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MODERN RECOMBINANT ERYTHROPOIETIN'S DRUGS AS THE PHARMACOLOGICAL INNOVATIONS FOR SPORTS MEDICINE

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The role of glycoprotein hormone of cytokines group erythropoietin in the regulation of erythropoiesis and mechanisms of the human body tissue oxygenation discussed in this article. Increasing the oxygen capacity of the blood with the help of endogenous erythropoietin markedly improves physical performance. This was the impetus for innovative scientific research to create recombinant human erythropoietin analogues by genetic engineering, which are successfully used in sports medicine as doping. The advantages and disadvantages of recombinant erythropoietin preparations, methods of detection and methods of concealment in doping control.
Keywords: erythropoietin, sports medicine, doping

СОВРЕМЕННЫЕ РЕКОМБИНАНТНЫЕ ПРЕПАРАТЫ ЭРИТРОПОЭТИНА – ФАРМАКОЛОГИЧЕСКИЕ ИННОВАЦИИ ДЛЯ СПОРТИВНОЙ МЕДИЦИНЫ

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Рассмотрена роль гликопротеинового гормона группы цитокинов эритропоэтина в регуляции эритропоэза и механизмов оксигенации тканей организма человека. Увеличение кислородной емкости крови с помощью эндогенного эритропоэтина выраженно повышает физическую работоспособность. Именно это стало стимулом для инновационных научных разработок по созданию рекомбинантных аналогов человеческого эритропоэтина путем генной инженерии, которые успешно применяются в спортивной медицине в качестве допинга. Рассмотрены преимущества и недостатки рекомбинантных эритропоэтиновых препаратов, методы их обнаружения и способы маскировки при допинг-контроле.
Ключевые слова: эритропоэтин, спортивная медицина, допинг

Dopes are drugs and methods that are used by athletes in order to improve efficiency during the training process and competitions. They can have a completely different and even opposite pharmacological effects (depending on the sport): from awareness-inducing to tranquilizing, from diuretic to cardiotropic. One of the most difficult to define dopes can be called erythropoietin dope [5].

General information about erythropoietin

Erythropoietin is a glycoprotein hormone (a cytokine precisely). It is main regulator of erythropoiesis: it stimulates the production of red blood cells from late progenitor cells (or rather erythropoietin binds to erythropoietin-sensitive receptors that are located predominantly on erythroblasts, and promotes proliferation of blastic active forms) and increases the outlet of reticulocytes from the marrow depending on the oxygen consumption [6].

Until tissue oxygenation is not affected, the concentration of erythropoietin, as well as the number of circulating erythrocytes, is constant. Production of erythropoietin is regulated at the level of transcription of its gene, and neither production nor erythropoietin metabolism do not depend on its concentration in plasma because the only physiological stimulus, that increases the amount of erythropoietin synthesizing cells, is hypoxia. Approximately 90% of erythropoietin is produced by the cells of glomerular capillaries and 10% – by the liver cells. In recent years was found that small amounts of erythropoietin are produced by astrocytes of nervous tissue. Erythropoietin plays

neuroprotective role during hypoxic and ischemic brain damage. [6] Erythropoietin is extremely active hormone that exerts its action in the body in picomolar concentrations. Small fluctuations in its concentration in the blood lead to significant changes in the rate of erythropoiesis. Its normal concentrations are ranging from 4 to 26 IU/litre. Therefore, until the concentration of hemoglobin is not below 105 g/litre, the concentration of erythropoietin is within the specified range, and it is impossible to identify its increase. Erythrocytosis leads to suppression of production of erythropoietin because of negative feedback mechanism. The half-life of erythropoietin is 69 hours. Thus, knowledge of the mechanism of influence of erythropoietin on erythropoiesis can help to understand its usage in sport [3, 4].

Erythropoietin dope

Erythropoietin belongs to the group of S2 - peptide hormones (the list of prohibited substances and methods). Expediency of its usage consists in ability to increase amount of red blood cells and, as a consequence, it increases oxygen capacity of the blood. Accordingly, more oxygen can be delivered to the tissues (especially muscle), it increases the efficiency of the organism. [5] Active usage of erythropoietin dope started from the time when it became possible to produce erythropoietin artificially. By the mid-1980s, the first recombinant erythropoietin was obtained using the introduction of the human EPO gene (that is localized on the seventh chromosome in the 11q-12q) in ovarian cells of hamsters. Recombinant human EPO is identical in amino acid composition to the natural human EPO. However, there are slight differences in composition of glycoside residues that influence the physical-chemical properties of the entire molecule of hormone [4]. Since 1988, people use alpha-EPO and beta-EPO. Their bioavailability is about 25%, when they are administered subcutaneously, the maximum blood concentration is in 12-18 hours, half-life is 24 hours (when we use intravenous injections – 5.6 h). Erythropoietin retard (NESP) has been used during past several years. It lasts longer in the organism than the other formations of EPO. Theta EPO is considered to be the most effective and least allergenic and it has the highest degree of purity. In fact, theta-EPO identical to human on 99%. Omega-EPO, which is derived from hamster kidneys, differs from the human more than any other EPO, so it is the easiest to detect. Apparently, the biological activity of these preparations depends on the carbohydrate component of the hormone [3].

In addition, recently (in 2008) the company Roche Pharmaceuticals announced the appearance of the third generation of erythropoietin. The preparation of this group was named Mircera (methoxy-epoetin beta). Basically, this substance is not different from previous erythropoietins apart from polyethylene glycol that is attached to the frame of the preparation and contributes to long half-life. Thus, Mircera can be located in the body 6 times longer than

darbpoetin alpha and 20 times longer than epoetin. This fact creates the possibility of large intervals between injections of the drug.

In general, all drugs are used according to the scheme: dosage varies between 50-300 IU per kg. The result is more or less noticeable after about two weeks of using. Majority of experts tend to believe that the drug should not be used for more than six weeks.

Advantages and disadvantages

As already mentioned, erythropoietin can increase the delivery of oxygen to the tissues and increase the efficiency of the organism. This type of dope is actively used in cyclic sports, where stamina plays a big role and when the same motion is repeated many times (running, swimming, skiing, skating, all kinds of rowing, cycling and others). However, there is a very serious risk when you use EPO, it is assumed that the cause of more than half a dozen deaths among Dutch cyclists was receiving erythropoietin (from 1987 to 1990, there were several deaths that were connected with using EPO among Dutch and Belgian cyclists). EPO increases percentage of red blood cells. Red blood cells form 40% of blood volume and it is quite normal. Athletes have this figure higher than normal men. Using of EPO leads to a very high concentration of red blood cells. The danger consists in such processes like sludging of blood, increasing of its viscosity, forming of thrombi – all these factors increase the load on the heart, and in the worst case they can cause thromboembolism which can be fatal. The risk of problems with blocked arteries becomes even greater when the athlete restricts itself in the consumption of liquid. Obviously, marathon runners and cyclists lose large amounts of fluid during competition. This loss of fluid can raise the concentration of hematocrit to exorbitant levels. Thus, athletes take risks when using EPO as dope. [1, 2].

Methods that are used for detection of EPO dope

All methods of detecting erythropoietin dope are either very laborious (require large expenditures of resources and the test material), or do not give a clear answer. The modern arsenal of methods for determining erythropoietin includes direct and indirect approaches.

The direct method is based on the identification of those minor differences that were found between natural endogenous erythropoietin and EPO that is produced by genetic engineering. In particular, some researchers have tried to use the differences in the distribution of electrical charge. Based on these differences, there were attempts to separate the two types of molecules using a method of capillary electrophoresis. Although this separation is possible in principle, this requires large volumes of urine (up to 1 liter, this is unacceptable, for obvious reasons, for the practice). Also, a few months before the Summer Olympics in Sydney, the French anti-dope laboratory has developed a new method for the detection of recombinant erythropoietin. This method is relatively

complicated and is based on isoelectric focusing patterning and a double blotting protocol (method for determination of macromolecules in a sample by hybridization with probes, eg. antibodies) [6].

Preference is given to indirect methods which require small amounts of blood or urine. Examples of indirect methods of detection of EPO are:

- deviations from normal levels of EPO in samples. This fact means that the excess levels of EPO have to be different from the variations of physiological or pathological nature. However, the use of this criterion is only possible if the variation range of the indicator is sufficiently narrow, in comparison with the values that are observed after the administration of exogenous drug. This is possible only when we use blood as a sample for the dope test;
- registration of biochemical parameters, the magnitude of which depends on the concentration of erythropoietin. Such an approach could be based on measurement of serum concentrations of soluble transferrin receptor (sTfR), the level of which is increased after the administration of recombinant EPO. However, this indicator undergoes similar changes after training in the midlands;
- definition of urinary fibrin degradation products and fibrinogen after administration of EPO.

At present, almost impossible to identify cases of exogenous administration of erythropoietin. Therefore, experts use the level of physiological changes in blood for monitoring that are detected after the administration of EPO. Thus, the International Cycling Union uses the criterion of the maximum value of hematocrit (50% for men). International Ski Federation as such a criterion use the maximum allowable values of hemoglobin (165 g/l for women and 185 g /l for men), as well as the level of reticulocytes that should be not more than 0.2%. The athlete disqualified from the competition in order to protect health in case of exceeding the specified limit values. However, hemoglobin, hematocrit can be influenced by many factors. In particular, both of these parameters may change significantly even after one average training. In addition, these indicators are characterized by considerable individual variability. Therefore, only one excess hematocrit value (greater than 50%) can not prove the fact of erythropoietin abuse in sport [1].

WADA (World Anti-Doping Agency) has introduced using blood passports for athletes to improve the control of the usage of erythropoietin dope. Blood passport is one of the developments of WADA, that is aimed primarily at identifying erythropoietin and its analogues: 30 different indicators form a single computer hematological profile of each athlete. This idea was approved by 10 countries, including Sweden, Norway, Canada and Germany. WADA recommends using apparatus of company “Sysmex” (Japan) or company “ERMA” to make blood tests [5].

In addition, some firms that produce recombinant erythropoietin offer to enter specific marker in a structure of the drug that would not affect the properties of the drug and would be easily detected with certain tests. Thus, it would be easy to track athletes that use this drug.

In general we can say that it is problematically to claim with 100% certainty that athletes use erythropoietin dope, because methods of indication of recombinant EPO are not so effective.

Methods of masking EPO

As I already said, recombinant erythropoietin differs from its natural analogue only by glycoside residues. Therefore pharmacists who are working to improve the various dopes lead the development in the direction of maximum similarity of glycoside residues of recombinant erythropoietin and natural human erythropoietin. Besides, athletes use various diuretics for the rapid withdrawal of the drug from the body. However, diuretics are agents that belong to the list S5 – diuretics and other masking agents [5].

There are a few very specific methods of dope masking, but they are less concerned to pharmacology: for example, athletes often use the technique of catheterization of the urinary bladder and introduction of the urine of another person, which does not contain the metabolites of the banned substance (but it is very problematic to perform this procedure due to the complexity of manipulation) [1].

Examples of the usage of erythropoietin dope

At the world championship in cross skiing in 2001, almost all the stellar Finnish team was caught on using EPO. After a year at the Olympic Games in Salt Lake City Larissa Lazutina and was suspected to use EPO. Four cyclists were caught on using new type of EPO – Cera in 2008: Bernhard Kohl was disqualified, as well as the Stefan Schumacher, Riccardo Ricco and Leonardo Piepoli. Race Results have been revised. Also Rashid Ramzi (twice world champion, runner at 1500 and 5000 meters) was caught on using Cera. Besides, Lance Armstrong was devoid of all awards for using EPO. Ukrainian athletes did not use EPO dope on world-wide competitions [1].

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