Potentiative effects of sulfhydryl compounds on carrageenin-induced oedema in rats and relationship to their potencies as inhibitors of angiostin-converting enzyme in vivo

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Summary. Carrageenin-induced oedema in rats was potentiated by oral administration of (4R)-3-[(2S)-3-mercapto-2-methylpropanoyl]-4-thiazolidinecarboxylic acid (SA291) and related sulfhydryl compounds, and the effect was closely correlated with their potencies as inhibitors of angiotensin-converting enzyme in vivo.

Recently, it was suggested that 1-(D-3-mercapto-2-methylpropanoyl)-L-proline (SQ14225), a potent orally active inhibitor of angiotensin-converting enzyme (ACE), might be useful for renal hypertension[2,3]. We have also found that (4R)-3-[(2S)-3-mercapto-2-methylpropanoyl]-4-thiazolidine carboxylic acid (SA291) has almost the same activity as SQ14225[4].

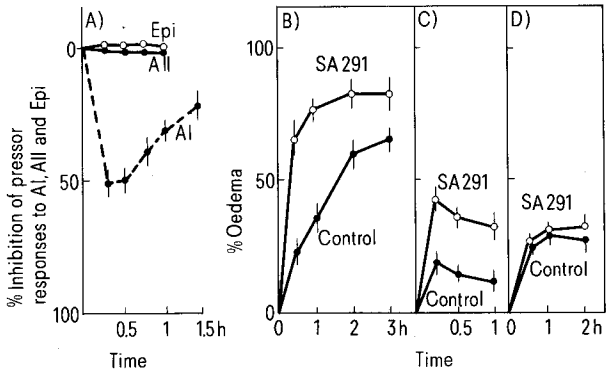

Fig. 1. Effects of oral administration of SA291 (1 mg/kg) on pressor responses to angiotensin I (AI), angiotensin II (AII) and 1-epinephrine bitartrate (Epi) in unanesthetized rats (A) and on rat paw oedema induced by carrageenin (B), bradykinin (C) and dextran (D). The experimental conditions are described in the text. Results are the mean±SE of 5–10 experiments.

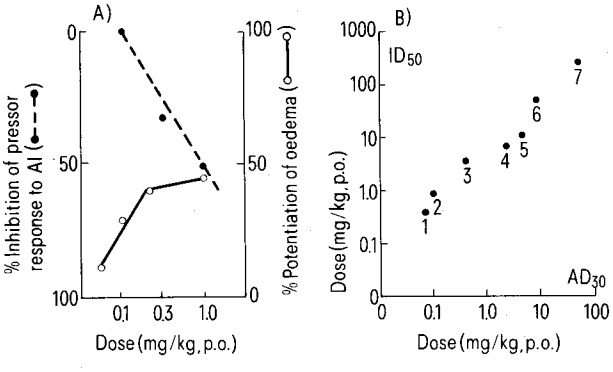

Fig. 2. *A* Dose-response curves for SA291 on the potentiation of the carrageenin oedema (○——○) and the inhibition of the pressor response to AI (●——●), and *B* relationship between the 2 effects caused by sulfhydryl compounds. Percent potentiation of oedema was determined by the difference between oedema (%) treated with compounds and control oedema (%) at 30 min after injection of carrageenin. AD_{30}, dose of compound producing 30% potentiation of the oedema. ID_{50}, dose of compound producing 50% inhibition of the response to AI. Compounds: 1, (2S)-1-[(2S)-3-mercapto-2-methylpropanoyl]-proline; 2, SA291; 3, (4R)-3-[(2S)-2-mercaptopropanoyl]-4-thiazolidinecarboxylic acid; 4, 1-(3-mercaptopropanoyl)-(S)-proline; 5, (2S)-N^2-[(2S)-2-mercaptopropanoyl]-tryptophan; 6, (2S)-N-[(2RS)-2-mercaptopropanoyl]-tyrosine; 7, tiopronin.

However, these sulfhydryl compounds have been shown to produce potentiative action on the effects of bradykinin in vivo and in vitro. Since ACE is thought to be identical with kininase II[5,6], the inhibition of angiotensin II formation and the bradykinin potentiation caused by these compounds may not be separable phenomena. On the other hand, it has been reported that locally given BPP_{9a}, a bradykinin potentiating peptide with inhibitory activity against ACE, potentiates the carrageenin-induced inflammation[7,8]. These findings prompted us to examine the effects of oral administration of SA291 and related sulfhydryl compounds, synthesized in our laboratory, on carrageenin-induced oedema in rats and the relationship to their potencies as ACE inhibitors in vivo.

Materials and methods. Male Wistar rats were used in all experiments. Inhibitory activities of test compounds against ACE were determined by bioassay[2]. Polyethylene cannulae were implanted into a carotid artery and a jugular vein of the rats (250–300 g) under ether anesthesia. After complete recovery from the anesthesia, angiotensin I (0.3 μg/kg), angiotensin II (0.03 μg/kg) and 1-epinephrine bitartrate (5 μg/kg) were infused into the jugular vein, and pressor responses before and after oral administration of test compounds were measured with a pressure transducer apparatus. Paw oedema was induced by injecting carrageenin (0.5 mg), bradykinin (1 μg) or dextran (0.1 mg) dissolved in 0.1 ml saline into the subplantar region of hind paw of the rats (160–180 g). The paw volume was measured by water displacement method. Increase in the volume caused by phlogogens was estimated by subtracting the volume of the contralateral paw which received an equal volume of saline, and was expressed as percent oedema relative to the normal paw volume. Test compounds were administered orally 30 min before phlogogens injection.

Results and discussion. Inhibitory activities of SA291 and related compounds against ACE in vivo were determined as shwon in figures 1, A and 2, A, and it was confirmed that SA291 was a potent orally active inhibitor of ACE. This sulfhydril compound, in oral dose of 1 mg/kg or less, greatly potentiated not only bradykinin oedema but also carrageenin-oedema in rats, while dextran oedema, which is mainly mediated by histamine and serotonin[9], was unaffected (figures 1, B–D and 2, A).

Other sulfhydryl compounds with inhibitory activity against ACE also potentiated the carrageenin-oedema, and the effects were closely correlated with their potencies as ACE inhibitors in vivo (figure 2, B). However, cysteine and glutathione with extraordinarily low inhibitory activity against ACE in vitro[4] did not produce any effect in these experiments (data not shown). These findings suggest that the carrageenin inflammation model may be employed as a convenient method for evaluation of orally active inhibitors of ACE in vivo. Since it has been shown that ACE is identical with kininase II[5,6] and that bradykinin plays an important role in pathogenesis of the inflammation[7,8,10,11], the potentiative effects of these ACE inhibitors on the carrageenin-oedema may be due to their inhibitory action on kininase II in the inflamed site. However, it remains to be clarified whether they inhibit kininase I as well as kininase II. Further investigations are now in progress.

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Experimental production of local osteomalacia[1]

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Summary. The newly deposited bone which was laid down on necrotic bone in the experimentally produced osteochondral chips in the knee joint of 16 sheep and rabbits remained unmineralized and undermineralized in which respect it resembled osteomalatic bone. Local factors which interfere with the mineralisation of a new covering bone should be considered in the pathogenesis of osteomalacia, in healing of aseptic bone necrosis and fractures, and incorporation and fate of bone transplants.

The recently developed histological staining techniques for osteoid tissue and individual bone matrix components in ordinary decalcified paraffin bone sections[2-4] allowed the author in the past 2 years to screen and evaluate the bone mineralisation process in a large number of specimens from human conditions and experimental material. Since the techniques are simple to perform and make it easy to distinguish between unmineralized osteoid tissue and calcified bone (figure 1), a number of interesting observations were made in a relatively short time. One of these - the existence of 'local osteomalacia'[5] forms the background of the experiments reported in the present communication.

In principle, it was found during our studies of human and experimental bone necrosis and bone-rebuilding processes that when the bone dies, the newly formed bone which after revascularisation of the area is deposited on the necrotic bone remains unmineralized or incompletely mineralized. The new wide seams and areas of unmineralized or hypomineralized osteoid tissue on the surface appear identical to those in human osteomalacia and metabolic bone disease. The existence of purely local factors which might be able to influence the normal mineralization process producing an osteomalacic bone seems to be a new and important element since at the present time it is commonly believed that osteomalacia is a systemic condition in which there is a general incapability to mineralize the newly formed osteoid tissue either due to the deficiency or malabsorption of the bone mineral or due to the deficiency of general humoral factors responsible for this process.

In order to confirm that lost or diminished vitality of the underlying bone is a factor responsible for 'local osteomalacia' the following experiment was set up: in 8 rabbits and 8 sheep bone necrosis was achieved under general anaesthetic by producing an osteochondral chip from the lateral femoral condyle, which was then left to float freely as a loose body in the knee joint. The bone in this fragment died between 4-12 days (as judged by the gradual degeneration and dissappearance of the bone cells from their lacunae) but at the same time revascularisation occurred, via adhesions from the synovia and joint capsule and layers of new bone were deposited on the dead trabeculi of the original bone. The animals were sacrificed at intervals between 8 h and 8 weeks after operation. The revascularized fragments were dissected out of joints, photographed and X-rayed and divided into 2 parts: one was decalcified in formic acid or EDTA and then stained by haematoxylin-eosin, toluidin blue and by the author's tetrachrome and PTAIH (phosphotungstic acid-iron haematoxylin) methods[2-4]; from the other half undecalcified sections were cut and stained by haematoxylin-eosin and von Kossa's silver method, and viewed under the fluorescent microscope for bone autofluorescence (Zeiss' fluorescent microscope, exciter filter = I, barrier filter = 53/44).

3 undecalcified sections from each group (from the specimens with most advanced bone-rebuilding process) were ground down to 80 µm thickness for the purpose of contact microradiography (Softex apparatus, 7.5 kV, 3 mA, 90-240 min, Kodak ES film V-6028).

The results have shown that, as it has been previously observed in human local osteomalacia[5] wide seams of newly deposited lamellar bone and to a certain extent woven bone which were laid down on cores of the old dead bone were unmineralized or grossly undermineralized (figure 2). This defective bone was found in all specimens 9 days after the operation and its microscopical appearance was identical to one type of the malacic bone seen in human metabolic bone disease. Because its incidence was locally limited being confined to the old dead trabeculi and because its undermineralisation was not caused by the lack of calcium and phosphorus in the circulating blood as witnessed by the normal calcification of the newly formed bone in other parts of the same specimen, this phenomenon is called local osteomalacia.

The new defective bone was represented on the surface by 7-9-µm wide seams of the unmineralized, 'normal' osteoid tissue underneath which, down to the junction with the dead bone, it had throughout the character of 'osteoid bone'[4]. This latter is a pathological undermineralized or demineralized bone tissue, neither osteoid nor bone, recently recognized as a common feature in human osteomalacia and numerous other conditions[4]. From about the 28th day after the experiment the mineralisation of this osteoid bone slowly advanced. At 8 weeks the calcification made a further progress though it was still incomplete. However, a 10-30-µm wide linear area at the site of the