Preparing Students for Future Therapies: The Development of Novel Agents to Control the Renin-Angiotensin System

Marc W. Harrold
School of Pharmacy, Duquesne University, Pittsburgh PA 15282

INTRODUCTION
Every year the FDA approves approximately 25 new chemical entities (NCE) which are subsequently marketed and introduced into medical and pharmaceutical practice. Many of these new compounds, such as ceftibuten (a new cephalosporin), glimepride (a new sulfonylurea), and moexepril (a new angiotensin converting enzyme inhibitor), are structurally and pharmacologically similar to current agents, while others, such as saquinavir (an HIV protease inhibitor), zafirlukast (a leukotriene receptor antagonist), and anastrozole (an aromatase inhibitor) represent new drug classes as well as novel approaches to the management of specific diseases. An elective course, entitled Advances in Drug Therapy, was developed to allow an increased emphasis on this latter group of compounds. Initially offered in the fall 1990 semester to fifth year students enrolled in a 0-5 BS curriculum, this course has been subsequently taught in either the spring semester to fourth year students or in the fall semester to fifth year students. With the development of a new 2-4 PharmD curriculum at Duquesne, this course will be taught in the future as an elective in the spring semester of the third professional year.

The objectives of this course are to: (i) introduce students to drugs and drug classes which are currently in development (clinical or pre-clinical) and which represent novel and promising approaches to drug therapy; (ii) discuss the biochemical and/or pathological basis for certain diseases and disorders and the potential approaches to drug intervention in these diseases and disorders; (iii) describe the rationale behind selecting a particular target for drug therapy from a variety of choices; (iv) compare the relative merits of current drug therapy with those of novel drugs or drug classes; (v) prepare students for their future practice by emphasizing drug therapies which will become available within the next five to ten years; and (vi) introduce students to current research areas in drug development and to identify unsolved problems in these areas. The course has a slight bent toward medicinal chemistry; however, the pharmacological and clinical aspects of the drugs are also presented. Particular attention is given to the underlying biochemical rationale for new therapies, and when appropriate, the advantages and disadvantages of investigational alternatives are compared to currently available therapies.

The following text is a summary of a two-hour lecture sequence presented within the Advances In Drug Therapy course. Discussions of recombinant DNA, the advantages and disadvantages of using peptides and proteins as pharmaceuticals, and the identification of drug targets immediately precede these lectures.

1Associate Professor of Medicinal Chemistry.
Fig. 1: The conversion of angiotensinogen to angiotensin II. The highlighted dipeptides are susceptible to cleavage by renin and angiotensin converting enzyme, respectively. The R in angiotensinogen represents the remaining 440 amino acids in this peptide.

Fig. 2: A general representation of peptide metabolism/formation. The depicted schematic can be either a single step reaction or part of a multistep pathway.

THE RENIN-ANGIOTENSIN SYSTEM

Identification of Drug Targets

The renin-angiotensin system is a complex, highly regulated pathway that is integral in the regulation of blood volume, electrolyte balance, and arterial blood pressure(1,2). It consists of two enzymes, renin and angiotensin converting enzyme (ACE), whose purpose is to release the octapeptide, angiotensin II, from its endogenous 452 amino acid precursor, angiotensinogen (Figure 1). Angiotensin II is a potent vasoconstrictor that increases total peripheral resistance through a variety of mechanisms: direct vasoconstriction, enhancement of both catecholamine release and neurotransmission within the peripheral nervous system, and increased sympathetic discharge. The result of all these actions is a rapid pressor response. Additionally, angiotensin II causes a slow pressor response resulting in a long term stabilization of arterial blood pressure. This long term effect is accomplished by the regulation of renal function. Angiotensin II directly increases sodium reabsorption in the proximal tubule. It also alters renal hemodynamics and causes the release of aldosterone from the adrenal cortex. Finally, angiotensin II causes hypertrophy of both vascular and cardiac cells. The overall result of all of these effects is to raise and/or sustain arterial blood pressure. Thus, the renin-angiotensin system is a suitable target for the development of antihypertensive agents.

Before continuing with the renin-angiotensin system, it is useful to consider a general approach to identifying potential drug targets. Consider the scenario depicted in Figure 2 where peptide C is converted to peptide D by an enzyme that can be either stimulated or inhibited by other regulators. If either peptide C or peptide D produces a beneficial effect in treating a disease or condition, there are several ways in which to increase these beneficial effects. These include directly providing the appropriate peptide, developing a more stable analog, developing a non-peptide mimetic, inhibiting the metabolism of the peptide (either directly or through a regulator), and/or stimulating an increased production of the peptide. If either of these peptides produces a detrimental effect which results in a disease or condition, there are again a variety of ways in which to block these effects. These include stimulating the metabolism of the offending peptide, inhibiting the biosynthesis of the peptide, and/or developing an antagonist to the peptide. This latter antagonist can be either a peptide or a non-peptide mimetic.

Returning to the renin-angiotensin system shown in Figure 1, the effects of angiotensin II are known to be detrimental to patients with hypertension. As discussed above, angiotensin II causes sodium and fluid retention, and significantly influences vascular tone and sympathetic nervous system activity. Using the general approach outlined above, several drug targets become obvious. These include renin inhibitors, ACE inhibitors, angiotensin II antagonists, and stimulators of the numerous exo- and endopeptidases which metabolize angiotensin II. Of these targets, stimulators of exo- and endopeptidases are the least promising since angiotensin II is naturally a short-lived compound. Thus, compounds which either inhibit the synthesis or block the effects of angiotensin II represent the most promising therapeutic drug targets.

A Comparison of Renin and Angiotensin-Converting Enzyme

Inhibition of either renin or ACE will result in a decreased formation of angiotensin II. The first orally active ACE inhibitor, captopril, was developed by Cushman et al.
inhibitors predate the interest in ACE inhibitors; however.

roid hormone, and glucagon stimulate renin release.

etostatin, atrial natriuretic factor, and angiotensin II inhibit renin release, while vasoactive intestinal peptide, parathy-

tary function is to cleave the leucine-valine bond located at residues 10 and 11 of angiotensinogen. It does not affect 

one of main attractions of renin inhibitors, specificity, has proven to be a significant hurdle to their clinical develop-

ment(5). Renin, being a very specific enzyme requires an octapeptide as the minimal acceptable substrate. This mini-

mum octapeptide substrate, His-Pro-Phe-His-Leu-Leu-Val-

Tyr, is similar to the eight amino acid sequence, His-Pro-
Phe9-His3-Leu10-Val11-Ile12-His13, which is found in angiotensinogen. In comparing the minimum substrate with the human sequence, it is seen that some variability is tolerated at the C-terminus of the peptide. The Val9-Ile12-

His3 sequence present in angiotensinogen can be replaced with other hydrophobic amino acids without the loss of substrate activity. While the goal is to develop an inhibitor of renin and not a substrate, known substrates of an enzyme are frequently used as starting points for the development of inhibitors.

Fig. 4: The effects of ACE on both angiotensin I and bradykinin.

Fig. 5: Statine and other dipeptide isosteres. By using appropriate R1 and R2 groups, these compounds can mimic any dipeptide.

Boger and coworkers(5) developed a renin inhibitor by using the minimum octapeptide substrate and replacing the labile Leu-Leu dipeptide with statine. Additionally, they replaced the two C-terminal residues with similar hydrophobic amino acids. The resulting compound, N-isovaleryl-His-Pro-Phe-His-Sta-Leu-Phe-NH2 (aka SCRIP), showed effective, though short-lived, inhibition of renin when given intravenously. Infusion experiments with SCRIP were the first to demonstrate that a small molecule renin inhibitor could maintain a lowered blood pressure for an extended period of time. Unfortunately, susceptibility to proteolytic cleavage limited the therapeutic utility of SCRIP and other analogous peptides. Thus, these peptides had to be modified.

In general, peptides are susceptible to proteolytic cleavage by both exo- and endopeptidases. Exopeptidases in-

clude both carboxypeptidases and aminopeptidases. The action of these enzymes can be blocked by simple modifications at either the N-terminus or the C-terminus of the
peptide. Conversion of the N-terminal amine to an amide, and conversion of the C-terminal acid to an ester, amide, or alcohol are commonly used modifications. Both of these modifications can be seen in SCRIP where the N-terminal histidine residue contains an isovaleryl amide, and the C-terminal phenylalanine residue contains a primary amide. The action of endopeptidases can be blocked in several ways. First, stable dipeptide isosteres can replace additional labile bonds. As shown in Figure 5, a number of stable dipeptide isosteres have been designed for this purpose(5,7). By use of appropriate substitutions at R₁ and R₂, these isosteres can substitute for any dipeptide. Second, the substitution of naturally occurring L-amino acids with D-amino acids at known cleavage sites will inhibit the action of endopeptidases. This strategy has successfully been used for several compounds, including gonadotropin-releasing hormone (GnRH) agonists and somatostatin agonists(8). Finally, endopeptidase activity can be minimized by reducing the size of the peptide. The octapeptide limit previously discussed pertains only to substrate activity. Simplification of SCRIP revealed that the N-terminal His-Pro-Phe sequence could be replaced with an acylated phenylalanine or tyrosine without any significant loss in inhibitor activity.

Application of many of the above concepts resulted in the clinical drug candidate enalkiren (A-64662), shown in Figure 6(9,10). The histidine residue, present in angiotensinogen and all previous inhibitors, was thought to be essential for enzyme recognition and was left unchanged. The acylated tyrosine protects the compound from aminopeptidase enzymes and also contributes to enzyme active site recognition. The remainder of the molecule is a stable dipeptide isostere. The cyclohexylmethylene and iso-butyl side chains are lipophilic and approximate the lipophilic side chains present in Leu₁₀ and Val₁₁ of angiotensinogen. Additionally, the use of a C-terminal alcohol instead of a C-terminal carboxylate protects enalkiren from carboxypeptidase enzymes.

Enalkiren has been extensively studied in preclinical and clinical experiments. It has been shown to be efficacious if given intravenously; however, it lacks significant reduction in blood pressure(10). Unfortunately, zankiren has since been withdrawn from clinical trials for undisclosed reasons². Despite this, other orally active renin inhibitors are still under development. Time will tell if this group of compounds ever reaches the market; however, their development serves to highlight both the potential and the problems of peptide-based, orally active drugs.

Peptide Mimetics and Angiotensin II Receptor Antagonists
Efforts to develop angiotensin II receptor antagonists began in the early 1970s. These initial efforts resulted in saralasin and other peptide based analogs. These compounds however lacked oral bioavailability and expressed unwanted partial agonist activity(2). More recent efforts have utilized peptide mimetics to circumvent these inherent problems with peptide based antagonists. The culmination of these efforts was the 1995 approval of losartan, a non-peptide angiotensin II receptor antagonist.

Peptide mimetics have been defined as molecules with no peptide bonds (i.e., no amide bonds between amino acids) and a molecular weight less than 700 Daltons(12). In comparison with peptide drugs, peptide mimetics have numerous pharmaceutical advantages. Foremost among these are increased bioavailability and increased duration of action. One needs only to look at the problems encountered in...
Fig. 8: A general process for the rationale design of peptide mimetics: (a) identification of crucial pharmacophoric groups, (b) determination of the spatial arrangement of these groups, and (c) use of a template to mount the key functional groups in their proper conformation. Those groups highlighted with an asterisk comprise the pharmacophore of the heptapeptide. Adapted from reference 12.

The development of orally active renin inhibitors to recognize the potential of these non-peptide alternatives.

The majority of known peptide mimetics have been discovered by random screening techniques; however, this process is costly, labor intensive, and unpredictable. A more logical and rationale approach is de novo peptide mimetic design(12). An example of this approach is illustrated in Figure 8. In this example, the overall process is divided into three basic steps (a-c). Initially, the amino acids which comprise the pharmacophore of the peptide must be identified. Thus, a knowledge of the structure-activity relationships for the peptide under consideration is essential. In Figure 8a, the side chains present on amino acid residues 1, 3, and 5 of a hypothetical heptapeptide are assumed to comprise the pharmacophore while the remainder of the peptide is assumed to provide the proper structural support for these key groups. In the second step of this de novo design process, the proper spatial arrangement of the pharmacophoric groups must be elucidated. Nuclear magnetic resonance spectroscopy, X-ray diffraction studies and molecular modeling programs which allow for energy minimization procedures and molecular dynamics simulation can be used to construct a model of the biologically active conformation. Returning to the example, the side chains representing the pharmacophore are assumed to be located on the inside of the peptide, while the remaining residues are assumed to be located on the outside of the peptide (Figure 8b). In the final step of the process, the pharmacophoric groups must be mounted on a non-peptide template in such a manner that they retain the proper spatial arrangement found in the original peptide. This is shown in Figure 8c where side chains 1, 3, and 5 of the original peptide are connected to a rigid template (represented by the polygon). A variety of aromatic ring systems (e.g., benzene, biphenyl, phenanthrene, and benzodiazepine) can be used to provide the rigid template, while appropriately placed alkyl groups can be used to enhance spacing and increase flexibility. Additionally, isosteres of the original pharmacophoric groups may be used to circumvent specific synthetic problems.

The de novo design process was successfully used to develop orally active angiotensin II receptor antagonists. Structure-activity studies revealed that the pharmacophore of angiotensin II (Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8) consists of the phenolic group of Tyr4, the His6 residue, the aromatic ring of Phe8, and the C-terminal carboxylate. The His6 residue is essential for receptor recognition, while the other three groups are important for agonist activity. The remaining groups in angiotensin II appear to have only supportive or structural roles(12). Losartan (Figure 9), an orally active, potent, and selective angiotensin II receptor antagonist, contains several of these pharmacophoric groups mounted on a biphenyl template. This biphenyl ring system provides rigidity and adequate spacing of the functional groups. The imidazole ring of losartan mimics the His6 residue of angiotensin II, while the hydroxymethyl group mimics the phenolic group of Tyr4. Additionally, the tetrazole ring functions as an isostere for the C-terminal carboxylate. Since losartan was designed as a receptor antagonist, it was not necessary to include every part of the pharmacophore (i.e., Phe8) in its structure.

Losartan is currently approved for the oral treatment of hypertension. Its efficacy for this indication is approximately equal to that of ACE inhibitors. It is available either alone (Cozaar) or in combination with hydrochlorothiazide (Hyzaar). Similar to most angiotensin receptor antagonists currently under study, losartan is selective for the angiotensin AT1 receptor. Antagonists at this receptor subtype...
have been shown to prevent and reverse all of the known effects of angiotensin II, including rapid and slow pressor responses, stimulatory effects on the peripheral sympathetic nervous system, CNS effects, release of catecholamines, secretion of aldosterone, direct and indirect renal effects, and all growth-promoting effects. In contrast, the physiological role of the AT₂ receptor has yet to be determined. Some concern has arisen that unopposed stimulation of the AT₂ receptor in conjunction with AT₁ receptor antagonism may cause long-term adverse effects. As a result, compounds which exhibit balanced antagonism at both receptor subtypes are currently being sought(2).

Losartan is rapidly converted in vivo (t₁/₂ = 1.5 - 2.5 hours) to EXP-3174 (Figure 9), a 5-carboxylic acid metabolite which is 10 to 40 times more potent than losartan and has a much longer duration of action (t₁/₂ = 6 - 9 hours). The overall blockade of the angiotensin II receptor is due to the combined effects of losartan and EXP-3174, while the once a day dosing is due to the long duration of EXP-3174. The most common side effects seen with losartan is dizziness. Unlike ACE inhibitors, and similar to renin inhibitors, losartan is specific in its actions and does not appear to affect bradykinin, prostaglandins, or other systems. As a result, cough and angioedema, two troublesome side effects frequently associated with ACE inhibitors, have not been seen with losartan (2, 4).

While losartan is an excellent example of what can be achieved through de novo mimetic design, the ultimate development of a therapeutically useful agent is not always as simple as the example in Figure 8. The limiting factor in the success of this process depends primarily on the validity of the conformational model generated in the second step (12). Additionally, the role of traditional analog design in this process cannot be understated. The development of losartan was achieved only after numerous stepwise modifications were applied to a initial lead compound (2). Despite these limitations, peptide mimetics have significant potential as therapeutic agents. To date, mimetics have been described for a variety of additional peptides including neurokinins (13), endothelin (14), and cholecystokinin-B (15, 16). As with all investigational therapies, time will ultimately reveal their overall contribution to drug therapy.

**SUMMARY**

Advances in Drug Therapy is an elective course designed to introduce students to drug therapies and drug classes beyond those that are currently used in practice. The lecture sequence presented above is part of this course and is representative of the types of subjects discussed within the course. These lectures allow students to identify and evaluate potential drug targets within the renin-angiotensin system. Even though students enrolled in the course would have discussed ACE inhibitors and losartan in previous courses, they would not have analyzed the relative advantages and disadvantages of ACE inhibitors versus renin inhibitors. Additionally, concepts discussed in the development of orally active renin inhibitors provide students with an understanding of the fundamental challenges present in the development of peptide-based enzyme inhibitors. Some of these same concepts can then be applied to analogous situations, such as the development of neutral endopeptidase inhibitors and endothelin converting enzyme inhibitors.

The description of losartan’s development has been retained in this lecture series despite its 1995 approval. This has been done for a variety of reasons. First of all, time constraints do not permit such a detailed discussion in the author’s medicinal chemistry courses. Additionally, the concepts presented in the design of losartan can later be applied to the study of other novel drug classes (e.g., non-peptide cholecystokinin antagonists). Finally, the success of losartan has prompted an increased interest in angiotensin II receptor antagonists. Additional compounds and new indications are currently being studied, and it is very likely that the clinical use of these antagonists will continue to increase.

In summary, pharmacy students as well as practicing pharmacists are continually confronted with an expanding number of therapeutic options to treat patients and their diseases. It is thus essential that pharmacy students be prepared for both current and future therapies. Elective courses such as the one described in this manuscript help prepare students for their future practice.

*Am. J. Pharm. Educ., 61, 173-178 (1997); received 1/28/97; accepted 4/2/97.*

**References**