Safer proteinase treatment of sciatica

A biochemical preview of chymopapain inhibitors

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As pointed out in the preceding review (Nachemson and Rydevik 1988), the most serious and common complications of chymopapain treatment of patients with sciatica due to disc herniation are cerebral or subarachnoid hemorrhages and allergic manifestations. Recent progress in biochemical and physiologic studies of cysteine proteinases and their inhibitors allows some speculation on how these complications in the future might be alleviated or avoided and on how the efficiency of proteinase treatment of patients with sciatica might be increased.

The biochemically active component of the two preparations Discase® and Chymodiactin®, which are currently used for the biochemical treatment of sciatica, is chymopapain (Buttle et al. 1986). This is a cysteine proteinase isolated from the latex of the papaya (*Carica papaya*) plant. The designation "cysteine proteinase" is based on the fact that the catalytically active center of chymopapain contains the amino acid residue cysteine as an essential component. Chymopapain is closely related to other cysteine proteinases, e.g., papain, which like chymopapain is present in large amounts in the papaya latex. Several human cysteine proteinases that closely resemble papain and chymopapain in structure and catalytic activity are present in high concentrations in human lysosomes. The best characterized of these human proteinases are cathepsins B, H, and L (Barrett 1987). The mammalian enzymes are known to be active in the intracellular catabolism of proteins and peptides and in extracellular degradation of connective tissue components (Delaissé 1984).

The proteolytic activities of human cysteine proteinases represent a potential threat to intact tissues, and several protein inhibitors of such proteinases that regulate their activities have recently been discovered. These inhibitors form a new superfamily of proteins comprising about 15–20 different inhibitors named the *cystatin superfamily* because it comprises cysteine proteinase inhibitors (Barrett et al. 1986). The structure and physiologic importance of several human cystatins have recently been elucidated, and it has been noted that the distributions of these inhibitors between human biological fluids vary considerably. For example, the cystatins in human cerebrospinal fluid, dominated by cystatin C *alias* γ-trace, have a total cysteine proteinase-inhibiting capacity of only 0.6 μmol/L, whereas the cystatins in human blood plasma, dominated by kininogen, have a total inhibiting capacity of about 12 μmol/L (Abrahamson et al. 1986). As judged from enzyme kinetic and concentration measurements, the two physiologically most important cysteine proteinase inhibitors of human extracellular fluids generally seem to be cystatin C and kininogen.

In hereditary cerebral hemorrhage with amyloidosis, the affected patients suffer at an early age from repeated cerebral hemorrhages (Jensson et al. 1987), and it has been noted that their cerebrospinal fluid concentration of cystatin C is very low (Grubb et al. 1984). Because it is known that injections of chymopapain into the cerebrospinal fluid result in cerebral hemorrhages (Garvin et al. 1965), it has been suggested that the endogenous release of an abnormally high amount of a cysteine proteinase is the pathophysiologic basis for hereditary cerebral hemorrhages with amyloidosis (Grubb et al. 1986). It is, of course, also possible that these patients produce an abnormal endogenous cysteine proteinase that is not inhibited in a normal way by their cysteine proteinase inhibitors.

Several of the human cystatins, like cystatin C and kininogen, have been observed to inhibit the plant proteinases chymopapain and papain just as efficiently as they inhibit the corresponding human proteinases, e.g., cathepsins B, H, and L (Abrahamson et al. 1986). These observations, in

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conjunction with the greatly varying total concentration of cystatins in different human fluids, might explain the great variation in the susceptibility of various human tissues to injections of chymopapain and other cysteine proteinases, which has been observed in animal experiments and in the clinical use of chymopapain (Garvin et al. 1965, Ford 1969). For example, whereas the low cysteine proteinase-inhibiting capacity of cerebrospinal fluid will be saturated already upon the injection of a low amount of chymopapain resulting in severe tissue damage with hemorrhages, the 20 times higher inhibiting capacity per volume of, e.g., blood plasma will allow injections of much higher amounts of chymopapain without detrimental effects. Not only can these recent biochemical observations explain the varying susceptibility of human tissues to chymopapain, but they also indicate a possible way of avoiding or alleviating serious side-effects of chymopapain treatment, such as cerebral hemorrhages. For if a human cysteine proteinase inhibitor like cystatin C is injected into the cerebrospinal fluid in a molar amount exceeding the amount of chymopapain used for disc injection, no damage of CNS tissue would result even if the entire dose of chymopapain was accidentally injected into the cerebrospinal fluid. Whether it is most suitable to inject a completely protective amount of, e.g., cystatin C into the cerebrospinal fluid before each chymopapain treatment or whether cystatin C should be injected only after the appearance of clinical and other signs of erroneous intrathecal administration of chymopapain must, of course, be decided by animal experiments and clinical experience. Although no commercial source of human

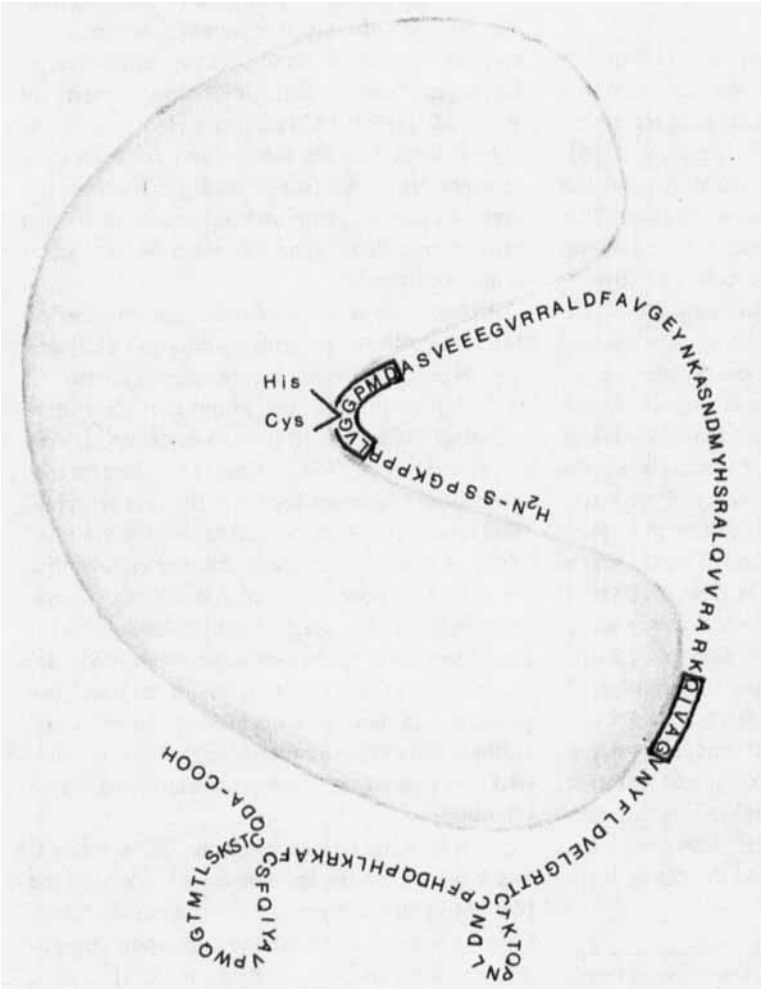

Figure 1. A model of the molecular mechanism by which cystatin C (and other cystatins) inhibits cysteine proteinases of the papain class. A "supersubstrate" region of cystatin C, comprising the amino acid residues 9–15, binds rapidly and strongly to the catalytically active site of the cysteine proteinase, thereby blocking it from reacting with other possible substrates. The binding of the "supersubstrate" region of cystatin C positions another region of cystatin C, called the "locking" region and comprising the amino acid residues 55–59, into a sensitive area of the cysteine proteinase, thereby interfering with its normal catalytic process. The one-letter code is used for the amino acid residues of cystatin C, and the red boxes mark the "supersubstrate" and "locking" regions. The cysteine and histidine residues of the active site of the proteinase interact closely with the indicated glycyl bond of the "supersubstrate" region of cystatin C. This glycyl bond is evolutionarily conserved in all known sequences of inhibitory cystatins.

cystatin C presently is available, experiments are in progress to produce substantial amounts of cystatin C by hybrid DNA techniques (Gassen personal communication 1987, Abrahamson personal communication 1987). Because the molecular mechanism by which cystatin C, and probably most other cystatins, inhibits cystein proteinases has been elucidated (Figure 1; Abrahamson et al. 1987), it will probably also be possible to produce small molecules with cysteine proteinase-inhibiting capacity that can be used as drugs.

Although the most serious complications of chymopapain treatment of patients with sciatica may be cerebral hemorrhages and other damage to the CNS resulting from uninhibited intrathecal cysteine proteinase activity, the most common side-effects are the allergic ones, as pointed out by Nachemson and Rydevik (1988). The reason for the allergic manifestations of chymopapain injections is, of course, that chymopapain is a nonhuman substance that will provoke an antibody response in a significant percentage of those individuals who have been exposed to it in food or at earlier injections. However, if the nonhuman cysteine proteinase chymopapain was replaced by a human cysteine proteinase, like cathepsin B, H, or L, or a mixture of such human proteinases, the allergic manifestations at cysteine proteinase treatment of patients with sciatica would probably diminish considerably. The availability of human cysteine proteinases would probably even allow repeated intradiscal injections over long periods of time without unacceptable risks of serious allergic manifestations. It is also conceivable that some of the human cysteine proteinases may be more efficient than chymopapain in reducing the intradiscal pressure – hence, in cysteine proteinase treatment of patients with sciatica.

In conclusion, progress in biochemical and physiologic studies of human cysteine proteinases and their inhibitors might lead to an increased success rate and a decrease in complications of cysteine proteinase treatment of patients suffering from sciatica due to disc herniation.

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