/week, n = 8, "Low-dose Group"), micro-dose rhEpo (20 IU/kg/week, n = 9, "Micro-dose Group") or saline (n = 8, "Control Group") in a double-blind between-groups design. A few days prior to treatment start, the subjects performed incremental cycling to determine their maximal oxygen (VO2max) uptake. The groups were randomised in strata according to their VO2max. Prior to rhEpo/saline administration on the first day and after the 4 weeks of treatment, the subjects underwent two cognitive performance tests; the Raven Standard Progressive Matrices (Raven), and the Number Finder (NUFI). The cognitive performance tests were administered using a computerised version coined APRO30 (Ability Profile) developed by Marine Profile (Halmstad, Sweden). Weekly semi-structured interviews were conducted by a blinded interviewer. All interviews and performance tests were carried out in the same room and under highly similar conditions. An observer was present in the room during the computer tests, and interference from the sorrounding environment was avoided.

Epo treatment

The first doses of rhEpo/saline were administered subcutaneously on three consecutive days in order to achieve a steady-state serum concentration. Hereafter, the weekly dose was divided into two injections per week for 4 weeks. The rhEpo was dissolved into 0.7 ml of saline. Once a week, a venous blood sample was drawn to assess blood haematocrit (Radiometer, ABL 80, Denmark). If the haematocrit reached ≥ 50%, the rhEpo dose was reduced to maintain the haematocrit at ~ 50% in order to minimise the risk of thrombosis. The subjects were instructed to take daily iron supplements of 100 mg, starting two weeks prior to the rhEpo treatment and continuing throughout the study period.

Cognitive evaluation

The Raven test

The Raven test is a non-verbal test based on figural stimuli. It contains complicated pattern solving and measures the reasoning and problem solving ability and the ability for visual analysis. The Raven test consists of 60 items, each containing a 3 × 3 matrix, in which one field is blank. The subject is asked to find the missing pattern from eight suggestions. The level of complexity increases throughout the test process.

All subjects were instructed in the same way and with the same words that they would be evaluated on both speed and accuracy. A programme calculated a score from 1 to 9 (best) for both speed and accuracy. Evaluation of this test was based on the sum of the two scores (ordinal scale, 2-18).

The Number Finder test

The Number Finder (NUFI) test measures the ability to seek information visually as well as the ability to perceive and organise visual information. In a matrix of 64 fields, each field is numbered randomly from 1 to 64. Each field is also identified with a corresponding number. When a number is shown on a screen, the subject must visually locate it in the matrix and enter the corresponding number. The subject has five minutes to find and enter as many numbers as possible. We evaluated this test on a ratio scale by counting the number of correctly found fields during the five-minute test period.

Interviews

The weekly semi-structured interviews were transcribed and any change in experienced cognitive performance was rated by three independent and blinded raters as suggested by Kvale and Brinkmann [16]. A scale ranging from -3 to 3 was used for the rating (-3: High experienced decrease in cognitive performance, 0: No change, 3: High experienced improved cognitive performance). When in doubt, the final score was based on a discussion between the raters. The subjects' responses were coded to identify what parameters the subjects were referring to when reporting experienced differences in their cognitive performance, e.g. ability to concentrate, or memory.

In order to assess different aspects of cognitive per-

formance, we decided to make use of both the Raven test, the NUFI test and self-reporting. We chose the Raven test because it has high validity and reliability, it is very often used for cognitive screenings and focuses on complex cognitive thinking. By evaluating speed and accuracy, we focused on the ability to perform a cognitive task under self-imposed time pressure given that the instruction for the test was to perform as fast and accurate as possible [17]. We chose a visual perception test, NUFI, because it measures the ability to perceive and organise visual information [18]. Finally, we chose self-reporting since qualitative information can give more precise information as to how the subjects experience the changes in cognitive performance and what kind of changes they experience.

Statistical analysis

The data are presented as mean ± standard error of mean (SEM) or as median [range]. For the NUFI test, one-way ANOVA was used to compare the groups' changes in score, and paired t-test was used to test for changes within each group. The scores from the Raven test and the interviews were not considered normally distributed, and non-parametric tests were therefore used. Specifically, the Kruskal-Wallis one-way ANOVA was used to compare the groups' changes in score, and the Wilcoxon Signed Ranks test was used to test for changes within each group. One-tailed p values were used. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 20.0).

Trial registration: ClinicalTrials.gov identifier: NCT03093506.

RESULTS

A total of 25 healthy men (age 27 ± 1.3 years, height 183 ± 1.3 cm, weight 82 ± 2.3 kg, Body Mass Index (BMI) 25 ± 0.6, VO_2max 3,518 ± 90 ml O_2/min) participated in this study. The three groups compared well. The total rhEpo-dose administered was ~32,000 IU/subject for the Low-dose Group and ~14,000 IU/subject for the Micro-dose Group. The weekly given dose after the initial three consecutive doses was ~5,000 IU/week for the Low-dose Group and ~2,200 IU/week for the Micro-dose Group.

One subject from each rhEpo treatment group received less than half the planned amount of rhEpo due to a high haematocrit value, and one dose was missed in another three subjects receiving low-dose and one receiving micro-dose rhEpo.

Blood tests

Before the treatment period started, the haematocrit (%) was 43.8 ± 0.8 for the Low-dose Group, 43.7 ± 0.7 for the Micro-dose Group and 42.9 ± 0.9 for the Control Group. The haematocrit increased significantly for the Low-dose Group and the Micro-dose Group, whereas the haematocrit for the Control Group remained the same during the treatment period (**Figure 1**).

TABLE 1

Raven Standard Progressive Matrices-scores.

		Score			
Group	n	pre-treatment, mean ± SEM	post-treatment, mean ± SEM	difference, median (range)	p-value[a]
Low-dose	8	14.3 ± 0.6	15.0 ± 0.8	0.5 (–1-3)	0.07
Micro-dose	9	13.4 ± 0.5	14.1 ± 0.6	0 (0-2)	0.04
Control	8	14.0 ± 0.2	14.3 ± 0.3	0 (–1-1)	0.18

SEM = standard error of the mean
a) Within-group differences (1-tailed).

TABLE 2

Number Finder-scores: correctly found fields.

		Score, mean ± SEM			
Group	n	pre-treatment	post-treatment	difference	p-value[a]
Low-dose	8	43.0 ± 3.6	47.1 ± 4.6	4.1 ± 2.1	0.047
Micro-dose	9	43.0 ± 2.6	41.6 ± 2.8	–1.4 ± 2.0	0.25
Control	8	40.6 ± 2.3	42.4 ± 3.0	1.8 ± 1.5	0.14

SEM = standard error of the mean
a) Within-group differences (1-tailed).

Raven

The scores before the initiation of rhEpo-treatment were not significantly different between the groups. After the treatment period, a significant improvement was only found for the Micro-dose Group (p = 0.04) (**Table 1**). However, the improvements observed in the three groups were not significantly different (p = 0.36).

Number Finder

The scores before the treatment period were not significantly different between the groups. After the treatment period, a significant improvement was found only for the Low-dose Group (p = 0.047) (**Table 2**). There was no significant difference in the improvements between the groups (p = 0.07).

Interviews

Nine subjects experienced improved cognitive performance and four subjects experienced decreased cognitive performance during the treatment period. In the Low-dose Group, 50% of the volunteers (four subjects) experienced improved cognitive performance, whereas this was the case in only 33% (three subjects) in the Mi-

TABLE 3

Semi-structured interviews-scores[a]: number of subjects with a given score for experienced change in cognitive performance.

	Score						
Group	**– 3**	**– 2**	**– 1**	**0**	**1**	**2**	**3**
Low-dose	0	0	1	3	2	1	1
Micro-dose	0	0	1	5	1	1	1
Control	0	1	1	4	1	1	0

a) Score – 3 = high decrease; score 0 = no change; score 3 = high increase.

cro-dose Group and 25% (two subjects) in the Control Group (**Table 3**). Two of the subjects participating in this study were given the highest possible score, 3 (high improvement in experienced cognitive performance), and they were in the Low-dose and the Micro-dose Group, respectively. However, none of the groups improved significantly in self-experienced cognitive performance (Low-dose Group p = 0.07, Micro-dose Group p = 0.10, Control Group p = 0.50), and there was no significant difference in the improvements between the groups (p = 0.26).

The results from the interviews suggested that those subjects who experienced an improved cognitive performance experienced the following: improved energy level, improved attention in focusing when performing complex reading tasks, improved ability to categorise and organise thoughts, better structure in thoughts, facilitation in decision-making processes and improved ability to maintain task focus. The subjects experiencing decreased cognitive performance experienced decreased energy level, decreased ability to maintain task focus and decreased memory.

DISCUSSION

Compared with placebo, we found no improvement of cognitive performance in complex cognitive tasks (Raven test), visual attention (NUFI test) or self-experienced cognitive performance in the two rhEpo-treated groups despite increased haematocrit levels in both. However, there was a within-group improvement in Raven score in the Low-dose Group and a within-group improvement in NUFI score in the Micro-dose Group. Some volunteers in each group experienced improved energy level, attention in focusing and ability to maintain task focus. However, this was not significantly different between the groups.

Previous studies have shown that in cases of anaemia, rhEpo improved cognitive performance by correcting the haematocrit [19]. In people without anaemia, high-dose administration of rhEpo (40,000 IU) has been shown to increase hippocampal activation during visual memory tasks [20] and to improve verbal fluency in human volunteers, also in the absence of haematological changes [2]. The impact of rhEpo on cognition may be due to expression of Epo receptors in the brain, even though it is known that Epo receptors are less expressed in healthy brains than in injured brains [2, 20]. There are several serious side-effects of rhEpo therapy [8, 10], and it is therefore important to consider the lowest effective dose. Only few human studies have examined the cognitive effects of low doses of rhEpo. So far, low-dose rhEpo treatment has, however, not been found to improve cognitive performance [11, 14]. Our findings support that without anaemia, low-dose rhEpo may not be sufficient to improve cognitive performance in healthy volunteers.

The primary limitation of the present study is the small sample size. This is due to the fact that the cognitive endpoint was not the primary endpoint. Accordingly, the conclusions of this study are hypothesis-generating rather than fact-establishing. This study was inspired by an earlier study from our unit [15] that was based on a small (n = 6), very homogenous group. However, the present study was made in a more heterogeneous and randomised group of healthy men with very different physical fitness levels and very different educational and socioeconomic backgrounds, since one of the aims of this study was investigation of the external validity in relation to the earlier study. However, the diversity of the group resulted in large standard deviations that made it less likely to detect small changes in cognitive performance.

CONCLUSIONS

In this small study, we found no significant effect of low-dose or micro-dose rhEpo treatment on visual attention, cognitive performance in complex cognitive tasks or self-experienced cognitive performance compared with placebo. To conclude whether low-dose rhEpo treatment has any effect on cognitive performance, further studies with larger sample sizes are needed.

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ACCEPTED: 12 July 2017.
CONFLICTS OF INTEREST: Disclosure forms provided by the authors are available with the full text of this article at www.danmedj.dk.

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