International Journal of Basic & Clinical Pharmacology
Shete MR et al. Int J Basic Clin Pharmacol. 2022 Sep;11(5):515-519
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20222046

Review Article

Pharmacokinetic availability of proteolytic enzymes after oral administration: a narrative review of the literature

Mohit R. Shete[1], Sanjay V. Popere[1], James John[2]*

[1]Department of Orthopaedics, Rajiv Gandhi Medical College and Chhatrapati Shivaji Maharaj Hospital, Thane, Maharashtra, India
[2]Department of Medical Services, SIRO Clinpharm Ltd., Thane, Maharashtra, India

Received: 06 July 2022
Accepted: 21 July 2022

***Correspondence:**
Dr. James John,
Email: james.john@siroclinpharm.com

ABSTRACT

Orally administered serine and cysteine proteolytic enzymes are used extensively in the therapy of various inflammatory conditions. However, due to their protein nature, there have been concerns about these enzymes undergoing digestion or biotransformation in the gut and the resultant amount of active enzyme reaching blood circulation and at the site of inflammation. Research has shown that orally administered serine and cysteine proteases are able to pass through the mucosal barrier of the gastrointestinal tract and reach the blood and lymph as intact, high molecular weight and physiologically active forms. These have been studied in in vitro, animal models and further confirmed in human studies. Despite high inter-individual variability, the maximum plasma levels of the free proteases follow dose linearity. They circulate bound to plasma anti-proteases and are detectable in clinically significant concentrations. Targeted studies also indicate that paracellular transport mechanism may play a significant role in the absorption of these molecules. We present a summary of the existing knowledge from these studies.

Keywords: Trypsin, Bromelain, Proteases, Enzymes, Absorption, Inflammation

INTRODUCTION

Proteolytic enzymes or proteases, derived from animal and plant sources, have been extensively used in therapy, especially of inflammatory conditions.[1] Cysteine proteases, commonly encountered in fruits, include enzymes like bromelain and papain. Serine proteases, like trypsin and chymotrypsin are secreted by mammalian pancreas. These enzymes are administered orally when used for medical treatment of inflammatory conditions.[2] Often, these enzymes are supplemented with other ingredients- most commonly bioflavonoids like rutin/rutoside, to provide additional radical scavenging and immunomodulating properties.[3] These enzyme-flavonoid combinations are widely used across the world as prescription drugs, over-the-counter drugs or as food supplements. The indications for their use include diverse conditions like acute and post-surgical trauma, sports injuries, chronic venous disease, rheumatoid and osteoarthritis, and other related conditions.[4-6]

These enzyme combinations are usually formulated as enteric-coated tablets, to overcome the degradation of the protein structure to amino acids or peptides in the acidic environment of the stomach. The strength of proteases provided in the preparations is described by F.I.P-units of the Federation Internationale Pharmaceutics, where one F.I.P.-unit is the amount of enzyme which converts one micromole of substrate in one minute under standard conditions.[2]

Although used widely and since many decades, there are often concerns regarding the extent of absorption of these enzymes. This is primarily due to the belief that intact macromolecular substances are not absorbed across the gastrointestinal tract. These molecules were expected to

get digested or bio-transformed in the gut with no active enzyme reaching blood circulation and at the site of inflammation. Their quantitative analysis was difficult, as multiple exogenous, endogenous, pathogenic, and pharmacological factors could influence their levels. Some of these factors include entero-hepatic circulation of endogenous proteases which cannot be differentiated from the exogenous ones, interaction of both endogenous and exogenous proteases with antiproteases in serum, lymph and interstitial fluid, redistribution of isotopes in case of radio-chromatographic measurements.[2,7]

However, with the availability of highly specific methods of analysis, it was possible to quantify the absorption of enzymes and other macromolecular proteins reaching the blood and measure the enzymatic activity exerted by these molecules. These include oral application of radio-labelled material in animals and quantitative determination in plasma by measuring proteases' intrinsic esterase activity on specific substrates. We have provided a summary of the findings from various *in vitro, in vivo* and human studies evaluating the pharmacokinetics of these proteases.[2]

ABSORPTION OF INTACT ENZYMES ACROSS GUT

In initial in vitro studies using isolated rat jejunum and ileum, it was found that after being filled with trypsin solution and incubation, trypsin was detected in increasing amounts over time in the serosal fluid.[8] In an *in vivo* study using rats, when 131-Iodine chymotrypsin was administered into rat intestine, it was observed that the radioactivity was distributed across various tissues.[9] The absorption of the plant enzyme Bromelain, after oral administration in humans, was initially demonstrated by increased ability of serum to digest casein.[10] Subsequently the bioavailability of Bromelain was evaluated in rats using 125-Iodination and measuring the plasma radioactivity after oral administration. The peak plasma level was attained at 1 hour post administration and the electrophoretic analysis revealed that the molecular weight at the peak of radioactivity to match the molecular weight of the purified enzyme.[11]

Seifert et al studied the absorption of Bromelain, labelled with 125-Iodine and administered to narcotized rats intra-duodenally.[12] During the six hours after the application, blood and lymph samples were evaluated for radioactivity. The absorption rate was estimated by calculating the difference between the I concentration applied and that still present in the gut at the end of the study.[12,13] Further, using rabbit antibodies to bromelain, the integrity of the enzyme was identified using agar-double diffusion technique. Using radio-chromatography, it was also identified that up to 40% of the administered dose of bromelain was absorbed in the high molecular form. From the results, it was also identified that there was no relationship between the molecular size and the absorption.

In another study, Seifert et al administered 125-Iodine labelled proteases amylase, trypsin, chymotrypsin, papain, and pan-creatin via enteric tubes to sedated rats.[13] Using methods similar to those employed for Bromelain, it was observed that all the studied proteases were absorbed in the high molecular forms. It was estimated that the absorption rate for trypsin was 26-34%. Menzel et al. and Steffen et al. reported similar results in rabbits and guinea pigs.

ACTIVITY OF ABSORBED ENZYMES IN HUMAN PLASMA

Multiple methods were evaluated to identify active protease concentrations in blood. This included application of antibodies raised in rabbits against bromelain, non-competitive one-species antibody ELISA using two rabbit antibodies and antibody capture method to measure the intrinsic proteolytic activity of the bromelain.

Castell et al conducted a study in human volunteers, confirming the presence of full-size immune reactive bromelain in human plasma samples after oral administration.[14] The identity of bromelain was confirmed using detection of specific protein bands by immunoprecipitation and gel electrophoresis technique and comparing with control individuals. In a randomized placebo-controlled study, 15 healthy males, aged 18-45 years, received enteric-coated film tablets containing 200 mg of Bromelain administered as 3 tablets five times over 12 hours of the day followed by a single dose of 5 tablets at night; this was repeated the next day too and on third day only 3 tablets were administered in the morning. During the study, all volunteers received standardized meals. Blood samples were collected before each administration, and the results were compared against 4 similar volunteers in the placebo group. The pharmacokinetic analysis of immunoreactive bromelain revealed that there was significant interindividual variability in the concentrations and proteolytic activities. Based on a mono-compartmental model, it was identified that the peak plasma levels (~5 ng/ml) for most subjects were achieved at around 48 hours, the elimination half-life was around 6 hours, and the total exposure (area under concentration curve) was about 82.2 ng/hr/ml. It was also estimated that the average amount of bromelain in plasma during the 3- to 51-hour period was around 10.8 micrograms.

Using a similar technique, another pharmacokinetic study was conducted, this time with a preparation (Phlogenzym) of the combination of trypsin, bromelain and rutoside in 20 healthy volunteers.[15] A cross-over design using two different doses was used. The lower dose comprised of 4 tablets four times a day, while the higher dose comprised 8 tablets 4 times a day. Both plasma concentration and enzymatic activity curves were evaluated for trypsin and bromelain. The concentrations were determined by ELISA and specific hydrolytic activities were assessed by previously validated method. Western blot analysis was

also done to further supplement the identification of trypsin and bromelain in the plasma. There was considerable inter-individual variation. For trypsin, the area-under-curve (AUC) values were 120 ng.hr/ml and 65 ng.hr/ml for the higher and lower doses, respectively, and corresponding changes of specific hydrolytic activities were observed; peak values of 13 ng/ml and 2.1 ng/ml trypsin were seen. Bromelain demonstrated a similar pattern, with AUC values of 295 ng.hr/ml and 142 ng.hr/ml; maximal concentrations were 33 ng/ml and 5.6 ng/ml, respectively. There was a significant (p value<0.0001) correlation between trypsin and bromelain concentration in plasma and their specific hydrolytic activities, indicating that hydrolytic activities had preserved following absorption. Additionally, the plasma values rutoside components were also, above the baseline values.

TISSUE DISTRIBUTION, METABOLISM AND ELIMINATION

In the studies conducted by Seifert et al with 125-I-labeled bromelain, tissue radioactivity (as a percentage of dose administered per gram tissue) in the different organs of the rat were calculated. The values were lung 0.14±0.025, liver 0.10±0.015, spleen 0.11±0.017, kidney 0.42±0.244, muscle 0.10±0.018, skin 0.15±0.032, thyroid gland 3.62±0561.[12] Kidneys and thyroid had the highest amounts, suggesting renal route of elimination and iodide accumulation, respectively. Similarly, in the studies conducted by Moriya et al with 131-I-labelled chymotrypsin, the radioactivity in the kidney increased gradually.[9]

If these enzymes remain free in the serum, they will get rapidly metabolized by the ubiquitous serum proteases. However, they are protected from this metabolism as most of the absorbed enzyme circulate bound to plasma proteins-the anti-proteinases, mainly α2-macroglobulin. The enzymes, however, retain the proteolytic activity even when complexed with the anti-proteinases. The resulting complexes are recognized by low density lipoprotein receptor-related proteins on the surface of blood cells and hepatocytes for rapid elimination These complexes eventually bind to cell receptors and are phagocytosed by the macrophages. In addition to protecting the enzymes from degradation in serum, the enzyme-anti-protease complexes are believed to contribute to the therapeutic effects of the enzymes by binding to different cytokines as well as the growth factors involved in acute or chronic inflammation and the ultimately leading to their elimination.[2]

In human volunteer studies, the elimination half-life of trypsin after oral administration, was estimated to be around 9-12 hours. In case of oral bromelain, a distribution and an elimination half-life were seen. Most subjects had a half-life of 6 hours, although it ranged from 8-14.5 hours in the rest.[2]

MECHANISM OF ABSORPTION

Since it became apparent that proteases are absorbed in their intact forms across the gastrointestinal tract, studies on the possible mechanism to explain this were conducted. A human colon carcinoma cell line-caco-2, which resemble normal small intestinal enterocytes was used in an ex vivo study.[16] The effects of proteases trypsin, chymotrypsin, papain and bromelain on the confluence and permeability of the differentiated caco-2 monolayer were examined using fluorescein (molecular weight 400) as a transport marker and by measuring the trans-epithelial electrical resistance (TEER). A time and concentration dependent effect was observed-decrease in TEER and increased transport of fluorescein across the monolayer. The effect was more prominent with papain and bromelain. These observations are indicative of loosening of the tight junction between epithelial monolayers. These findings were replicated with fluorescent transport markers with a molecular weight of up to 600,000 Daltons. The effects were even found to be reversed after a few days. To create an environment resembling the physiological conditions due to the presence of water layer, mucus, food, albumin and antiproteases, mucin and albumin were added to the caco-2 monolayer. The activity of trypsin and chymotrypsin was widely unaffected, while mucin decreased the activity of papain and albumin increased the activity of bromelain.

Table 1: Summary data from studies.

Study design/model	Ingredient	Summary findings
***In vitro*-isolated rat jejunum and ileum[8]**	Trypsin	Trypsin was detected in increasing amounts over time in the serosal fluid
***In vivo*-rats[9]**	Chymotrypsin administered into intestine	Distributed across tissues, high levels in kidney
Human study[10]	Bromelain administered orally	Increased ability of serum to digest casein
***In vivo*-rats[11]**	Bromelain administered orally	Peak plasma level attained at 1 hour in intact form, high levels in kidney
***In vivo*-sedated rats[12]**	Bromelain administered intraduodenally	Up to 40% of bromelain was absorbed in intact form
***In vivo*-sedated rats[13]**	Amylase, trypsin, chymotrypsin, papain, and pancreatin administered via enteric tubes	26-34% of trypsin absorbed

Continued.

Study design/model	Ingredient	Summary findings
Randomized doble-blind placebo-controlled study in 19 healthy males[14]	Repeated oral dose of enteric-coated Bromelain	Intact immunoreactive bromelain demonstrated in serum. Peak plasma levels (~5 ng/ml) achieved around 48 hours, elimination $t_{½}$ ~6 hours
Randomized low vs high dose study in 20 healthy volunteers[15]	Repeated oral administration of low and high doses of fixed dose combination of trypsin, bromelain and rutoside	Dose-dependent linear increase of the maximum plasma levels of trypsin and bromelain; proteolytic serum activity paralleled the plasma concentration.
***In vitro*-caco-2 cell line[16]**	Trypsin, chymotrypsin, papain and bromelain	Decrease in trans-epithelial electrical resistance and increased transport of fluorescein (indicative of loosening of the tight junction between epithelial monolayers) with all enzymes. More prominent with papain and bromelain.
***In vitro*-caco-2 cell line with albumin and mucin[16]**	Trypsin, chymotrypsin, papain and bromelain	Trypsin and chymotrypsin showed similar results as above. Albumin increased the activity of bromelain.

Based on these findings, a self-enhanced paracellular diffusion of proteases was hypothesized.[2] This could easily be expected in the presence of locally widened intercellular junctions. This corroborated with the known strong mucolytic activity of bromelain, trypsin and papain. Their capability to cleave amino acid binding sequence of mucus glycoproteins and degrade extra-cellular matrix components have been used to isolate cells in various experimental techniques. In fact, it was noted that oral administration of enzymes resulted in several folds higher concentration across the gastrointestinal tract than those observed in in vitro studies. This further strengthens the paracellular diffusion hypothesis. Another possibility in this regard is the role played by protease-activated receptors (PAR) especially PAR-2 which is predominantly cleaved by serine proteases like trypsin. PAR-2 has been demonstrated to modulate epithelial permeability and may play a role in transport of molecules across gut.[2]

CONCLUSION

Orally administered proteolytic enzymes are able to reach the circulation in intact, high molecular weight and physiologically active forms. This has been demonstrated in various in vitro, *in vivo* and clinical studies. The human pharmacokinetic studies have revealed a dose-dependent linearity in the maximal plasma levels attained. The pharmacokinetics of these enzymes are further characterized by slow absorption and rapid and extensive binding to plasma proteins (antiproteases). Paracellular diffusion is believed to be the major mechanism for transport of intact enzymes across the gastrointestinal tract.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Paradis ME, Couture P, Gigleux I, Marin J, Vohl MC, Lamarche B. Impact of systemic enzyme supplementation on low-grade inflammation in humans. Pharma Nutri. 2015;3(3):83-8.
2. Lorkowski G. Gastrointestinal absorption and biological activities of serine and cysteine proteases of animal and plant origin: review on absorption of serine and cysteine proteases. Int J Physiol Pathophysiol Pharmacol. 2012;4(1):10-27.
3. Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharm J. 2017;25(2):149-64.
4. Bakhshi GD, Rangwala ZS, Chauhan AR. Systemic enzyme therapy in chronic venous disease: a review. Int J Res Med Sci. 2021;9:2869-76.
5. Leipner J, Iten F, Saller R. Therapy with proteolytic enzymes in rheumatic disorders. Bio Drugs. 2001;15(12):779-89.
6. Bakhshi GD, Tiwari S, Prajapati RK, Rudrappa GH, Uppin MI, Vinchhi PJ et al. Systemic Enzyme Therapy with Trypsin-Bromelain-Rutoside combination to counter post-operative wound inflammation-a randomized active-controlled multi-centre trial. Eur J Pharm Med Res. 2019;6(8):493-500.
7. Kolac C, Streichhan P, Lehr CM. Oral bioavailability of proteolytic enzymes. Europ J Pharma Biopharm. 1996;42:222-32.
8. Faudemay F, Laporte JC, Trémolières J. Passage de la trypsine à travers la paroi intestinale de rat *in vitro*. Nutr Metab. 1973;15:207-12.
9. Moriya H, Moriwaki C, Akimoto S, Yamaguchi K, Iwadare M. Studies on the passage of α-chymotrypsin across the intestine. Chem Pharm Bull. 1967;15:1662-8.
10. Miller JM, Opher AW. The increased proteolytic activity of human blood serum after the oral administration of bromelain. Exp Med Surg. 1964;22:277-80.
11. White RR, Crawley FEA, Vellini M, Rovati LA. Bioavailability of I-bromelain after oral administration to rats. Biopharmaceut Drug Dispos. 1988;9:397-403.
12. Seifert J, Ganser R, Brendel W. Absorption of a proteolytic enzyme originating from plants out of the

gastro-intestinal tract into blood and lymph of rats. Z Gastroenterol. 1979;17:1-8.
13. Seifert J, Siebrecht P, Lange JP. Quantitative Untersuchungen zur Resorption von Trypsin, Chymotrypsin, Amylase, Papain und Pankreatin aus dem Magen-Darm-Trakt nach oraler Applikation. Allgemeinmedizin. 1990;19:132-7.
14. Castell JV, Friedrich G, Kuhn CS, Poppe GE. Intestinal absorption of undegraded proteins in men: presence of bromelain in plasma after oral intake. Am J Physiol. 1997;273:G139-46.
15. Donath F, Roots I, Mai I, Maurer A, Wood G, Kuhn CS, Friedrich G. Dose-related bioavailability of bromelain and trypsin after repeated oral administration. Eur J Pharmacol. 1997;52:A146.
16. Pinto M. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Biol Cell. 1983;47:323-30.

Cite this article as: Shete MR, Popere SV, John J. Pharmacokinetic availability of proteolytic enzymes after oral administration: a narrative review of the literature. Int J Basic Clin Pharmacol 2022;11:515-9.