British Journal of Global Ecology and Sustainable Development
Volume-35, December- 2024
ISSN (E): 2754-9291

A NOVEL APPROACH TO CERULOPLASMIN STABILITY: PUTTING PROTEASE INHIBITORS INTO PRACTICE

Polyarush Svetlana Vitalievna
Candidate of Biological Sciences,
LBM Medical Centre. Tashkent, Uzbekistan

Ryskulov Farrukh
Junior Researcher, II&HG, Tashkent, Uzbekistan

Masaidova Iroda Bakhtier kizi
Junior Researcher, II&HG, Tashkent, Uzbekistan
E-mail:iroda_2727@mail.ru

Abstract

Ceruloplasmin (CP) is a multi-subunit protein that is central to copper metabolism in the human body [7; P. 722–732]. This protein not only performs a transport function by delivering copper to tissues, but also serves as an important marker of inflammatory processes and pathologies associated with impaired copper metabolism [3; P. 6904–6910, 8; P. 27–36]. Additional studies have shown that plasma ceruloplasmin levels can be altered in a variety of diseases, including hereditary and autoimmune disorders [5; P. 887–905]. This emphasises the critical importance of accurate quantification of its levels in clinical practice, as these data can have a significant impact on diagnosis and subsequent treatment [1; P. 1–18].

Various techniques are currently used to analyse SR levels, among which enzyme-linked immunosorbent assay (ELISA) and biochemical spectrophotometry are the most common. These methods provide results with high sensitivity and specificity [6; P. 294–301]. However, the use of immunological methods such as ELISA can sometimes lead to overestimated results [2; P. 561–573]. This is due to the possibility of antibody interaction with apoforms of ceruloplasmin, which distorts the true concentration of this protein in serum [4; P. 2254–2260].

In view of the above, the aim of our study is to improve the accuracy of ceruloplasmin level measurements. We intend to look at the use of proteolysis inhibitors that may help to stabilise the structure of the octameric ceruloplasmin molecule and thereby prevent potential distortions of assay results caused by proteolytic cleavage of the protein.

Keywords: Ceruloplasmin, Proteolysis, Protease inhibitors, Enzyme-linked immunosorbent assay (ELISA), Biochemical spectrophotometry, Copper metabolism, Diagnosis, Protein stability, Pathology, Inflammatory processes.

Introduction

Purpose of the study

The aim of this study is to comprehensively evaluate the effect of proteolysis inhibitors on the stability of the octameric molecule of ceruloplasmin, and to determine their ability to prevent proteolytic cleavage of this protein using various methods of quantification of its level (SR). Ceruloplasmin, as an important marker that plays a key role in copper metabolism and inflammatory processes, requires highly accurate assay methods to ensure the validity of clinical diagnoses. Since proteolytic cleavage can distort test results, the use of proteolysis inhibitors seems to be a relevant and necessary step to improve the reliability of diagnostic methods.

Objectives of the study

1- To evaluate the efficacy of proteolysis inhibitors. The primary objective is to evaluate the ability of various protease inhibitors, such as phenylmethylsulfonyl fluoride (PMSF) and a complex cocktail of inhibitors, in preventing proteolytic cleavage of ceruloplasmin. This will help to establish the extent to which these substances can preserve the integrity of the protein molecule during analysis.
2- Analysing the collected data. An equally important task is to analyse the collected data to identify patterns in the change in the stability of the octameric ceruloplasmin molecule depending on the application of different inhibitors. This step will allow the establishment of links between experimental conditions and results, which will help to further understand the mechanisms underlying the stability of receptors and proteins.

Materials and Methods

In order to achieve the aims and objectives of the study, specific materials and carefully selected methods were used to ensure the validity of the results.

- Materials:

- Serum samples. A total of 80 serum samples from patients with known genetic disorders were used for analysis. These samples provided the basis for comparative studies and evaluation of the effect of inhibitors on ceruloplasmin levels under different conditions.
- Protease inhibitors. The following inhibitors were used:
 - cOmplete™, Mini Protease Inhibitor Cocktail Tablets (Roche), a specialised cocktail that provides broad protection against proteolysis.
- PMSF (phenylmethylsulfonyl fluoride, SIGMA, USA) is a classical inhibitor used in laboratory practice to prevent protease activation.

Methods

Various methods were employed in this study to achieve the aims and objectives, which included both the preparation of inhibitor solutions and the statistical analysis of the

Journal Zone Publishing, Ilford, United Kingdom

data obtained. These methods were chosen to ensure high accuracy and reliability of the results and to be able to identify patterns in the change in ceruloplasmin stability.

1. Preparation of inhibitor solutions:

- PMSF working solution: To begin the preparation of the phenylmethylsulfonyl fluoride (PMSF) working solution, it was necessary to measure exactly 0.087 grams of this substance. This instrument is important for qualitative analysis because its effect on proteolysis by vesicle is quite significant. WHEN the drug is deficient, the results of the assay may be uncertain or unreliable. PMSF was carefully dissolved in 1 ml of isopropanol, and it was necessary to stir the solution thoroughly on a vortex to achieve complete dissolution of the substance. In some cases, the application of a small amount of temperature, up to 42°C, would improve solubility. The resulting solution was necessarily stored in a dark place to protect it from photoreactions and to prevent degradation of the active substance by light.
- Inhibitor cocktail working solution: To prepare the inhibitor cocktail working solution, it was necessary to take one tablet of COMPLETE MINI PROTEASE INHIBITOR COCKTAIL and dissolve it in 1.5 ml of chilled buffered saline or deionised water. This process also involved thorough mixing to ensure complete dissolution of the tablets and efficient distribution of the inhibitors in the solution. The use of precisely chilled buffer solution or deionised water is a key aspect, as this preserves the activity of the inhibitors and prevents their irreversible denaturation. Proper preparation of solutions is the basis for reliable performance of all further analyses.

2. Statistical analysis:

- After obtaining the ceruloplasmin stability data, the collected results were processed using various statistical methods. This not only allowed us to assess the accuracy and reliability of the results obtained, but also carried out the identification of patterns related to the effects of inhibitors on the stability of ceruloplasmin macromolecules. Methods of descriptive statistics such as mean values, standard deviations and confidence intervals were applied, as well as more sophisticated statistical tests including t-test and ANOVA to determine the significance of differences between groups.

All these steps contributed to an in-depth analysis of the data obtained, allowing reasonable conclusions to be drawn about the effect of inhibitors on ceruloplasmin stability under different conditions and finally generalising the results for further application in clinical practice.

Results

Two types of inhibitors aimed at preventing proteolytic cleavage and dissociation of ceruloplasmin globules were used in our study. To evaluate the efficacy of these inhibitors, we tested 10 serum samples from patients divided into three groups: the first group contained no inhibitors, the second group was treated with the protease inhibitor PMSF, and the third group was treated with a complex cocktail of inhibitors.

Journal Zone Publishing, Ilford, United Kingdom

The results of quantification of ceruloplasmin concentration showed that in the samples without inhibitor application, the SR levels ranged from 23.3 to 29.7 mg/dl. In the group with the addition of PMSF, the level decreased significantly, reaching minimum values of 17.4 to 24.1 mg/dl, indicating its inhibitory properties. In the group treated with the complex cocktail of inhibitors, the level of ceruloplasmin was also lower, but its values ranged from 20.9 to 27.2 mg/dl. This confirms that the addition of inhibitors has a positive effect on the stability of the ceruloplasmin molecule.

Analysis of the data showed that the levels of ceruloplasmin in samples containing protease inhibitors were significantly lower than in the control group containing no inhibitors. This indicates that the inhibitors do contribute to preventing the dissociation of ceruloplasmin globules, which in turn leads to more accurate and reliable results when quantifying SR.

It should be noted that the greatest reduction in ceruloplasmin levels was recorded when PMSF was used, confirming its high inhibitory activity. At the same time, the efficiency of the complex cocktail of inhibitors was slightly lower, but it also showed a positive effect on globule stability.

Conclusions

Thus, the results of our study confirm that the use of proteolysis inhibitors, such as PMSF and complex inhibitor cocktail, has a significant positive effect on the stability of the octameric ceruloplasmin molecule. The use of these inhibitors in the analysis of ceruloplasmin levels provides more objective and reliable data, which is important for clinical diagnosis.

Discussion

The results of our study supported the hypothesis of a positive effect of protease inhibitors on the stability of ceruloplasmin obtained from patients' serum. Measured levels of ceruloplasmin in samples without the use of inhibitors confirmed that standard assay procedures may expose samples to undesirable changes due to proteolysis. This opens up important aspects for clinical diagnosis, as the use of inhibitors, such as PMSF and a complex cocktail of inhibitors, can significantly improve the accuracy and precision of the assay.

Comparing the results obtained with available data in the scientific literature, the reduction of ceruloplasmin levels in samples using PMSF is consistent with numerous studies where this inhibitor has demonstrated its effectiveness in stabilising proteins. Works on the stability of complex protein molecules also emphasise the importance of preventing proteolytic cleavage in order to obtain correct results when determining the levels of specific proteins. This in turn points to the need for the use of inhibitors in routine practice, especially in clinical laboratories.

It is interesting to note that the greatest decrease in the concentration of ceruloplasmin was observed in the group with PMSF, which may indicate its high inhibitor activity. At the same time, the effect of a complex cocktail of inhibitors was less pronounced, which

Journal Zone Publishing, Ilford, United Kingdom

may be due to differences in the mechanisms of action of the components included in the cocktail. Combination inhibitors may have limited efficacy in targeting specific proteases or may not provide complete stability of the molecule under near clinical conditions.

In addition, some limitations of our study should be considered. First, the sample size of ten samples may not be representative of the entire patient population and may not account for possible individual differences in response to inhibitor treatment. Second, the type and storage conditions of samples prior to analysis may also have an impact on ceruloplasmin stability and hence the results. Future studies should focus on increasing the sample size and looking at different factors that may affect protein stability.

Finally, further research could focus on other types of protease inhibitors and their effects on the stability of other proteins similar to ceruloplasmin. It is also worth considering more advanced analytical techniques, such as proteomics, to better understand the interaction between inhibitors and protein molecules, which may open new horizons in the field of molecular diagnostics and therapeutics.

Thus, the results of our study not only emphasise the need for the use of inhibitors to improve the accuracy of assays, but also open new opportunities for further research into the stability of biomolecules that play a key role in clinical practice.

References

1. Coverdale, J.P.C., Harrington, C.F., Solovyev, N. Review: Advances in the Accuracy and Traceability of Metalloprotein Measurements using Isotope Dilution Inductively Coupled Mass Spectrometry // Crit Rev Anal Chem. 2023. C. 1–18.
2. Del Castillo Busto, M.E., Cuello-Nunez, S., Ward-Deitrich, C., Morley, T., Goenaga-Infante, H. A fit-for-purpose copper speciation method for the determination of exchangeable copper relevant to Wilson's disease // Anal Bioanal Chem. 2022. T. 414. C. 561–573.
3. El Balkhi, S., Poupon, J., Trocello, J.-M., Massicot, F., Woimant, F., Laprévote, O. Human Plasma Copper Proteins Speciation by Size Exclusion Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry. Solutions for Columns Calibration by Sulfur Detection // Anal Chem. 2010. T. 82. C. 6904–6910.
4. El, B.S., Trocello, J.-M., Poupon, J., Chappuis, P., Massicot, F., Girardot-Tinant, N., et al. Relative exchangeable copper: A new highly sensitive and highly specific biomarker for Wilson's disease diagnosis // Clin Chim Acta. 2011. T. 412. C. 2254–2260.
5. Linder, M.C. Ceruloplasmin and other copper binding components of blood plasma and their functions: an update // Metallomics. 2016. T. 8. C. 887–905.
6. MacIntyre, G., Gutfreund, K.S., Martin, W.R.W., Camicioli, R., Cox, D.W. Value of an enzymatic assay for the determination of serum ceruloplasmin // J Lab Clin Med. 2004. T. 144. C. 294–301.
7. Sandahl, T.D., Laursen, T.L., Munk, D.E., Vilstrup, H., Weiss, K.H., Ott, P. The prevalence of Wilson's disease: an update // Hepatology. 2020. T. 71. C. 722–732.

Journal Zone Publishing, Ilford, United Kingdom

8. Solovyev, N., Ala, A., Schilsky, M., Mills, C., Willis, K., Harrington, C.F. Biomedical copper speciation in relation to Wilson's disease using strong anion exchange chromatography coupled to triple quadrupole inductively coupled plasma mass spectrometry // Anal Chim Acta. 2020. T. 1098. C. 27–36.

Journal Zone Publishing, Ilford, United Kingdom