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The Influence of *D*-Penicillamine on Enzymatic Activities: Glucose-6-phosphate Dehydrogenase. Correlation with Serum Levels Measured in Humans

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Summary: The influence of *D*-penicillamine on glucose-6-phosphate dehydrogenase of yeast (pure enzyme), human hemolysate, and human skin homogenate were determined. In high concentrations, *D*-penicillamine inhibits glucose-6-phosphate dehydrogenase activity (concentrations above 6.7 mmol/l, i. e. 1 g/l). In low concentrations, *D*-penicillamine exerts an indirect influence by removing some inhibiting metal ions, such as zinc. In human skin homogenates, an activating action of *D*-penicillamine on glucose-6-phosphate dehydrogenase activity occurs due to the chelation of metal ions.

Der Einfluß von D-Penicillamin auf Enzymaktivitäten: Glucose-6-phosphat-Dehydrogenase

Zusammenfassung: Der Einfluß von *D*-Penicillamin auf die Glucose-6-phosphat-Dehydrogenase-Aktivität von Hefe (Reinenzym), von Hämolysat menschlicher Erythrocyten und vom Homogenat menschlicher Haut wurde untersucht. In Konzentrationen ab 6,7 mmol/l (= 1 g/l) entfaltet *D*-Penicillamin eine direkte Hemmwirkung auf die Glucose-6-phosphat-Dehydrogenase-Aktivität der Hefe und des Hämolysates. Niedrigere Konzentrationen beeinflußten die Glucose-6-phosphat-Dehydrogenase-Aktivität durch Entfernung hemmender Metallionen (Zinkionen), was zu einer Aktivierung führte. Im Hauthomogenat ließ sich nur eine derartige Aktivierung nachweisen. Das Zustandekommen der verschiedenen Wirkungen von *D*-Penicillamin auf die Glucose-6-phosphat-Dehydrogenase-Aktivität wird diskutiert.

Introduction

D-Penicillamine (β,β'-dimethylcysteine) is used in human therapy for an increasing number of diseases. The mechanisms whereby this interesting substance exerts its various therapeutic actions, are, however, not always fully understood. There is increasing interest in the changes in enzymatic activities evoked by *D*-penicillamine.

Biochemically, *D*-penicillamine acts as a chelating agent (e. g. chelation of copper ions in *Wilson*'s disease), splits disulfide bonds (exchange reactions), and reacts with aldehyde groups (e. g. with aldehyde groups of pyridoxal-phosphate and tropocollagen). Important enzymes and enzyme systems may be influenced by *D*-penicillamine via all three of the above mechanisms. So far, an anti-collagenase effect of *D*-penicillamine has been reported (1) as well as an inhibitory action on alkaline phosphatase activity (2). The latter effect was attributable to a chelation of important ions. In further investigations, changes in glucose-6-phosphate dehydrogenase activity were encountered under the influence of *D*-penicillamine. As a contribution to the various biochemical actions of *D*-penicillamine, the results of these studies will be reported here.

Methods and Materials

Enzyme sources: Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity has been investigated from three sources: from yeast (pure enzyme preparation, commercially available), from human hemolysate, and from human skin homogenates (Blendor: Ultraturrax). Details of preparations have been described elsewhere (3).

D-Penicillamine: *D*-Penicillamine (Biochemie, Vienna) dissolved in saline was added to the solutions with Glucose-6-phosphate dehydrogenase activity. Incubation was performed at 37 °C for 1 h. In every instance, enzymatic activity was compared to a control assay containing saline instead of *D*-penicillamine. Final concentrations of *D*-penicillamine ranged between 6.7 µmol/l (i. e. 1 µg/l) und 335 mmol/l (i. e. 50 g/l).

Zinc ions: As the activity of glucose-6-phosphate dehydrogenase is known to be highly susceptible to the presence of zinc or other metal ions (4, 5), additional investigations were performed with *D*-penicillamine and zinc ions. $Zn(NO_3)_2 \times 4H_2O$ (M = 261.5) was used. The effects of *D*-penicillamine

(concentration range 6.7 µmol/l to 335 mmol/l) on the activity of glucose-6-phosphate dehydrogenase of yeast (pure enzyme) and of human hemolysate were determined in the presence of zinc ions (360 µmol/l which corresponds to 100 mg zinc nitrate per litre). Furthermore, the inhibitory action of zinc ions (concentration range 3–360 µmol/l, i. e. 1–100 mg zinc nitrate per litre) was recorded with and without *D*-penicillamine (concentration 67 µmol/l), using the pure enzyme preparation only.

Determination of enzymatic activity: Glucose-6-phosphate dehydrogenase activity was assayed in the following way: 0.05 ml of 31 mmol/l glucose-6-phosphate, 0.1 ml of 10 mmol/l NADP, and 1.0 ml of the test or control solution were added to 2.0 ml of a 5 mmol/l triethanolamine buffer pH 7.6 containing 5 mmol/l ethylenediamine tetraacetate. Immediately after mixing and temperature adaption (25 °C) extinction at 340 nm was read; readings were repeated after 1, 2, and 3 min. Mean extinction difference per min was multiplied by 506 in order to calculate the U (= µmol × min^{-1}) glucose-6-phosphate dehydrogenase activity per litre.

Results

Pure enzyme from yeast

Control activities in ten experiments with pure glucose-6-phosphate dehydrogenase from yeast ranged between 45 and 50 U/l. *D*-Penicillamine caused an inhibition in concentrations above 1 g/l (6.7 mmol/l). In this study, a significant inhibition ($P < 0.001$) was found with two concentrations: 10 and 50 g/l *D*-penicillamine (67 and 335 mmol/l). The dose-response curve is shown in figure 1.

The presence of zinc ions (360 µmol/l) inhibited glucose-6-phosphate dehydrogenase activity by 68% ($P < 0.001$).

The presence of increasing amounts of *D*-penicillamine depressed the above-described inhibitory action of zinc ions. However, when the concentrations of *D*-penicillamine exceeded 670 µmol/l, inhibition increased again. With 67 mmol/l *D*-penicillamine and 360 µmol/l zinc ions, inhibition of glucose-6-phosphate dehydrogenase activity reached 95%. This value proved to be significantly higher ($P < 0.001$) than the inhibition produced by zinc ions alone. For details, cf. figure 1. The dose-response curve depicting the inhibition of glucose-6-phosphate dehydrogenase activity by zinc ions in concentrations between 3.0 µmol/l and 1.8 mmol/l (1 and 500 mg/l zinc nitrate) was the same as published in a previous paper (5). The presence of 67 µmol/l *D*-penicillamine weakened this inhibitory action over the whole concentration range; the encountered changes measured about 5% with the lower zinc concentrations and 10% with the higher zinc concentrations. A statistical significance could not be obtained ($P > 0.05$).

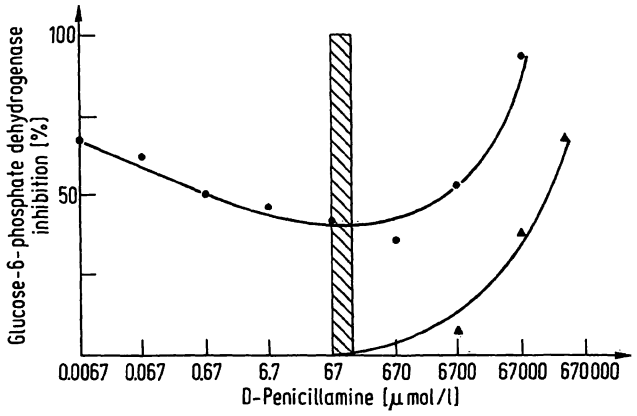

Fig. 1. Changes in glucose-6-phosphate dehydrogenase activity (pure enzyme from yeast) in the presence of *D*-penicillamine (concentrations between 1 µg/l and 50 g/l, i. e. 6.7 nmol/l and 335 mmol/l) and zinc ions ($Zn(NO_3)_2 \times 4\ H_2O$, 100 mg/l, i. e. 360 µmol/l) *with* *D*-penicillamine in the above mentioned concentrations.
●——● Zinc ions and *D*-penicillamine
▲——▲ *D*-penicillamine
The hatched column indicates therapeutic serum levels of *D*-penicillamine.

Hemolysate

In five different hemolysates, control activities measured 55 to 65 U/l. The inhibitory action of *D*-penicillamine was found to be lower and amounted to a significant value in the highest concentration (335 mmol/l, ≙ 50 g/l; 27% inhibition; $P < 0.001$), only. Details are shown in figure 2.

Zinc ions in a concentration of 360 µmol/l provoked a complete inhibition of glucose-6-phosphate dehydrogenase activity of human hemolysates.

The presence of *D*-penicillamine in increasing concentrations produced an increasing deterioration of the above-described inhibitory action of zinc ions. With *D*-penicillamine in concentrations between 0.67 and 67 mmol/l, the inhibitory action of zinc ions (360 µmol/l) was no longer significant (only 10% inhibition; $P > 0.05$). In the assays with 335 mmol/l *D*-penicillamine and 360 µmol/l zinc ions, glucose-6-phosphate dehydrogenase activity of the hemolysates was significantly inhibited (29% inhibition; $P < 0.001$). This value corresponded to the inhibition obtained with 335 mmol/l *D*-penicillamine without zinc ions (cf. fig. 2).

Human skin homogenates

In five fresh human skin homogenates, control values of glucose-6-phosphate dehydrogenase activity ranged between 17 and 35 U/l. In old homogenates (stored overnight at - 30 °C), glucose-6-phosphate dehydrogenase activities measured only half of the above-mentioned activities.

In concentrations between 67 µmol/l and 67 mmol/l, *D*-penicillamine provoked an activation of glucose-6-phosphate dehydrogenase activity in both fresh and old human skin homogenates. Significant activation was found with concentrations of 670 µmol/l and above ($P < 0.001$). As shown in figure 3, this effect of activation was more pronounced in „old" homo-

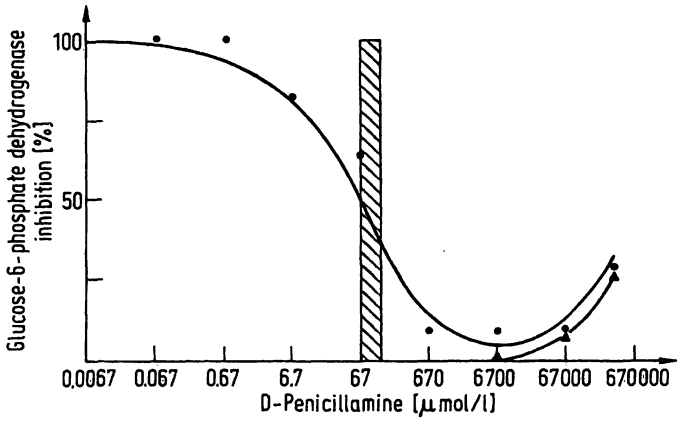

Fig. 2. Changes in glucose-6-phosphate dehydrogenase activity of human hemolysate in the presence of *D*-penicillamine (concentrations between 1 µg/l and 50 g/l, i. e. 6.7 nmol/l and 335 mmol/l) and zinc ions ($(Zn(NO_3)_2 \times 4\ H_2O$, 100 mg/l, i. e. 360 µmol/l) *with* *D*-penicillamine in the above-mentioned concentrations.
●——● Zinc ions and *D*-penicillamine
▲——▲ *D*-penicillamine
The hatched column indicates therapeutic serum levels on *D*-penicillamine in humans.

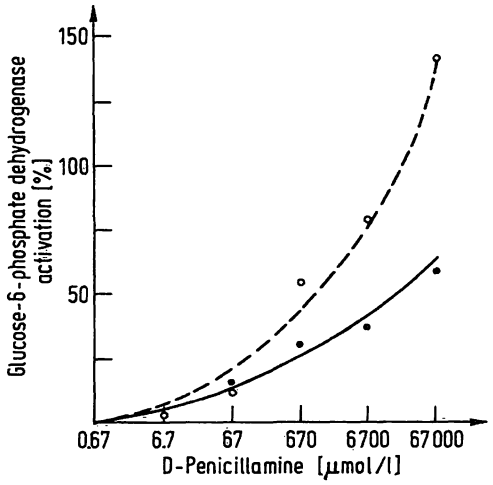

Fig. 3. Changes in glucose-6-phosphate dehydrogenase activity of human skin homogenates in the presence of *D*-penicillamine (concentrations between 0,1 mg and 10 g/l, i. e. 6.7 nmol/l and 67 mmol/l). Two types of homogenates are depicted: low control activity 5.9 U/l (o——o) and high control activity 17.2 U/l (●——●).

genates with low control activity. Figure 3 presents the results of a typical experiment with one skin preparation (fresh and old homogenate).

Discussion

In pure enzyme preparations as well as in the human hemolysate, a direct inhibitory action of *D*-penicillamine on glucose-6-phosphate dehydrogenase activity was seen. However, high concentrations of *D*-penicillamine were needed to provoke such an effect (cf. fig. 1 and 2). The occurence of such inhibitory action can be ascribed to the well-known reaction of *D*-penicillamine with sulfhydryl groups. Via this mechanism, an influence on active sites of the enzyme protein may be exerted, leading to an inhibition of enzymatic activity.

Zinc ions could be shown to inhibit both pure glucose-6-phosphate dehydrogenase from yeast and glucose-6-phosphate dehydrogenase from hemolysate. However, the glucose-6-phosphate dehydrogenase of hemolysate was more sensitive. The presence of *D*-penicillamine has been found to block partially the inhibitory action of zinc ions on glucose-6-phosphate dehydrogenase activity. With higher concentrations of *D*-penicillamine, however, this effect is lost, most probably due to the fact that the direct inhibitory action of *D*-penicillamine is beginning to have an effect.

In the skin homogenates, no inhibitory action of *D*-penicillamine on glucose-6-phosphate dehydrogenase activity could be found. On the contrary, an activation of this enzymatic activity occurred with increasing concentrations of *D*-penicillamine. As glucose-6-phosphate dehydrogenase is an enzyme with high susceptibility to zinc and various other metal ions (4) it seems logical to assume that *D*-penicillamine activates glucose-6-phosphate dehydrogenase by removing metal ions (chelating effect). More difficulties arise when one tries to explain why *D*-penicillamine provokes a stronger activation of lower enzymatic activity (compare the two curves in fig. 3). May be the enzyme in the "old" preparation is more susceptible to the influence of metal ions; another explanation could be given by the assumption of a direct influence of *D*-penicillamine on the enzyme protein in an altered state (artificial S-S bridges after prolonged storage).

For practical therapeutical purposes, a direct influence of *D*-penicillamine does not seem to be of importance; the concentrations needed for such effect are not reached in human therapy. On the other hand, pharmacokinetic studies (6, 7) show that serum levels of 10 to 20 mg/l are attained with some commonly used regimens of *D*-penicillamine therapy. As shown in figures 1 and 2, such concentrations may influence enzymatic activities by chelating metal ions. Such metal ions either can act as inhibitors (as it is the case with glucose-6-phosphate dehydrogenase), or as activators (cf l.c. (2)).

Relatively high concentrations of *D*-penicillamine are reached in the skin (6, 7). Levels of 40 to 60 µg/g may be reached. Again, no direct effect of *D*-penicillamine on glucose-6-phosphate dehydrogenase activity can be assumed but an indirect effect (chelation of metal ions) is most likely to occur. The absence of direct (inhibitory) *D*-penicillamine effects in skin homogenates may be ascribed to binding to proteins or other substances in the homogenates.

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