Personal and Historical Perspectives

History of the Design of Captopril and Related Inhibitors of Angiotensin Converting Enzyme

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In describing the development of captopril and related antihypertensive drugs, we hope to resurrect a more specific meaning for the term "drug design," namely, the logical process whereby molecules are constructed for precise fit with a macromolecular receptor. Thus defined, drug design requires direct or indirect knowledge of the nature of the receptor, which in this case is the active center of a peptidase known historically as angiotensin converting enzyme (ACE). We believe that the development of captopril and related ACE inhibitors truly merits the use of the term drug design and that the therapeutic usefulness and specificity of the resulting drugs are a direct consequence of logical design. Captopril was developed first and foremost as a highly specific enzyme inhibitor; its antihypertensive activity was a consequence of this specific primary action.

Another overused word that is appropriate for our historical discussion is "collaboration," a term with shades of meaning quite different from those of "cooperation," and one that admirably describes our eventual working relationship. Our scientific backgrounds and approaches to problems were just different enough to be highly complementary. More importantly, we freely discussed and developed key ideas to such an extent that today we cannot always determine their precise origin. Our collaboration, however, did not spring into existence overnight. We first met in 1966 in New Brunswick, N.J., at the Squibb Insitute for Medical Research, the only scientific institution with which either of us has ever been associated. We both worked in the main research facility in New Brunswick, Ondetti in the Chemistry Department and Cushman in the Biochemistry Department. Cushman had recently completed a biochemistry thesis on microbial oxygenases at the University of Illinois and was shortly to be the lone biochemist in Zola Horovitz's Pharmacology Department. Ondetti was an established expert in peptide synthesis who had moved to Squibb New Brunswick in 1960, after working 4 years for Squibb in his native Argentina. Both of us, however, had participated in projects that had yielded important new discoveries and were thus prepared in our scientific studies to act on opportunities that history and serendipity might make available.

The first such opportunity came in 1968. Cushman had been studying the poorly characterized lung ACE for about a year. This project, begun after a frustrating year of looking for fluoroacetate synthesizing enzymes from Australian plants, was suggested by Dr. John Vane, a consultant who had been hired by our new Research Director, Dr. Arnold Welch. By 1968, this program had resulted in one of the first simple assay methods for measuring ACE activity, and ACE of rabbit lung had been partially purified and characterized. Before 1968, Ondetti had been engaged in very successful projects with Dr. Miklos Bodanszky on the synthesis and properties of the gastrointestinal hormones secretin and cholecystokinin (pancreozymin) and was continuing a long-term collaboration with Dr. Bernard Rubin, who at that time was concentrating on gastrointestinal pharmacology. In 1968, Dr. Y.S. ("Mick") Bakhle demonstrated[1] that dog lung ACE was inhibited by a mixture of peptides from the venom of the Brazilian viper *Bothrops jararaca*, a mixture first described in 1965 by Sergio Ferreira[2] as bradykinin-potentiating factor (BPF). Vane had provided us with a preprint of Bakhle's important paper and suggested that we might collaborate with Dr. Ferreira, another of his long-time colleagues, on the isolation of venom peptides. The events of 1968 did not lead to a collaboration with Ferreira, but they were the starting point for our own collaboration. Because we had one of the few adequate assays for measuring ACE activity, we could isolate ACE-inhibitory venom peptides, whereas Ferreira, working with Lewis Greene at the Brookhaven National Laboratory, isolated peptides by following their bradykinin-potentiating activity.

By the mid 1960s, the initial enthusiasm of scientists for studying the renin-angiotensin system had waned considerably, and it was generally considered to be of minor importance for blood pressure regulation.[3] Renin, an easily measured component of the system that is secreted into the blood, was expected, like an endocrine hormone, to be elevated in the blood if it were indeed a causative factor in hyper-

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tension. The only specific inhibitors of the system, renin antibodies, did not have any consistent effects on blood pressure in animal models. Later, the use of peptidic angiotensin receptor antagonists with short durations of action and residual agonistic activities would also lead to an underestimation of the role of the renin-angiotensin system in hypertension. Development of specific ACE inhibitors from snake venom would finally provide the pharmacological tool necessary to overcome the prevailing dogma. Further study of these agents would lead to a better understanding of the nature of the active site of ACE, an understanding that was essential for the later design of simpler, orally active inhibitors such as captopril.

In the competition for isolation of snake venom peptides, Ferreira and Greene (Ferreira et al[4]) isolated and characterized the first peptide, a bradykinin-potentiating pentapeptide that they called BPP_{5a}; it also inhibited ACE and transiently lowered blood pressure in animal models. We isolated, characterized, and synthesized six longer ACE-inhibitory peptides with sequences quite different from the pentapeptide. The term "we" was not altogether appropriate at that point in time (circa 1971–1973) since our collaborative interaction was not yet complete. We published our work separately at this time, treating the chemical[5] and biochemical[6] work as distinct projects. The pentapeptide BPP_{5a}, because of its susceptibility to enzymic degradation, was short-lived in hypertensive animal models. The most potent of the larger peptides that we had sequenced, a nonapeptide, was very stable; its name, teprotide, reflects the four proline residues that help to confer this stability. Teprotide and a large number of analogues were thoroughly characterized in vitro and in vivo. These important pharmacological studies were not carried out as part of Squibb's existing cardiovascular program. Instead, Bernie Rubin, a member of the "peptide team," made an important transition from gastrointestinal pharmacology back to cardiovascular pharmacology and developed several important test systems for evaluating ACE inhibitors. From all of this work at least two key results emerged. Teprotide was shown to be an effective antihypertensive drug, albeit one with limited use because of its expense and lack of oral activity. Structure–activity studies with analogues of teprotide and BPP_{5a} helped to refine our emerging hypothetical model of the active site of ACE, which we already considered to be a zinc metallopeptidase. The terminal sequence Trp-Ala-Pro of BPP_{5a}, or the related but more stable sequence Phe-Ala-Pro, was found to be optimal for binding to the active site of ACE. These important structure-activity studies were published jointly,[7] as were nearly all subsequent papers during the next decade, a clear indication that a true state of collaboration had been achieved. But none of this yet was drug design.

Our limited program might easily have died a quiet death after 1973 when, in spite of promising clinical results,[8,9] teprotide was discontinued as a clinical candidate because of lack of commercial interest. It seemed to us, on the basis of preclinical and clinical results already obtained with teprotide, that ACE inhibitors had great potential for use as antihypertensive drugs, but we had not a clue as to how to develop an agent with the appropriate pharmokinetic properties, including oral absorption. We had already begun, not entirely voluntarily, to shift our attention to other areas, Ondetti to antibiotics and Cushman to prostaglandins. Despite the success of the program, the lack of commercial interest had prompted some of the members of the company's management team to press for change.[10] Fortunately, we were strongly supported by a few key directors, including Zola Horowitz and Arnold Welch, who recognized the great scientific and commercial potential of our work and were not afraid to take chances. We had, since 1970, randomly tested about 2,000 reasonably diverse chemical structures for specific inhibition of ACE and had found a small collection of metal binding compounds and other nonspecific agents.[11] This exercise was not completely in vain; it showed how rare, indeed, were specific inhibitors of ACE, but it also demonstrated that these, whether designed or stumbled upon, could be readily identified using a simple guinea pig ileum test system developed by Dr. Rubin and his colleagues.[11,12] Success in this simple in vitro test was also highly predictive of activity in vivo, including antihypertensive activity. A specific inhibitor such as teprotide would inhibit contractions induced by angiotensin I and augment those induced by bradykinin, in both cases due to inhibition of ACE within the tissue, but it would not affect contractions induced by a wide variety of other agents. Thus, in 1974 we had all of the test systems necessary to develop an orally active ACE inhibitor if we could find some clever way to obtain one.

The initial conception that led directly to the development of captopril is, fortunately, well documented. On the afternoon of Wednesday, March 13, 1974, we got together to discuss a paper by Byers and Wolfenden[13] that had been published a year earlier. A brief summary of their work had appeared on a card in a series called *Drugs in Prospect*, published by Paul de Haan, Inc., a subscription service that highlighted literature compounds with possible drug action. The card described "L"-benzylsuccinic acid, by far the most potent inhibitor of carboxypeptidase A that had ever been developed. The authors, in a somewhat theoretical exercise, ascribed the potent inhibitory activity of this compound to the fact that it was a "biproduct analog" that combined, in a single molecule, binding interactions characteristic of both products of the enzyme's action. The majority of the compound's structure was analogous to an aromatic amino acid product, with only a single succinyl carboxyl group taken to be analogous to the corresponding, newly liberated carboxylic acid function of the second hydrolytic product. It was widely known how an amino acid product would bind to the active site of

carboxypeptidase A, but what about this succinyl carboxylate? Byers and Wolfenden discussed but dismissed rather too easily the suggestion that the carboxylate function might bind to the catalytically functional zinc ion present at the active site of this well-characterized peptidase; however, in our discussion, we did not at all dismiss this important possibility. A number of properties of ACE suggested to us that it too was an exopeptidase with an active site similar to that of carboxypeptidase A, presumably including the presence of a zinc ion, although this had not yet been directly demonstrated. The major difference between the two exopeptidases, we thought, was that the active site of ACE had evolved to accommodate a dipeptide residue rather than a single amino acid residue as the leaving group for the peptidolytic reaction that it catalyzed. With this simple hypothetical model in mind, instead of a substituted succinic acid derivative, we envisaged, as an inhibitor of ACE, a similar compound extended by the addition of an amino acid residue, a substituted succinyl amino acid derivative. Benzylsuccinic acid is an analogue of the amino acid phenylalanine, which, as a terminal amino acid of a peptide substrate or as a product, binds very effectively to the active site of carboxypeptidase A. Since we needed an analogue of a dipeptide that would bind effectively to ACE, Ala-Pro was the obvious choice from our studies with the *B. jararaca* peptides. The compound suggested from such deliberations was D-2-methylsuccinyl-L-proline, although we decided to first make the much simpler molecule succinyl-L-proline, an analogue of the dipeptide Gly-Pro.

From this moment of conception on March 13, 1974, only a year and a half passed before the first synthesis of captopril.[14] Our thought processes, however, had been conditioned by results obtained during the preceding 6 years. Succinyl-L-proline had disappointing potency as an ACE inhibitor, since it had about 30,000 times less activity than our eventual goal, captopril. The key result with this prototype compound, however, came in Dr. Rubin's guinea pig ileum test. Unlike the 2,000 or so random compounds that we had previously tested, succinyl-L-proline had the properties of a specific ACE inhibitor: it inhibited contractile actions of angiotensin I and potentiated those of bradykinin, without having any effects on contractile actions of angiotensin II or those of several other smooth muscle agonists. This stimulated the synthesis of the D-2-methyl derivative of succinyl-L-proline, which proved to be about 15 times more inhibitory than succinyl-L-proline, potent enough to demonstrate its oral activity for inhibition of the hypertensive actions of angiotensin I or augmentation of the hypotensive actions of bradykinin. This first demonstration of an orally active ACE inhibitor occurred on March 31, 1975, only a year after our initial conception. In our attempts to improve the inhibitory activity of such prototype compounds, we avoided theoretical concepts such as "transition state analog" or Byers and Wolfenden's "biproduct analog" and searched from the beginning for a working model that would explain inhibition on the basis of specific chemical interactions of compounds with the active site of ACE. We proposed and tested five probable active site interactions of the prototype D-2-methylsuccinyl-L-proline. By far the most important of these was the presumed interaction of the succinyl carboxyl group with the zinc ion at the active site of ACE. Much of our work during the year and a half leading to captopril was taken up by attempts to replace this succinyl carboxylate by other functional groups that might interact more effectively with an enzyme-bound zinc ion. Hydroxamate and phosphonate groups were more effective than the succinyl carboxylate, and a wide variety of other substitutions led, as expected, to loss of inhibitory activity. However, when the carboxylate was replaced by a simple sulfhydryl function, a 2,000-fold increase in inhibitory potency was achieved.

On the path from succinyl-L-proline to captopril, we synthesized and tested only about 60 compounds in a logical sequence designed to confirm the active site interactions that we had proposed and to develop a compound with optimally effective binding.[15] With the benefit of hindsight, it is obvious that captopril was only two steps removed from succinyl-L-proline, but these steps might not have been taken without the simple but logical structure-activity studies that we carried out at this time. Captopril, the final product of these logical studies, is one of the simplest chemical structures as well as one of the most optimized drugs to be studied in the clinic for any indication. Our project in 1974 had not yet become a crash program, and at the chemical level, personnel included only ourselves and two assistants, Emily Sabo and Hong Son Cheung. We were still concentrating on the optimization of the structure of captopril rather than trying to make additional inhibitors of more complex structure. Since 1975, several hundred compounds have been synthesized that are dipeptide analogue ACE inhibitors related to captopril, and the only useful structural change has been the addition of certain hydrophobic residues onto the proline ring.[16] Our original hypothetical model, however, predicted active site interactions beyond those exploited in the design of dipeptide analogues such as captopril. The active site of ACE was known to interact specifically with at least the last three carboxyl terminal amino acids of peptide substrates or inhibitors, with the optimal sequence being Phe-Ala-Pro. We made early but unsuccessful attempts to produce tripeptide analogue inhibitors of ACE by substituting onto sulfhydryl or carboxylic acid prototypes a phenylalkyl side chain that might mimic the corresponding residue of an antepenultimate aromatic amino acid in a substrate. Our observation that substituted glutaryl proline derivatives were equal to or better than the corresponding succinyl prolines was one of the starting points for the development by the Merck group[17] of the next major class of specific ACE inhibitors, a series of tripeptide analogue inhib-

itors, including enalapril and lisinopril, that were reported in 1980. Later, tripeptide analogue inhibitors with hydroxyphosphinyl zinc ligands were developed at Squibb; and these, like all of the other clinically important ACE inhibitors, are structurally related to the tripeptide sequence Phe-Ala-Pro.[16,17] Thus, our original model for the active site of ACE, a purely theoretical construct, has led to the development of a series of highly optimized enzyme inhibitors that have markedly changed our understanding of the pathophysiological importance of the renin-angiotensin system and provided excellent therapy for a growing list of cardiovascular disorders, particularly hypertension and heart failure.

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