Cerebral Ischemia, Matrix Metalloproteinases, and TNF-α: MMP Inhibitors May Act Not Exclusively by Reducing MMP Activity

To the Editor:

We thank Drs Pfefferkorn and Rosenberg for their enlightening paper1 on matrix metalloproteinases (MMPs) after ischemic stroke.

MMPs are attributed an important role in the pathophysiology of cerebral ischemia. The gelatinases MMP-2 and MMP-9 are of particular interest in this respect. In numerous experimental settings, the reduction of gelatinase activity has been demonstrated to be associated with improved outcome. Beside natural MMP inhibitors (tissue inhibitors of MMPs),2 monoclonal antibodies,3 and genetic approaches,4 the broad-spectrum MMP inhibitors BB-94, BB-1101, and KB-R7785 have proven to reduce ischemic damage.5,6,7,8

In addition to their inhibitory effects on MMPs, broad-spectrum inhibitors impede the activity of other metalloendopeptidases such as tumor necrosis factor α (TNF-α) converting enzyme (TACE), which cleaves membrane-bound pro-TNF-α to active soluble TNF-α.9,10 TACE is inhibited by BB-94,11,12,13 BB-1101,14,15 and KB-R7785.16 TNF-α has been proven to display negative effects after cerebral ischemia,17 and its neutralization ameliorates ischemic lesions.17,18,19,20 TNF-α contributes to the opening of the blood–brain barrier (BBB) by a mechanism involving soluble guanylyl cyclase and protein tyrosine kinase.21 Therefore, broad-spectrum MMP inhibitors could have contributed to BBB protection via reducing TNF-α activity and not exclusively via inhibition of MMPs. Unfortunately, the literature on the impact of MMP inhibitors after stroke does not provide any insight into the possible involvement of TNF-α in this context.

We investigated the specific MMP-2/MMP-9 inhibitor I, N-((1,1′-biphenyl)-4-ylsulfonyl)-N-phenylalanine (Calbiochem),22 in focal cerebral ischemia. Experimental procedures were carried out in accordance with guidelines of the German law governing animal care and the European Communities Council Directive (86/609/EEC). Protocols were approved by the Ethics Committee for Animal Research of the Bavarian government.

Wistar rats (250 to 300 g; Charles River, Sulzfeld, Germany) were subjected to 90 minutes middle cerebral artery occlusion (n=38) or sham surgery (n=6) as described by Longa et al,23 with modifications previously described in detail.24 After reperfusion, the MMP-2/MMP-9 inhibitor I was locally infused into the internal carotid artery over 30 minutes. Nine animals received a dose of 1 mg/kg body weight or vehicle, respectively. A second consecutive group received 10 mg/kg or solvent (n=8 each). Four additional animals were used for quantification of plasma levels. They received a dose of 1 or 5 mg/kg (n=2, respectively). Blood specimens were collected before, immediately after, 30 minutes, 90 minutes, and 12 hours (n=1 per dosage) or 24 hours (n=1 per dosage) after infusion. Plasma levels were determined by isocratic (54% CH3CN/46% TFA (0.1%), 1 mL/min) high-performance liquid chromatography on a 5 μm LiChrospher 100 RP-18 column (250×4 mm) by uv detection (254 nm) using α-[(1,1′-biphenyl)-4-ylsulfonyl]amino-N-hydroxy-(αR)-benzenepropanamide (MMP-2/MMP-9 inhibitor II; Calbiochem) as internal standard. Deproteination was carried out by addition of 2 aliquots of ice-cold CH3CN to the plasma samples.

Results revealed no differences in physiological parameters. Plasma concentrations of MMP-2/MMP-9 inhibitor I are displayed in the Table. There were no significant differences in lesion volumes, midline shift, body weight, and neurological examination between inhibitor-treated animals and controls.

In conclusion, broad-spectrum MMP inhibitors have been repeatedly reported to have beneficial effects after cerebral ischemia. In our study, an MMP-2/MMP-9 inhibitor failed to influence the effects of transient focal cerebral ischemia. Neither a pharmacodynamic nor a pharmacokinetic malfunction of this inhibitor can be ruled out. Nevertheless, these data, together with the present literature, suggest that therapeutic success due to synthetic MMP inhibitors may possibly not be attributed exclusively to its effects on MMP-2 and MMP-9. This hypothesis concurs with the finding of Pfefferkorn and Rosenberg that BB-94 reduced BBB opening without influencing zymographically determined MMP-2 and MMP-9 levels.8 Since broad-spectrum MMP inhibitors affect other enzymes like TACE as well, the effects on TNF-α activity should not be underestimated in the discussion of these drugs.

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Response:

We appreciate the comments of Dr Dittmar and colleagues on the role of selective MMP-2/MMP-9 inhibitors in cerebral ischemia. Most of the inhibitors used in the neurological studies have been broad-spectrum agents such as the one that we used.1 The finding that an MMP-2/MMP-9 inhibitor failed to affect the endpoints they tested suggests that other MMPs or possibly tumor necrosis factor-alpha converting enzyme (TACE) are also involved. The emphasis of the earlier studies on MMP-2 and MMP-9 (gelatinase A and B, respectively) was based on the availability of gelatin zymography, a highly sensitive quantitative method to detect gelatinases.2 It is not surprising to find that selective blockade of MMP-2 and MMP-9 is not sufficient to block ischemic damage since over 20 MMPs have been discovered.3 Other MMPs implicated in stroke include MMP-3 (stromelysin-1) and MMP-14 (membrane-bound MMP).4–5

The current MMP inhibitors, including the one tested by Dittmar and colleagues, are poorly soluble.6 Little is known about the penetration of these agents into the brain. Because they are poorly soluble, a diluent such as DMSO, which may be neuroprotective by itself, is used. The authors do not specify the diluent used. Another potential drawback of this study is that the selected endpoints did not address the effect on the blood-brain barrier (BBB), which is the major site of action of the MMP inhibitors. It would be interesting to know whether the MMP-2/MMP-9 inhibitor had any effect on the BBB, which may be separate from its effect on lesion size.

Computer-aided drug design has increased the number of selective MMP inhibitors.7 The challenge will be to compare the ones with promising drug profiles against the broad-spectrum inhibitors and other classes of agents that interfere with the expression or action of the MMPs, such as the tetracycline derivatives doxycycline and minocycline.8 Studies such as that described by Dittmar and colleagues will aid greatly in the selection of optimal agents to control the neuroinflammation associated with MMP expression in stroke.

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