



REVIEW ARTICLE

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Role of protease inhibitors in Insulin therapy of Diabetes: are these beneficial?

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ABSTRACT

For the treatment of diabetes, currently available insulin replenishment is the most successful therapy which improves insulin receptor sensitivity, reduces glucotoxicity and lipotoxicity. However, insulin injection therapy has disadvantages such as pain, inconvenience of multiple injections, allergic reactions, hyper-insulinemia, and insulin lipodystrophy at injection site. Non-invasive delivery of insulin would eliminate side effects, compliance problems to treatment adherence and other complications associated with insulin injection therapy such as pain caused by injection, psychological barriers associated with multiple daily injections such as needle anxiety and possible infections. Several approaches have been developed to improve bioavailability of insulin, such as use of protease inhibitors to prevent enzymatic degradation of insulin, enhancement of membrane permeability or widening of tight junctions to improve absorption of insulin, and development of novel insulin carriers. Protease inhibitors such as aprotinin, bacitracin and camostat are known to improve insulin absorption and bioavailability as evident by several experimental and clinical studies. However, these are not approved for use in combination with insulin to treat diabetes. This may be due to the adverse effects associated with use of protease inhibitors as well as lack of sufficient clinical data. Moreover, there are reports which indicate that there is no clear-cut benefit of protease inhibitors when these were used in combination with insulin to treat the diabetes. This review article mainly focuses on role of different protease inhibitors to improve therapeutic usefulness of insulin. Moreover, the risks or adverse effect associated with protease inhibitors have been discussed.

Keywords: protease inhibitors, lipotoxicity, insulin

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INTRODUCTION

Recently biomolecules such as proteins and peptides have gained much interest in therapeutics due to their potency, selectivity, biocompatibility and biodegradability (1-3). However, big molecular size (4), polarity (5), and rapid inactivation by endogenous enzymes result in low permeability, absorption and bioavailability of such molecules (6-9). These obstacles must be overcome in order to achieve successful therapeutic uses of proteins and peptides. Among the proteins, insulin has been investigated extensively but without much success so far due to inherent problems associated with this peptide such as rapid enzymatic degradation (10-11), poor permeability (12-14) and absorption (15-17).

Several approaches have been developed to improve bioavailability of insulin, such as use of protease inhibitors to prevent enzymatic degradation of insulin (18-41), enhancement of membrane permeability or widening of tight junctions to improve absorption of insulin (42-58) and development of novel insulin carriers with improved absorption of insulin (59-65). This review article mainly focuses on use of different protease inhibitors to improve therapeutic usefulness of insulin. Moreover, the adverse effects associated with the use of protease inhibitors have also been discussed.

PROTEASE INHIBITORS AND INSULIN ABSORPTION

Pretreatment with protease inhibitors such as aprotinin, bacitracin, camostatmesilate and sodium glycocholate or co-administration with insulin were found to improve the hypoglycemic effect of insulin either by inhibiting insulin degradation or by promoting insulin absorption or both. Yamamoto et. al., 1994 investigated effects protease inhibitors such as aprotinin, bacitracin and soybean trypsin inhibitor on the pulmonary absorption of insulin by means of an in-situ pulmonary absorption experiment.

Significant and continuous hypoglycaemic effects were found when insulin was co-administered with protease inhibitors (66). They further investigated effects of protease inhibitors on the intestinal absorption of insulin in situ in closed small and large intestinal loops in rats (67). They observed no marked hypoglycemic effect when insulin alone was administered into small or large intestinal loops however a significant hypoglycemic effect was observed when insulin was co administered with protease inhibitors such as bacitracin, camostatmesilate and sodium glycocholate (67). Researchers have evaluated the stability of insulin in rat lung homogenate with or without various protease inhibitors so as to explore the effectiveness of for insulin delivery from the lung (23). They noticed that Insulin rapidly degraded in lung homogenate. Protease inhibitors such as aprotinin, bacitracin, soybean trypsin inhibitor and sodium glycocholate effectively reduced insulin degradation in lung homogenate in order of bacitracin >aprotinin> soybean trypsin inhibitor > sodium glycocholate (23).

Metabolism of insulin and their protection by various protease inhibitors, in fresh caecal contents prepared from non-fasted rats were investigated by Tozaki et. al. (27). They observed that degradation of insulin was inhibited by protease inhibitors such as camostat and aprotinin in rat caecal contents, which was consistent with the high chymotrypsin activity of these contents. They concluded that protease inhibitors might be useful for increasing the stability of these peptides in the large intestine, thereby improving their large-intestinal absorption to the systemic circulation.

Morimoto et. al., 2000 evaluated the permeability of insulin to characterize the tracheal epithelial barrier in in vitro experiments using excised rabbit trachea (29). They noticed that tracheal permeability of insulin was significantly increased by glycocholate, bestatin and aprotinin. They concluded that the tracheal absorption of peptide drugs through the respiratory tract may contribute to the systemic delivery of these drugs following the pulmonary administration of these drugs by intratracheal insufflation and instillation.

Radwant and Aboul-Enein evaluated role of several absorption enhancers including protease inhibitors to protect insulin-loaded Poly (ethylcyanoacrylate) nanospheres after oral administrations to streptozotocin-induced diabetic rats (31). The addition of protease inhibitor to insulin-loaded PECA nanospheres significantly reduced the blood glucose level after po administrations.

Liu et. al., investigated effects of protease inhibitors on the absorption of insulin in-situ from closed small and large intestinal loops in the presence or absence of luminal contents (33). They observed a significant hypoglycaemic effect of insulin in the large intestinal loop in the presence or absence of luminal contents when insulin was co-administered with protease inhibitors in the order of leupeptin>sodium glycocholate>bacitracin>bestatin>cystatin. They noticed that hypoglycemic effects of insulin were amplified a little in small intestinal loop whereas prominent hypoglycemic effects were found in large intestinal loop thus suggesting that protease inhibitors could increase the efficacy of insulin if co administered especially to the large intestine (33).

Park et. al., developed human insulin microcrystals and administered to rat via pulmonary route with or without protease inhibitors (35). They observed around 43% drop in blood glucose level after intratracheal instillation of insulin microcrystals which was further dropped to 49-56%, 53-57% due to presence different amount of protease inhibitors such as soybean-trypsin inhibitor and aprotinin respectively. They suggested that the use of insulin microcrystals and protease inhibitors would be useful to improve the hypoglycemic effect in pulmonary route (35). Jelvehgari et. al., investigated effect of oral insulin microspheres containing protease inhibitor in rats (37). They observed that enzymatic degradation of insulin was decreased due to protection from trypsinic degradation in the microspheres. They reported enhanced insulin absorption and biological response along with high loading efficiency, pH responsiveness and prolongation of insulin release (37).

APROTININ AND INSULIN

Aprotinin is a monomeric polypeptide consisting of 16 different amino acid types and is derived from bovine lung tissue (68). This is approved and marketed in Europe to reduce bleeding during complex surgery, such as heart and liver surgery. It is mainly used as an antifibrinolytic molecule that inhibits trypsin and related proteolytic enzymes also.

ANIMAL STUDIES AND EX VIVO EXPERIMENTS OF APROTININ AND INSULIN

Several researchers have investigated effect of aprotinin on insulin absorption or degradation in ex vivo setup. Deurloo et. al., reported absorption enhancement of intranasally administered insulin by protease inhibitor in rabbits and rats (69). Bendayan et. al., evaluated effect of aprotinin on insulin absorption by the rat ileal epithelium in presence of aprotinin by co administering insulin and aprotinin into the lumen of the ileum of rats (70). Analysis of blood samples from the inferior vena cava, at different time points has demonstrated an increase in plasma insulin followed by a decrease in blood glucose levels (70).

Kraeling and Ritschel evaluated absorption of insulin from colon release capsule dosage form in beagle dogs with aprotinin as protease inhibitor (71). Beagle dogs were administered with i.v. insulin, p.o. insulin microemulsion and colonic release capsule dosage forms. The pharmacological availability for the p.o. microemulsion and colon release capsule dosage form was found to be 2.1 and 6.2%, respectively (71).

Morishita et. al., investigated role of aprotinin on hypoglycemic effects of insulin after co-administration to the duodenum, the jejunum, the ileum and the colon using an in situ loop method (72). Insulin solution was administered to the various loops of fasted rats with or without aprotinin. They observed an obvious hypoglycemic effect of insulin co-administered with aprotinin in the ileum loop (72). Bendayan et. al., evaluated effect of aprotinin on insulin absorption by co-administering insulin and aprotinin to the duodenum and colon of diabetic rats (70). Blood analysis made at several time points has demonstrated a rapid increase in circulating levels of insulin followed by significant and consistent decreases in blood glucose. Moreover, levels of circulating insulin were higher for longer time when the administration was performed in the colon (70). Cilek et. al., evaluated effect of aprotinin on oral absorption of insulin from lecithin-based microemulsion in non-diabetic and streptozotocin-induced diabetic rats. Microemulsions of recombinant human insulin were co administered with aprotinin intragastrically by a cannula to diabetic and non-diabetic rats. They observed slight increase in insulin bioavailability when it was co-administered with aprotinin (34).

EFFECT OF APROTININ ON HYPOGLYCEMIC EFFECT OF INSULIN IN HUMAN SUBJECTS

Several clinical studies have demonstrated better hypoglycemic effect of insulin when it was co-administered with aprotinin. Berger et. al., investigated the effect of aprotinin on the absorption of regular insulin in healthy human (73). An increase in the rate of insulin entry into the circulation leading to overall increased amount of insulin was noticed when insulin was injected subcutaneously together with aprotinin. They concluded that this could be possibly by an inhibition of the local degradation of exogenous insulin at the injection site (73). Similar type of investigation was done by Freidenberg et. al., who observed improved hypoglycemic effect of conventional doses of subcutaneously administered insulin in diabetic patients when they received aprotinin also along with insulin (74). However, insulin alone did not produce hypoglycemic effect especially when administered subcutaneously. These findings suggest excessive degradation or sequestration of insulin at the site of injection.

Absorption kinetics and biologic effects of insulin co administered with aprotinin in 52 male non-obese healthy human volunteers were investigated by Berger et. al., (75). They observed that aprotinin caused a marked acceleration of the insulin absorption process. In another similar study carried out by Linde and Gunnarsson 1985, in normal-weight Type 1 diabetic patients, insulin absorption was increased significantly due to co-administration of aprotinin (76). They concluded that subcutaneously injected soluble insulin is more rapidly absorbed by addition of aprotinin to the insulin solution in Type 1 diabetes. Owens et. al., also evaluated influence of aprotinin on regional absorption of soluble human insulin. Soluble human insulin and aprotinin admixture was injected subcutaneously into the Anterior abdominal wall, and into the thigh in normal human subjects (77). They observed that admixture of insulin with aprotinin led an acceleration of the early phase of absorption from subcutaneous tissue.

BACITRACIN AND INSULIN

Bacitracin is a mixture of related cyclic polypeptides produced by organisms of the licheniformis group of *Bacillus subtilis* var Tracy. Its unique name derives from the fact that the bacillus producing it was first isolated in 1943 from a knee scrape from a girl named Margaret Tracy. Bacitracin is a widely used metalloproteinase antibiotic with a potent bactericidal activity against gram positive bacteria. Bacitracin is known to inhibit proteases (78) and metalloproteinases (79, 80).

EFFECT OF BACITRACIN OVER DEGRADATION, ABSORPTION AND UPTAKE OF INSULIN

Bacitracin has been used along with insulin in several ex vivo experimental and clinical studies. It was found to affect the degradation, absorption and uptake of insulin. Hammons et. al., demonstrated that at physiological concentrations of insulin bacitracin inhibited the degradation of specifically bound insulin by enzymes located in the rat adipocyte plasma membrane (81). They observed an increase in the amount of intact insulin specifically bound to the plasma membrane as bacitracin inhibited ¹²⁵I-insulin degradation by isolated plasma membranes. In another similar study, addition of bacitracin caused significant reduction of degradation of insulin in isolated hepatocytes (82). Bonser et. al., 1983 investigated effect of bacitracin on ¹²⁵I-labelled insulin-receptor interactions in isolated rat hepatocytes (83). They observed that bacitracin did not alter insulin receptor affinity or number while increased cell-associated radioactivity with bacitracin was due to surface-bound insulin. Lönnroth et. al., evaluated

effect of bacitracin on intracellular accumulation of insulin in rat adipocytes (84). They observed that bacitracin markedly increased cell-associated specifically bound 125I-labelled insulin without altering the affinity of the binding sites. They concluded that bacitracin inhibited both extracellular as well as intracellular degradation of insulin (84).

Peavy et. al., investigated effect of bacitracin on intracellular insulin degradation using an isolated rat hepatocyte and observed that insulin degradation was due to cell-mediated processes and bacitracin significantly affected hepatocyte insulin metabolism (85). In another similar experiment, Fleig et. al., investigated effect of bacitracin on the nature of cell-associated radioactivity in studies on the binding and degradation of 125I-insulin in cultured rat hepatocytes (86). They concluded that bacitracin increase insulin internalization and act predominantly at the cell surface as proportion of surface-bound to internalized intact insulin remained unchanged. Yagil et. al., investigated effect of bacitracin on the binding and processing of 125I-labeled insulin in a proximal tubular epithelium-like opossum kidney cell line (87). They observed that addition of bacitracin inhibited insulin degradation significantly and delayed the time of appearance of products in the medium compared with control cells. In another similar experiment carried out by Dahl et. al., presence of bacitracin caused around two-third increase in intracellular radioactivity (88). Shen et. al., investigated in vitro effect of protease inhibitors over biodegradation of insulin in lung cytosol and subcellular pellets of normal or diabetic rats (89). They observed that cytosolic insulin degradation was strongly inhibited by bacitracin. They concluded that co-administration of protease inhibitors would be a useful approach for improving the pulmonary absorption of insulin (89).

CAMOSTAT MESILATE AND RELATED COMPOUNDS

Camostat mesilate is a serine protease inhibitor which is used in the treatment of some forms of cancer, liver fibrosis, and pancreatitis and is also effective against some viral infections (90-92). Uses of camostat mesilate and related compounds for the enhancement of insulin absorption or uptake due to inhibition of proteases have been investigated by several researchers. Takeyama et. al., developed ointment containing gabexate mesilate or nafamostat mesilate, which was applied to the rat skin around the insulin injection site (20). They observed that insulin degradation at the subcutaneous injection site was decreased. Tozaki et. al., evaluated the effect of camostat mesilate on colon-specific delivery of insulin (30). A slight decrease in plasma glucose levels was observed following the oral administration of these pellets containing 12.5 IU of insulin compared with the same dose of insulin solution. They observed that camostat mesilate caused decrease in plasma glucose levels in a dose-dependent manner (30). Del Curto et. al., investigated an oral dosage form intended for time-dependent colon delivery of insulin along with a camostat mesilate. They observed better hypoglycemic effect of insulin due to prevention of insulin degradation by proteases (36).

UNDESIRABLE EFFECTS OF PROTEASE INHIBITORS

Protease inhibitors have been used extensively along with insulin in order to increase the absorption or uptake of insulin by inhibiting its enzymatic degradation. However, there are some reports which demonstrate that concomitant administration of protease inhibitors along with insulin did not show any improvement in insulin absorption. For instance, Lunetta et. al., reported that aprotinin administration along with insulin in type I diabetes patients did not affect blood glucose levels when compared with insulin alone (93). These findings do not support the hypothesis that the administration of aprotinin together with insulin can be clinically useful in conventional insulin treated diabetic patients. However, this could be due the fact that aprotinin was administered for relatively very short period of time i.e. 24 hours only. Moreover, dose of aprotinin was also less than routine dose.

There are reports that protease inhibitors have detrimental effects over insulin therapy such as nephrotic syndrome and proteinuria (94), lipohypertrophy and glomerulonephritis (95). Dandona et. al., reported detrimental effect of aprotinin when it was administered with insulin in insulin dependent diabetic patients. They observed that aprotinin induced lipohypertrophy and glomerulonephritis which was reversed after withdrawal of aprotinin (94). Boag et. al., also reported occurrence of lipohypertrophy and glomerulonephritis in insulin-dependent diabetic patients which was associated with the use of aprotinin (95).

It is interesting to note that protease inhibitors were found to improve the subcutaneous insulin resistance (96), however there are contradictory reports as well which states that protease inhibitors co-administered with insulin impaired the insulin sensitivity and oral glucose tolerance (97).

CONCLUSION

Protease inhibitors have been used extensively along with insulin in order to increase the absorption or uptake of insulin by inhibiting its enzymatic degradation. Several clinical trials have demonstrated that concomitant administration of protease inhibitors and insulin had better hypoglycemic effects as compared to patients which were administered with insulin alone. This was associated with improved absorption of insulin in presence of protease inhibitors. However, there are some contradictory reports as well, which demonstrate that concomitant administration of protease inhibitors along with insulin did not show any improvement in insulin absorption. This could be due the fact that aprotinin was administered for relatively very short period of time i.e. 24 hours only. Moreover dose of aprotinin was also less than routine dose. Nevertheless, role of protease inhibitors in insulin therapy of diabetes remains dubious. Their definite beneficial effect on insulin therapy warrants more clinical studies in larger settings.

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