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Therapeutic Applications of Enzymes

By A. De Barbieri

The idea of employing enzymes for therapeutic purposes follows:

- a) the improvement of our knowledge about the mechanism of the activity, specificity, and biochemical meaning of enzymes;
- b) the improvement of the techniques for the preparation, conservation, and assay of enzymes.

The basic requirements for the therapeutic application of enzymes are:

1. Possibility of a safe administration of the enzymes.
2. Possibility of an effective activity in the organism.

The first requirement is very important in order that the biological activities, brought about by the administration of enzymes, are to be related only to their activity, not to that of contaminating substances which could be present in the enzyme preparation.

Meanwhile, in case of parenteral administration, the enzyme tolerance has to be considered not only from the pharmacological but also from the immunological viewpoint, keeping in mind the protein nature of the enzymes, their antigenic activity and the consequent possibility of sensitization of the host. In this connection it is to be pointed out that the antigenicity of several enzymes, at present employed in therapy (hyaluronidase, cytochrome C, trypsin and chymotrypsin), is low and almost practically negligible.

As it is well known, the antigenicity of a protein is not a function of the whole molecule, but is restricted to some specific regions of the same, which are therefore called "antigenic determinants". Protein antigenicity is for that reason based on the number of antigenic determinants of the protein itself.

In general, the differences between enzyme proteins of different species are small, provided that they act in the same cycle or type of chemical reactions, independently from the animal species where these reactions

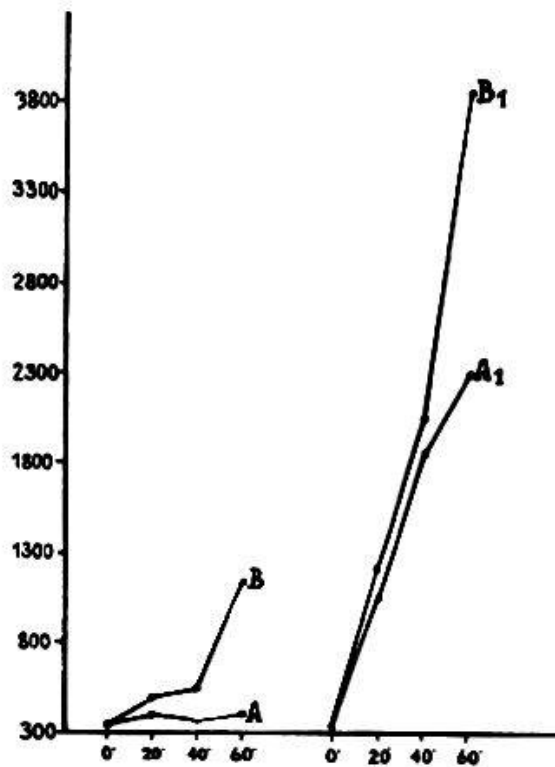


Fig. 1. Fibrinolytic activity of plasma of rabbits, injected with crystallized trypsin or TEP: 5 mg/kg intravenously. - A curve: $\frac{1}{20}$ ml of plasma after trypsin injection; B curve: $\frac{1}{10}$ ml of plasma after trypsin injection; A₁ curve: $\frac{1}{20}$ ml of plasma after TEP injection; B₁ curve: $\frac{1}{10}$ ml of plasma after TEP injection.

Abscissae: Average of the products of two diameters of the lysis areas expressed in millimeters. Ordinates: Time of taking blood samples.

take place, and provided that such species are not too far in the taxonomic classification as, from the chemical viewpoint, such differences lie only in the sequence of some aminoacids in the peptide chain; from this follows that the antigenic determinants of such proteins are very scanty, therefore they cannot affect the immunological behaviour very deeply.

Two types of therapeutic applications of enzymes can be considered: 1. external or exocellular application, 2. internal or intracellular application. The first type of application refers to the hydrolyzing enzymes with the already well-known applications of hydrolyzing enzymes (amylase, lipase, proteases) to the digestive pathology, to secure a more complete and physiologic digestion of food. The recent applications of proteases and ribonuclease to the external pathology, i.e. to the digestion of necrotic tissues and pathologic material (pus, mucous, fibrinous exudates), are to be considered in this connection. Moreover, the recent use of fibrinolytic enzymes for the lysis of intravascular thrombi belongs to this type of application. In fact human fibrinolysin obtained by activating profibrinolysin with streptococcal streptokinase was introduced in therapy lately.

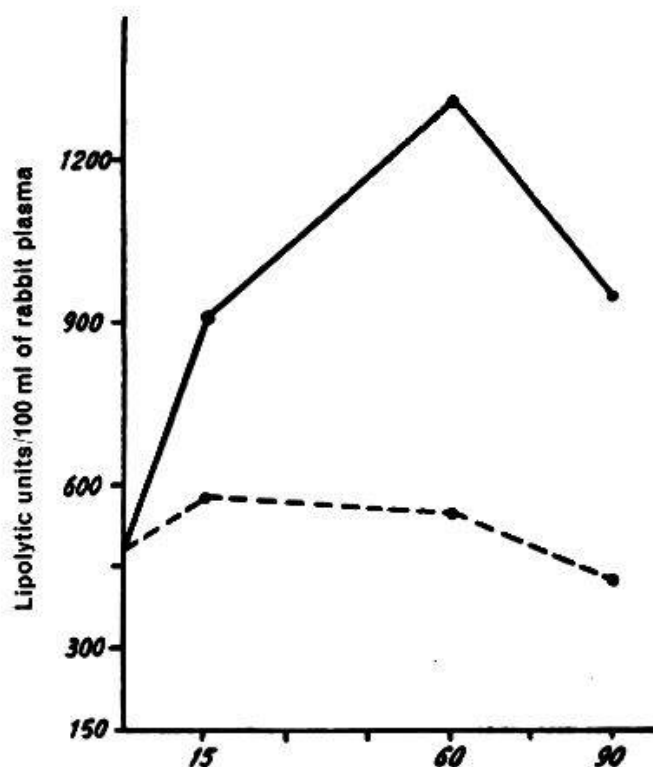


Fig. 2. Behaviour pattern of plasma lipase after intravenous injection to rabbits of 5 mg/kg of: Crystallized trypsin -----, TEP —————

On the contrary, it is impossible to obtain a fibrinolytic effect by means of trypsin injection, as this enzyme is bound very quickly to its specific plasma inhibitor, to yield a proteolytically inactive complex. According to our personal experience (*De Barbieri and Scevola, 1960*), it is, however, possible to obtain a fibrinolysis by means of trypsin when this enzyme is injected as a complex with substances, which, being devoid of the requirements of a substrate for trypsin activity, cannot neutralize its specific activity, though being able to modify its molecule so that it becomes less capable of combining with its specific inhibitors. One of these substances is, e.g., heparin.

The diagrams of fig. 1 show the fibrinolytic activity of the plasma of rabbits, intravenously injected with 5 mg/kg of crystallized trypsin (110 Anson units/mg). A and B curves were obtained respectively by placing $\frac{1}{20}$ and $\frac{1}{10}$ ml respectively of rabbit plasma on the surface of the fibrin film according to *Astrup's* and *Müllertz's* technique (the single points of the curve represent the product in mm of two perpendicular diameters of the lysis areas). A₁ and B₁ diagrams show the same phenomenon, obtained with 0.05 and 0.1 ml of rabbit plasma intravenously injected with 5 mg/kg of the trypsin-heparin complex (TEP). The trypsin-heparin complex is endowed with a plasma lipase promoting activity, as shown by fig. 2, where the lower curve indicates the behaviour of the

plasma lipase after intravenous injection of 5 mg/kg of crystallized trypsin in rabbits, and the upper curve the same phenomenon after injection of 5 mg of TEP.

The second type of enzyme application, which I called internal or intracellular, concerns the parenteral administration of enzymes to affect metabolic patterns. In this latter case the second condition, i.e. the effective activity displayed by enzymes in the organism, acquires an outstanding importance.

Enzymes must move across the cell wall and have a part in the biochemical enzymatic process of the cells to develop their therapeutic activity. This implies that enzymes spread all through the body from the site of their introduction, reach the site where they display their activities, get across the cells to enter into the metabolic enzymatic pattern of the cells themselves. Leaving out of consideration the fact that present views on cellular physiology tend to abandon a strictly material idea of the cell membrane and to consider it as a cell organ with specific selective functions, which can allow different substances to get across the cell wall, inside the cell and vice versa, as a consequence of various situations, not only in relation to the chemical structure or to the molecular size of the compounds, many facts would appear inexplicable, if we denied the possibility that protein molecules (hence enzymes) could get across the cells.

How would it, otherwise, be possible to explain the biological activities of many proteins (protein hormones, bacterial toxins, etc.)? Recent experiments (*De Barbieri and Scevola, 1959*) showed that bovine cytochrome C, labelled by means of fluorescein isocyanate, can be found in the mitochondria of heart, liver and kidneys of the injected guinea-pigs. Moreover, one should take into account the observed increase of cytochrome C and succinoxidase in mitochondria and microsomes of the heart in the injected animals, which we interpreted (*De Barbieri and Scevola, 1957*) as a consequence of the biosynthesis of the relevant enzymes brought about by cytochrome C, which acts as a substrate inducer through an enzymatic adaptation.

The anti-inflammatory activity of trypsin and chymotrypsin prompted their employ in the therapy, by parenteral administration. I think it unnecessary to mention the many clinical papers published on this subject, already well known. The mechanism of the anti-inflammatory activity of proteolytic enzymes has been widely studied from the physiological viewpoint, but it has not yet been cleared from the biochemical viewpoint.

The time allowed does not enable me to discuss this problem. I must,

however, mention an interesting observation, i.e. the documented anti-inflammatory activity (we, too, could personally control this fact) of trypsin and chymotrypsin, directly injected into rat ileum. Considering that the split products of these enzymes are inactive (*Guzzon and Scevola, 1957*), one must admit the crossing of the enzymes through the intestinal barrier. This fact further supports the possibility of penetration of enzymes.

The use of enzymes in therapy not only started a new therapeutic trend, but it pointed out new problems for scientific research.

Summary

After a brief outline of the requirements for the therapeutical applications of the enzymes, both from the pharmacological and the immunological viewpoint, the author considers the possibilities of therapeutic application of enzymes, which he records as follows: exocellular or external, endocellular or internal. The first type of application refers to the hydrolyzing enzymes, widely employed in the digestive pathology, and to proteases and nucleases (ribo- and desoxyribonuclease) for topical application (necrotic tissue digestion). Moreover fibrinolytic enzymes, recently introduced into the therapy of thromboembolic diseases, are considered. The author calls attention to a series of researches, which he carried out for pointing out the particular fibrinolytic activity of a trypsin-heparin complex, in which the enzyme displays a remarkable fibrinolytic activity, as a consequence of its resistance to proteolytic inhibitors, thus acquiring the capacity of activating plasminogen. The second type of therapeutic application of enzymes considers the possibility of an endocellular action of the enzymes, parenterally injected, as pointed out by experiments carried out with fluorescent enzymes, where localization in mitochondria is shown. These facts show the possibility that enzymes cross the cell walls, entering into the cellular biochemical pattern, thus displaying therapeutic effects.

Zusammenfassung

Nach einer kurzen, sowohl den pharmakologischen als auch den immunologischen Standpunkt berücksichtigenden Darstellung der Voraussetzungen für die therapeutische Anwendung der Enzyme, erörtert der Autor die Möglichkeiten ihrer Anwendung, die er wie folgt einteilt:

1. extracelluläre oder externe;
2. intracelluläre oder interne.

Die extracelluläre Anwendung trifft für die hydrolytischen Enzyme zu, die bei Verdauungskrankheiten besonders häufig indiziert ist, sowie für die Proteasen und Nucleasen (Ribo- und Desoxyribonucleasen) zur örtlichen Behandlung nekrotischen Gewebes. Hierzu gehören auch die fibrinolytischen Enzyme, die neuerdings zur Therapie thromboembolischer Erkrankungen eingeführt wurden. Der Autor weist besonders auf eine Reihe von Untersuchungen über die fibrinolytische Wirksamkeit eines Trypsin-Heparin-Komplexes hin, in welchem das Trypsin eine bemerkenswerte fibrinolytische Aktivität entfaltet; dies infolge seiner Resistenz gegenüber proteolytischen Inhibitoren und seiner Fähigkeit, Plasminogen zu aktivieren.

Die zweite Verwendungsart betrifft die Möglichkeit intracellulärer Wirkung von parenteral eingeführten Enzymen, wie sie bei den Experimenten mit fluoreszierenden, sich in den Mitochondrien lokalisierenden Fermenten gezeigt wurden. Diese Untersuchungen beweisen die Möglichkeit des Eindringens der Enzyme in das Zellinnere, wo sie an den biochemischen Vorgängen teilnehmen und damit einen therapeutischen Effekt ausüben.

Résumé

Après avoir décrit brièvement les caractéristiques nécessaires pour l'emploi des enzymes en thérapeutique, aussi bien du point de vue pharmacologique que du point de vue immunologique, l'auteur considère deux possibilités d'emploi en thérapeutique, c'est à dire: extracellulaire ou externe, intracellulaire ou interne. Le premier type concerne les enzymes hydrolytiques, employées dans le traitement des troubles de l'appareil digestif, les protéases et les nucléases (ribo- et désoxyribonucléases) (emploi topique pour la digestion des tissus nécrotiques). Les enzymes fibrinolytiques, récemment introduites en thérapeutique dans le traitement des maladies thromboemboliques, sont ensuite considérées. L'auteur souligne une série de recherches, qu'il vient d'exécuter sur l'activité fibrinolytique d'un complexe trypsine-héparine, dans lequel la trypsine acquiert une activité fibrinolytique remarquable, comme conséquence de sa résistance aux inhibiteurs protéolytiques, et du pouvoir d'activation du plasminogène. Le deuxième type d'emploi, considère la possibilité d'une action intracellulaire des enzymes, injectées par voie parentérale, ce qui est démontré par les expériences avec les enzymes fluorescentes, qui vont se localiser dans les mitochondries. Ces faits prouvent la possibilité de pénétration des enzymes dans les cellules, où ils peuvent expliquer des actions biochimiques, et par suite, des effets thérapeutiques.

Riassunto

Dopo una breve introduzione, in cui vengono delineate le caratteristiche richieste per l'impiego degli enzimi in terapia, sia dal punto di vista farmacologico che da quello immunologico, l'autore passa a considerare le possibilità d'impiego degli enzimi in terapia che vengono suddivisi in due tipi: 1. esocellulari o esterne, 2. interne o endocellulari. Il primo tipo di impiego si riferisce agli enzimi idrolitici largamente impiegati nella terapia dei disturbi dell'apparato digerente, nonché alle proteasi e alle nucleasi (ribo- e desossiribo-) per applicazione topica (digestione di tessuti necrotici). Inoltre vengono presi in considerazione gli enzimi fibrinolitici di recente introdotti nella terapia delle malattie tromboemboliche. L'autore richiama l'attenzione su una serie di ricerche da lui condotte per mettere in luce la particolare attività fibrinolitica di un complesso tripsina-eparina in cui l'enzima esplica una notevole attività fibrinolitica, in conseguenza della resistenza verso gli inibitori proteolitici, e per cui acquista il potere di attivare il plasminogeno.

Il secondo tipo di impiego degli enzimi in terapia considera invece la possibilità di un'azione endocellulare degli enzimi iniettati per via parenterale, come si rileva da esperienze con enzimi fluorescenti dei quali si dimostra la localizzazione nei mitocondri. Queste esperienze provano la possibilità che hanno gli enzimi di penetrare entro le cellule e quindi, partecipando al biochimismo cellulare, di esplicare effetti terapeutici.

De Barbieri A. and Scevola M. E.: Attività biologiche di una tripsina complessata. Atti Soc. lombarda Sci. med. biol. 15, 37 (1960).

De Barbieri A. and Scevola M. E.: Sul comportamento nell'organismo del citocromo c fluorescente. G. Biochim. 8, 18 (1959).

De Barbieri A. and Scevola M. E.: The action of cytochrome c on the enzymic activities of the succinoxidase system of heart. G. Biochim. 6, 44 (1957).

Guzzon V. and Scevola M. E.: Rapporti fra potere proteolitico della tripsina e sua attività antiinfiammatoria. Boll. Soc. ital. Biol. sper. 33, 813 (1957).