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In vitro alpha amylase inhibitory effect of some clinically-used drugs

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Twenty six medicinal compounds used for treating various diseases and representing different chemical and pharmacological classes were tested for their potential alpha amylase inhibitory activity *in vitro*. Three of these (tetracycline, enalapril and captopril) were found to have a substantial alpha amylase inhibitory activity. The effect was shown to be dose dependent and the IC_{50} was determined (tetracycline = 0.59 mM, enalapril = 0.29 mM, and captopril = 0.78 mM) and compared to that of acarbose (0.0062 = mM). The potential pharmacological implications of this effect are discussed.

1. Introduction

The use of many drugs is associated with a range of side effects. The seriousness and occurrence of the side effects of certain drugs vary between individuals and some side effects may require several years to become evident. In some cases side effects of drugs have been exploited to treat other conditions. A typical example is the exploitation of the sedative effect of antihistamines as a sleeping-aid (Qidwai et al. 2002). Therefore, understanding how drugs interact with physiological systems other than their main targets is quite important as it sheds light on how they work, and what would be the other potential effects of the drug on the body. However, achieving this might not be an easy task if the side effects were mild and occurred over a long period of time.

Moreover, understanding the potential interaction of clinically used drugs with other targets may provide lead compounds for treating other conditions. Investigating potential side effects is an essential part of clinical trials which are performed for proving the efficacy and safety of new drugs (Hammer 1986). Approaches to assessing side effects could include routine clinical laboratory testing in addition to physical examinations of patients. However, it is uncommon to study potential interactions of drugs under development with the various body enzymes. In this paper we have screened 26 drugs in common clinical use for their potential interaction with the enzyme alpha amylase. Alpha amylase is the main enzyme in humans that is responsible for the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose and glucose) (Alexander 1992). Although the activity of the enzyme has not been directly involved in the etiology of diabetes, alpha amylase inhibitors have long been thought to improve glucose tolerance in diabetic patients (Lebovit 1998). Extensive efforts have been made over the past decades to find a clinically effective alpha amylase inhibitor with the aim of obtaining better control of diabetes (Jung et al. 1996).

More recently alpha glucosidase inhibitors (in particular acarbose) have been marketed. However, this drug has been associated with GIT side effects with flatulence being the most often encountered (Lebovit 1998).

In this study we have screened a group of 26 drugs representing several pharmacological categories for alpha amylase inhibitory activity. The purpose of this screening was to gain a better understanding of the action and side effects of the drugs examined. Moreover this screening might help in the development of effective alpha amylase inhibitors.

2. Investigations, results and discussion

All the available drugs were initially subjected to the alpha amylase inhibitory test, at the relatively high concentration of 0.5 mg/ml. These included seven pharmacological and pharmaceutical classes i.e. non-steroidal anti-inflamatories (NSAI), anti-depressants, thiazide diuretics, antihistamines (AH), antibiotics (macrolides, aminoquinolones, penicillins, cephalosporins and aminoglycosides), angiotensin converting enzyme inhibitors (ACEI) and beta blockers. The results so obtained (expressed as percentage

Table: Percentage inhibition of alpha amylase under the studied conditions

Drug	Percentage inhibition	Drug	Percentage inhibition
Naphazoline	13.6	Fluoxetine	4.7
Chlorpheniramine	− 11.4	Hydrochlorothiazide	3.8
Captopril	99.5	Ibuprofen	− 5.1
Tetracycline	97.9	Metronidazole	3.9
Enalapril	99.9	Clindamycin	1.6
Ketotifen	77.6	Atenolol	3.5
Diclofenac sodium	4.74	Timolol	3.9
Orphinadrine citrate	2.6	Propranolol	4.6
Amoxycillin	− 9.25	Clarithromycin	3.13
Antazoline	− 3.73	Azithromycin	2.75
Fluconazole	7.4	Ampicillin	2.8
Ciprofloxacin	4.7	Cloxacillin	3.5
Astemizole	2.1	Cephalexin	2.6

inhibition under experimental conditions) are presented in the Table. From this data it is clear that only three of the drugs examined (namely; tetracycline, enalapril and captopril, showed significant (more than 95%) alpha amylase inhibitory activity. Ketotifen showed only mild inhibitory activity (77.6%). Every effort was made to ensure that the observed effect was a specific one and not simply a result of non-specific inactivation of the enzyme by changes in pH. Measurements of the pH of the incubate showed insignificant changes which supports the conclusion that the inhibition is a specific one.

It was interesting to note that both enalapril and captopril which belong to the ACEI showed such a high activity, implying that this effect was a characteristic of the ACEI group of compounds. However, it is rather unexpected for such small molecules which are significantly different in structure from starch (the usual substrate for amylase) to act as competitive inhibitors according to the classical concept of enzyme inhibitors i.e. pseudosubstrates. The best known (and most clinically used) alpha glucosidase and amylase inhibitor, acarbose, is a pseudosubstrate for alpha amylase.

Moreover the inhibition was shown to be concentration dependent, and dose response curves (*in vitro*) could be obtained for the three drugs (Fig. 1). The Fig. shows typical pharmacological dose response curves that would permit the estimation of the inhibitor concentration that would produce 50% inhibition under the experimental conditions used (IC_{50}). The estimated IC_{50} values were 0.59 mM, 0.29 mM, and 0.78 mM for tetracycline, enalapril and captopril, respectively. Both of the ACEI (enalapril and captopril) were found to be more potent than tetracycline. However, in comparison to the standard alpha amylase inhibitor (acarbose), all three drugs were found to be significantly less potent (IC_{50} for acarbose = 0.0062 mM). Perhaps, this relatively low potency in addition to the low doses used for of ACEI are the reasons why such inhibitory effects are not easily noticeable when these drugs are given in practice for their intended use.

In order to obtain more direct evidence of the potential interaction between alpha amylase and these drugs, a solution of alpha amylase was titrated with a solution (0.5 mg/ml) of each drug separately in a UV cell. The addition of each of the drugs examined (ACEI and tetracycline) produced a progressive hypochromic shift in the UV spectrum of alpha amylase close to that previously reported for the binding of cyclodextrins to alpha amylase (Fig. 2), thus providing direct evidence of binding of these small molecules to alpha amylase. However it is less likely that these drugs bind to the same active site of the substrate as does the typical pseudo-substrate inhibitor acarbose (Koukiekolo et al. 2001). Therefore, ACEI and tetracycline may bind to some remote binding sites which however influence the structure of the enzyme making it less active. The presence of such secondary binding sites for amylases is supported by several literature reports (Qian et al. 1995; Talamond et al. 2002; Alkazaz et al. 1996).

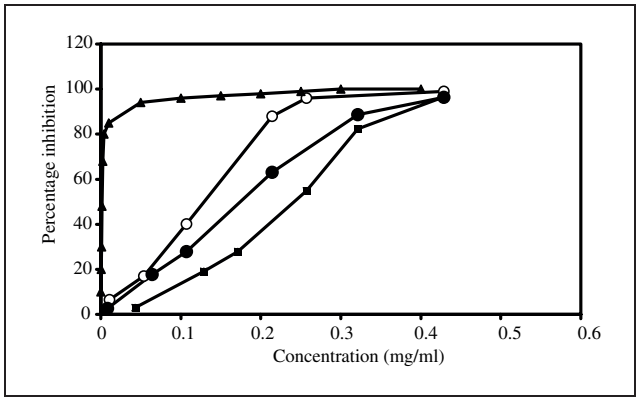

Fig. 1: Dose-response curves for tetracycline HCl (■), captopril (●) and enalapril (○) compared to that of acarbose (▲). Standard error for the mean of three measurements (at each point) was less than +/– 4.9%.

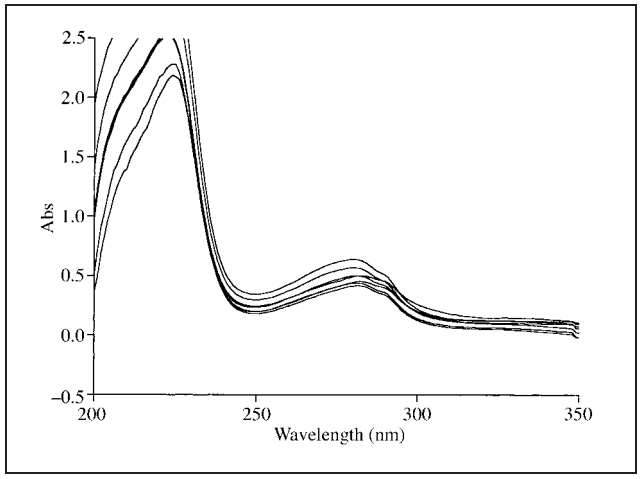

Fig. 2: Overlaid UV spectra of amylase solution titrated with (tetracycline). Similar results were obtained for the titration of alpha amylase with captopril and enalapril

Two secondary binding sites in addition to the active site have been described previously using x-ray structure analysis (Qian et al. 1995). One of these binding sites was found to be 20Å away from the active site while the second site was found to be close to the calcium binding site (Qian et al. 1995). Calcium has been known to be essential for the activity of alpha amylases since the early studies on these enzymes. Quite recently a unique calcium-sodium-calcium metal triad in the structure of alpha amylase (calcium binding site) was shown to play a major rule in activating the enzyme (Marchius et al. 1998). Removal of the metal from its site was shown, using x-ray crystallography, to induce conformational changes that involve 21 residues leading to helix winding and order to disorder transition. This finding represented a structural-level explanation of the calcium dependency of the enzyme. Therefore it is likely that small inhibitor molecules such as ACEI and tetracyclines produce their inhibitory effect through binding to calcium in its binding site and possibly leading to order–disorder transition in the enzyme structure.

At the pharmacological level some literature reports may support the observed alpha-amylase inhibitory effect of these drugs. It is interesting that acute pancreatitis is a common side effect for both tetracycline and the ACEI (Borgia et al. 2001; Lorenzo et al. 1999) in spite of the wide chemical and pharmacological differences between the two classes of compounds. Signs of acute pancreatitis disappeared once treatment with the drugs was discontinued (Borgia et al. 2001). Some studies have also reported that treatment with oxytetracycline resulted in low alpha-amylase activity in pancreatic tissue and duodenal fluids (Lorenzo et al. 1999). Moreover, in a study that examined the effect of salivary alpha amylase on various antibiotics, only tetracycline and chloramphenicol were shown to be influenced by the presence of alpha-amylase (Eke et al. 1984). These literature reports support the hy-

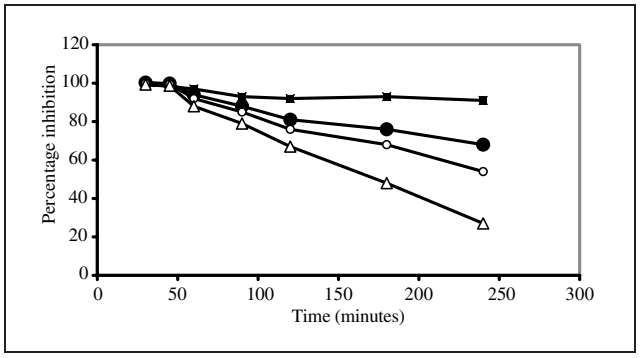

Fig. 3: Plot of the percentage inhibition of acarbose (■), enalapril (●), captopril (○) and tetracycline (△) against time of incubation. Standard error for the mean of three measurements (at each point) was less than +/− 4.5%

pothesis of similar effects of tetracylines and ACEI on alpha amylase at the pharmacological level in terms of side effects.

The effect of these drugs on the hydrolysis of starch by alphaamylase was also studied as a function of time. Fig. 3 shows a plot of the calculated percentage of inhibition against time of incubation. The plot indicates that both enalapril and captopril are slightly more effective inhibitors than tetracycline. Unlike the standard amylase inhibitor, acarbose, the activity of the three drugs appeared to decrease substantially with time over a period of 4 h. However, their effect *in vivo* would be expected to be modified by the presence of other enzymes, absorption through the GIT, GIT movement and other physiological processes. Therefore the actual *in vivo* pharmacological consequences of amylase inhibition by these drugs needs to be further characterized, particularly in the light of reported links between the clinical use of these drugs and acute pancreatitis. The exact mode of binding of these small molecules to alpha amylase and consequent inhibition of the enzyme represents another interesting point that deserves further investigation.

3. Experimental

All pharmaceutical compounds tested (Table) were kindly provided by Dar Al Dawa, Naur, Jordan. Other chemicals (iodine, potassium iodide, soluble potato starch and porcine pancreatic alpha amylase) were obtained from Sigma (USA). Iodine solution was prepared by dissolving 0.254 g I_2 and 4.0 g KI in 1 L of distilled water. Starch solution was prepared by dissolving 1 g of starch in 10 ml of distilled water, gently boiling, cooling and making up to 100 ml with distilled water. Amylase solution was prepared by transferring 6 µl of the standard porcine pancreatic amylase suspension (40 mg/ml) to 8 ml of phosphate buffer (pH 6.9).

Alpha amylase inhibitory activity was based on the starch-iodine method described by Hansawasdi et al. (2000) with some modifications. Briefly, control and test solutions were prepared as follows: 0.3 ml of amylase solution were transferred to a sample tube containing 0.3 ml of the drug solution to be tested (substituted by the solvent for controls) and 0.6 ml phosphate buffer (pH 6.9). Initial experiments were performed on solutions having concentrations of 0.5 mg/ml but several dilutions of such solutions were tested to obtain dose response curves. The mixture was incubated at 37 °C for 15 min. 0.4 ml aliquots of this incubate were transferred to sample tubes containing 3 ml starch (1 g%) and 2 ml of phosphate buffer (pH 6.5) and the mixture was re-incubated for 45 min. At time zero and at the end of the incubation period 0.1 ml of the reaction mixture was withdrawn from each tube after mixing and discharged into 10 ml of iodine solution. Solutions were thoroughly mixed and the absorbance measured immediately at 565 nm. Percentage inhibition was calculated according to the formula:

$$[(A_0 - A_t)_{control} - (A_0 - A_t)_{sample}/(A_0 - A_t)_{control}] \times 100\%$$

where A_0 and A_t are the absorbance values at zero time and at the end of the incubation respectively. Each experiment was repeated three to four times and the average value was used for the relevant plots.

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