THE ROLE OF POLYAMINES AS THE ONCOMARKERS IN THE BLOOD

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Introduction

- Polyamines (PAs) (putrescine (Put), spermine (Sm) and spermidine (Sd)) are polyfunctional low-molecular nitrogen compounds what are present in low concentrations in the blood of healthy people.
- Transport function of PAs in organism carried erythrocytes in which polyamines are in a free state and do not form complexes with macromolecular substances [1].
- Red blood cells can capture excess PAs produced in the body [2].
- The concentration of PAs in whole blood depends on their content in the elemental elements since it is known that the level of PAs in plasma and serum is very low. In serum and plasma the content of Put and Sd is higher than Sm. Sd prevails in whole blood [3].
- Tumor development generally leads to the accumulation of PAs in the body. Therefore concentration of PAs in the blood varies in some pathological processes. It is established that this concentration is increased at different stages of the disease. In this regard the concentration of PAs in the blood, especially in erythrocytes, is considered an indicator of cell proliferation. This indicator is of clinical importance for monitoring the treatment of cancer patients.

Discussion

- The data on the content of PAs in the blood differ significantly from one author to another, what as can be seen depends on the sensitivity of the methods for determining them, on the small number of studies conducted and on other causes.
- However it should be noted that almost all authors did not detect free Put in the whole blood of healthy people, and some authors found it in very low quantities with LC-MS/MS and enzymatic methods.
- Paul Elworthy and Edward Hitchcock (1989) reported the cause of a low amount of Put. They suggested that Put is carried by plasma proteins after binding with transglutaminase. Low concentration in the blood makes it inaccessible for quantification [7].
- There were no significant differences in the concentrations of PAs in the blood, depending on sex and age. Both sexes had elevated levels of Sd. The range of concentrations of Sm and Sd was the same for both men and women.

Conclusion

- Dynamics of changes in PA levels in the blood during the study and treatment is relevant for the further development of an effective method for the determination of PAs.
- The determination of statistically significant differences in the Spd/Spm ratio for PAs in the blood could indicate a specific type of tumor. Until now, this kind of comparison of changes in this indicator with the type of tumor has not been carried out. This requires further study.

Review of some methods for determining the content of polyamines in the blood

- Large amounts of Sd and Sm and only trace amounts of Put are present in the blood and are generally in free form, so do not hydrolyze them before analysis [4].
- A large number of methods for the determination of PAs and their derivatives in blood are known: high performance liquid chromatography (HPLC), thin layer chromatography (TLC), liquid chromatography with tandem mass spectrometry (LC-MS/MS) [5], and electrophoretic method. One typical method is the HPLC method. However this method is known to be complex and time-consuming in spite of high reproducibility of results, high resolution and sensitivity. This method requires the use of special expensive equipment, instruments and expensive highly chemically pure reagents [1, 4, 6].
- Vèronique Ducros et al. (2009) believe that the HPLC method does not show sufficient sensitivity, especially for the minor polyamine, which is Put in erythrocytes. They developed the first LC-MS/MS method for the determination of PAs in erythrocytes. Sensitivity of this method allows to reduce the sample size of red blood cells. The specificity of LC-MS/MS analysis makes it possible to identify and quantitatively determine Put in a five-fold reduced volume of the injected sample, in contrast to the HPLC method with fluorescent detection. But this method, like most chromatographic methods, has significant drawbacks: it takes a lot of time for analysis and limited storage of chromatogram samples over time [5].
- Isobe et al. (1987) developed a rapid and reliable enzymatic analysis of PAs in whole blood, based on a combination of substrate specificity of polyminoxidase (PAO) from *Aspergillus terreus* and putrescine oxidase (PUO) from *Micrococcus rubens*. The principle of this method is a two-stage enzymatic reaction. The quinone dye, obtained from H2O2 formed in the oxidation reaction, is determined spectrophotometrically. This method makes it easy to determine the total amounts of Put, cadaverine, Sd and Sm in the blood, and apparently surpasses other methods in the simplicity and speed of the analysis. With this method one researcher can perform at least 20 measurements within 1 hour [4].
- However the authors of the US patent Tagaki K. and MiZunami-shi (2005) believe that the method of Isobe et al. is problematic due to diagnostic accuracy with respect to certain types of diseases. They proposed a method for the determination of PAs in erythrocytes with high accuracy, in which only PAO having substrate specificity for Sm and Sd, or PAO having substrate specificity for Sm is used. The eluate from red blood cells, separated and purified from blood, reacts with these enzymes, and formed H2O2 is determined by using a highly sensitive chromogen, such as a diphenylamine-based chromogen [6].
- Sd and Sm are more important as indicator substances than other PAs *in vivo*. Thus the determination of the total amount of Sd and Sm in erythrocytes is effective as an oncomarker [6].

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