«IN VITRO» AND «IN VIVO» INHIBITORY EFFECT OF COPPER SULPHATE UPON THE ACTIVITY OF BLOOD CATALASE

E. Yordanova

Catalase represents a metalloenzyme, hemo-protein with 250 000 molecular weight. Ferriprotoporphyrin 9 (type 3) forms its prosthetic group, containing trivalent iron which never alters its valency during enzyme reaction. Catalase is the most active of all enzymes hitherto known. A single catalase molecule decomposes 5 000 000 hydrogen peroxide molecules per minute at temperature 0° C and pH=6.6.

The biological role of the enzyme consists in splitting and elimination of toxic hydrogen peroxide, produced during metabolic processes in the human organism (2, 6).

Numerous and rather different mechanisms exist through which the organism regulates the activity of its enzymes. In principle, such a regulation is manifested either by enhancing the enzyme activity by means of various substances denominated activators, or by suppressing it by means of inhibitors, acting upon the enzyme reactions under adequate conditions (3, 4, 5, 6, 7, 8). The problem of enzyme activity inhibition is very important and is subject to numerous up-to-date biochemical and enzymological studies.

A number of inhibitors of the catalase activity are known — mainly metal ions — which react with the iron in the prosthetic group of the enzyme (1).

Taking into consideration the circumstance of the frequently occurring iron metabolism disorders in systemic blood affections and the fact that catalase is an iron-containing enzyme, playing an essential role in redox reaction processes of the human organism, we set out to investigate the inhibitory effect of copper sulphate, exerted upon the activity of erythrocyte catalase under «in vitro» and «in vivo» conditions.

Material and Methods

«In vitro» studies were performed on twenty healthy individuals. Catalase activity of the blood in the course of the experiment was determined repeatedly, namely: a) before the influence of copper sulphate — initial value of the enzyme, and b) threefold investigation following treatment of the blood with 0.1, 0.5 and 1% water solution of copper sulphate. The concentrations mentioned above were obtained by the addition of 10, 50 and 100 mg copper sulphate substance to a determined standard solution with the purpose to preserve unaltered its constituents' ratio.

«In vivo» studies were conducted on twenty-one practically healthy subjects under the following experimental setting: a) determination of the initial catalase activity; b) administration per os of 1% water solution of copper sulphate over a period of seven days, 3.5 ml daily, at weekly dose 0.6 mg per kilogram body weight; c) re-examination of the blood catalase at 2 hours, 3, 7 and 12 days after cessation of copper sulphate application. The periods of investigation indicated above were arbitrarily selected to the end of obtaining a better idea about the duration of the enzyme's activity inhibition and recovery.

Catalase investigation was carried out after the titration method of Bach and Zubkova.

In both experimental settings, also the catalase number and the catalase index were investigated (catalase activity of 10^6 erythrocytes). The catalase index was determined on the basis of the number of erythrocytes, as well as on the basis of the venous hematocrit in part of the examinees.

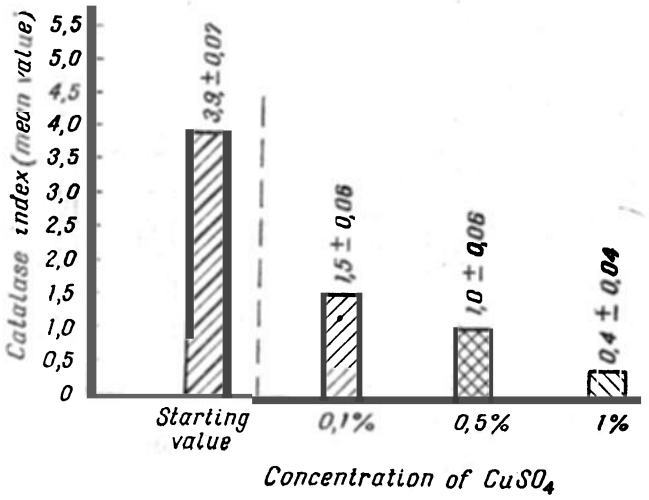
Results and Discussion

In the ten parallel investigations on the activity of blood catalase performed in one and the same subject, a very good reproducibility of data was established. The mean catalase index was 4.5 ± 0.07, whereas the mean catalase number — 15.0 ± 0.05. The latter data confirm the reliability of the method employed.

I. Results from Investigations «in Vitro»

The starting mean value of the catalase index was normal: 3.9 ± 0.07 (ranging from 3.5 to 4.6). It was used as a standard value in the assessment of the data obtained after influencing the enzyme reaction with copper sulphate. After addition of the smallest copper sulphate amount employed (10 mg), a substantial inhibition of the catalase activity occurred. The mean catalase index was lowered more than two times as compared to the starting one, reaching the values 1.5 ± 0.06. Upon treatment with copper-sulphate dose exceeding 50 mg, the degree of enzyme inhibition was intensified. The mean catalase index reached up to 1.0 ± 0.06, i. e. a value three times lower than the starting one. Maximal enzyme activity inhibition was recorded after treatment with the highest concentration — 100 mg copper sulphate (1%) at which the mean catalase index reached its lowest value — 0.4 ± 0.04. The attempts to use still higher concentrations of the inhibitor (3%) failed, owing to the fact that very low values of the catalase number were produced, rendering rather difficult its recording.

The data submitted corroborate in an indisputable manner the powerful inhibitory effect of copper sulphate on the blood catalase activity «in vitro». The inhibition of the enzyme activity was manifested in all concentrations employed in the experiment, and rised in proportion to the concentration of the inhibitor. The latter dependence between copper sulphate and enzyme activity is presented in Diagram 1.



Diagr. 1

The statistical elaboration, performed after the method of variational analysis, reveals a great reliability of the results between the different settings of the experiment «in vitro» ($P < 0.001$).

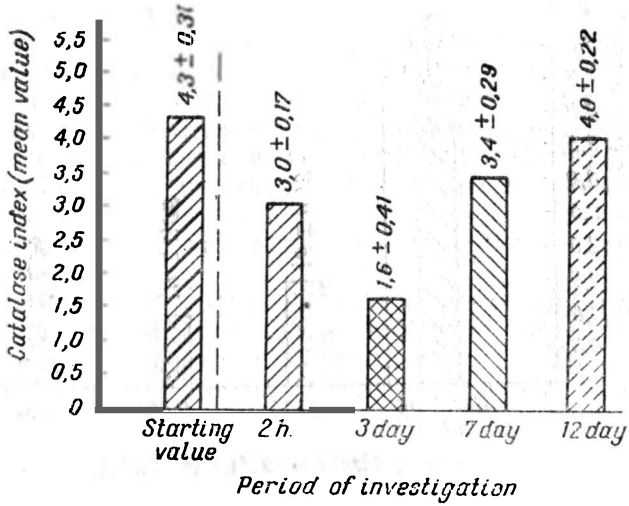
II. Results from Investigations «in Vivo»

Peroral application of copper sulphate was carried out in compliance with its routinely adopted therapeutical dosage.

The mean starting value of the catalase index was normal — 4.3 ± 0.31 (ranging from 3.5 to 5.5). After 7-day application of copper sulphate, the catalase activity was reliably lowered ($P < 0.005$), while the mean catalase index reached 3.0 ± 0.17. The latter value refers to investigation of catalase, carried out at 2 hours from the last administration of the inhibitor. Despite the cessation of copper sulphate, the activity of catalase progressively diminished and at 3 days, the enzyme activity reached its maximal inhibition. The mean catalase activity was lowered three times, as compared to the starting one (mean value 1.6 ± 0.41). At 7 days, reduction of the catalase activity inhibition was established, which began restoration, however, without reaching full normalization ($P > 0.05$). The mean catalase activity rised up to 3.4 ± 0.29. Only at 12 days after discontinuing copper sulphate application, the catalase activity showed full normalization and reached its starting level (mean catalase index — 4.0 ± 0.22) ($P < 0.05$).

Diagram 2 illustrates the results of the investigations «in vivo».

The analysis of the concrete data concerning the different subjects investigated demonstrate that catalase activity inhibition during experiments «in vivo» runs a course mainly in tw direoctions, namely:



Diagr. 2

1) Maximum inhibition of the enzyme activity occurs at 3 days after discontinuing the effect of the inhibitor, proved in 15 cases of the series under investigation (71.5%). At 12 days, the inhibitory effect of copper sulphate was already absent in them and the enzyme activity disclosed full normalization. Such a regularity lends itself to graphic expression, as illustrated in Scheme 1.

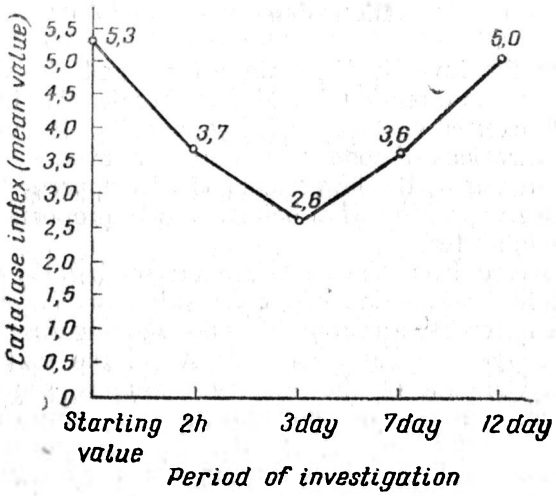
2) Maximum inhibition of the enzyme activity occurs later — at 7 days after discontinuation of copper sulphate — disclosed by five of the examinees (23.8%). In the latter group, the catalase activity was normalized similarly at 12 days. Graphically expressed, this pattern of inhibition is illustrated in Scheme 2.

There is a single exception of the two basic patterns of inhibition described above (4.7%). It concerns a case in which a rather prolonged inhibition of enzyme activity was recorded, with normalization occurring not earlier than the 20th day after the stopping of copper sulphate.

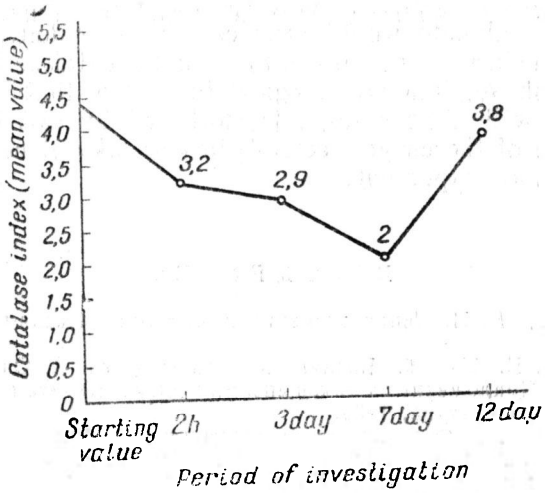
In two cases of the series, catalase activity was traced daily, over a period of 12 days subsequent to the cessation of copper sulphate application, in order to mark the time, by days, of the enzyme inhibition. In accordance with the regularity outlined, in the latter two cases, catalase activity inhibition with an identical 10—12 days duration was likewise established.

Furthermore, the summarized and specific data from the experiments «in vivo» confirm the inhibitory effect of copper sulphate, being manifested after 7-day-long application and persisting for about 10—12 days after its discontinuation.

The comparative study of the degree of enzyme inhibition, after exerting effect with the highest concentration of 100 mg, respectively 1% water



Scheme 1



Scheme 2

solution of copper sulphate, «in vivo» and «in vitro», in ten of the subjects under investigation, shows inhibition «in vitro» two times stronger than «in vivo». Such differences in the two experimental settings are logical, since the conditions under which the inhibitor exerts its effect are likewise principally different.

Discussion

The data from the investigations «in vitro», and «in vivo» submitted in the paper are mono-directional and corroborate the inhibitory effect of copper sulphate exerted on blood catalase activity. It should be stressed that all the concentrations of copper sulphate employed by us in the experimental setting «in vitro», the lowest comprised, suppress the enzyme activity, with the degree of inhibition being directly proportional to the concentration of the inhibitor.

The data recovered from the experimental setting «in vivo» similarly corroborate the inhibitory action of copper sulphate. The maximum inhibitory effect in the latter case occurs in the period ranging from 3 to 7 days after the cessation of copper sulphate. The activity of the enzyme is fully restored at 12 days. The data of the «in vivo» studies show that in this particular instance, a process of reversible enzyme inhibition takes place, with duration of the inhibitory effect about 12 days with ensuing full normalization of catalase activity. This finding of ours may have certain practical bearing upon the therapeutical use of copper sulphate in certain conditions of anemia, due to copper sulphate. On the basis of the results obtained, it might be presumed that the continuous therapeutical application of copper sulphate would produce also a prolonged inhibition of the activity of such an important enzyme as the catalase. Bearing in mind the facts listed above, it appears logical to assume that short-term therapeutical courses with copper sulphate, followed by rest intervals during which restoration of the enzyme activity would take place, should be considered much more expedient.

REFERENCES

1. Григорянц, Н. Н. Микроэлементы в медицине, Ивано-Франковск, УССР, 1969, 208.
2. Диксон, М., Е. Уебб. Ензимы, Москва (под ред. А. Опарина), 1966, 277.
3. Кирчев, П. Приложение на някои ензими в диагностиката и терапията, София, Медицина и физкултура, 1963.
4. Лундегорд, Х. Физиология на растенията, София, Земиздат, 1967, 226.
5. Юркевич, В. В. Биолог. наук. Москва, 81, 1970, 9, 115.
6. Abderhalden, R. Clinical Enzymology, D. Van Nostr. Comp., Toronto—New York — London, 1961, 8.
7. Masszi, I., T. Mecher, Kiserl, Orvost. Budapest, 15, 1963, 6, 567.
8. Tudhope, G. R. *Clin. Chim. Acta,* 25, 1969, 3, 379.

ИНГИБИТОРНОЕ ДЕЙСТВИЕ СЕРНОКИСЛОЙ МЕДИ НА АКТИВНОСТЬ КАТАЛАЗЫ КРОВИ IN VITRO И IN VIVO

Евг. Йорданова

РЕЗЮМЕ

Изучили ингибирующее действие сернокислой меди на активность каталазы крови in vitro и in vivo. Установили, что в обеих опытных постановках сернокислая медь ингибирует активность энзима. Ингибиция выражена очень сильно в опытной постановке in vitro; ее степень прямо пропорциональна концентрации сернокислой меди.

В исследованиях in vivo установили более слабое ингибирующее действие сернокислой меди, достигающее максимума на 3-й и на 7-й день со дня удаления ингибитора. Через 12 дней ингибирующего энзимного действия сернокислой меди наступает нормализация в активности каталазы, что говорит об обратимости энзимной ингибиции.

Исходя из полученных данных, автор делает практический вывод в связи с осторожным терапевтическим применением сернокислой меди, поскольку продолжительное его применение вызовет и более продолжительную ингибицию такого важного энзима как каталазу.