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Evidence for a Neuroprotective Effect of Pyrid-3-yl-sulphonyl-urea in Photochemically Induced Focal Ischaemia in Rats: Magnetic Resonance Imaging Evaluation

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Abstract

A neuroprotective effect can be obtained with *N*-[(4-cycloheptylaminopyrid-3-yl)sulphonyl]*N*′-cycloheptyl urea (BM27), a pyrid-3-yl-sulphonylurea structurally related to torasemide, a loop diuretic. We have investigated the neuroprotective effect of BM27 by magnetic resonance imaging and use of the photothrombotic model of cerebral infarction in the rat. This method enables non-invasive quantification of the extent of the cerebral oedema from T2-weighted spin-echo images.

This article reports the evolution of the extent of oedema with time (0·5, 1, 2, 4, 6, 24 and 48 h, 7 and 15 days and 1 month after induction of the lesion) in rats pretreated with 5 mg kg^{-1} BM27 or an appropriate control. At all times, the rats treated with BM27 had, on average, smaller lesions than control rats (30% decrease between 2 h and 6 h).

These results strongly suggest a significant ($P < 0{\cdot}01$) but modest neuroprotective effect of BM27 in ischaemic cerebral stroke. Further investigations should be performed to determine if BM27 or its analogues are of clinical interest.

Torasemide (Figure 1) is a loop diuretic synthesized by Delarge (1988). The diuretic action of this pyrid-3-yl-sulphonylurea is attributed to inhibition of the luminal $Na^+/2Cl^-/K^+$ co-transporter of the thick ascending limb of Henle's loop. Plangger (1992) later demonstrated the anti-oedema activity of torasemide at high doses in experimental brain oedema induced in nephrectomized rats. Torasemide derivatives have been synthesized to develop new potent neuroprotective and anti-oedema compounds (Masereel et al 1992, Masereel 1993). One, *N*-[(4-cycloheptylaminopyrid-3-yl) sulphonyl]*N*′-cycloheptyl urea, or BM27, (Figure 1) has promising anti-oedema and neuroprotective properties in various animal models (Masereel et al 1994; Le Bars et al 1996).

In this study we evaluated the effect of BM27 in the photothrombotic model, a standard experimental model of stroke (Watson et al 1985). In this animal model, a local photochemical reaction is induced to produce platelet-rich thrombi and acute blood–brain barrier breakdown (Dietrich et al 1987a, b). As the focal ischaemic cerebral lesion is associated with brain oedema, magnetic resonance imaging is ideally suited to investigating the phenomena of infarct growth. This method enables non-invasive, real time quantification of the extent of the cerebral oedema from T2-weighted spin-echo images (Verlooy et al 1993; Lee et al 1996; Loubinoux et al 1997; Mottet et al 1997).

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Materials and Methods

BM-27 (Figure 1) was synthesized as previously described (Masereel et al 1992) and dissolved in dimethylsulphoxide (5 mg mL^{-1}).

Treatment

Experiments were performed on adult male Wistar rats, 250–300 g. Animals were treated intraperitoneally 30 min before induction of local photothrombosis. The treated group received a single injection of BM27 (5 mg kg^{-1}, n = 6) and the

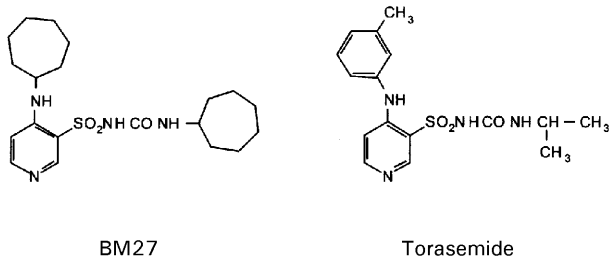

Figure 1. Chemical structure of BM27, a torasemide derivative. The hydrophobicity of torasemide (log P torasemide +0·47, log P BM27 +2·06) has been enhanced by replacement of the *N*-isopropyl and *m*-toluyl groups with cycloheptyl groups.

control group received vehicle only (DMSO, n = 5). Rats were anaesthetized by intraperitoneal administration of chloral hydrate (1·2 mg/10 mL NaCl 0·9%; 0·5 mL/250-g rat) and immobilized in a stereotaxic frame. The head was shaved and a midline incision was made to expose the skull. Muscle and connective tissue were kept intact. The cool light source applied was a quartz-halogen illuminator (Fiber-lite high-intensity illuminator, model 180, Dolan-Jenner Industries). The intensity of the light emitted from the light guide (B-624 type, Dolan-Jenner) was confirmed by means of a light-meter (Minolta, Japan). The light guide (0·19-cm diameter) was positioned close to the surface of the right side of the skull at co-ordinates (relative to the bregma) anterioposterior −4·5 mm and lateral 2 mm. The photoreagent rose bengal (Acros Organics, 4 mg mL^{-1} in sodium chloride 0·9%, filtered through a 0·22-μm filter, 2·5 mL kg^{-1}) was injected into the femoral vein. Immediately after injection of the dye, the light was switched on for 5 min. The light was then interrupted and the scalp and leg wound were sutured.

MRI experiment

Lesion development was monitored by imaging the rats between 0·5 h and 1 month post-insult. The brains were then removed from the skull for histological analysis (paraffin-embedded brain section). All measurements were performed on a 4·7 Tesla (200 MHz, ^{1}H) 40-cm inner diameter bore system (Bruker Biospec, Ettlingen, Germany). Images were obtained with a 7-cm i.d. linearly polarized ''bird-cage'' transmitter-receiver coil. Eight contiguous transverse slices, each 2 mm thick, were acquired with a multislice fast T_2-weighted spin-echo technique. The repetition time, the echo train length and the effective echo-time were, respectively, 3086 ms, 16 and 113 ms. Ten averages were acquired, leading to a total acquisition time of 4 min 23 s. The field of view was 5 cm and the matrix size 128^2. The ischaemic lesion area, in pixels, was computed from the hyperintense area in the slice with the largest lesion by use of an algorithm based on an automatic growing method and morphological operators.

Results

Temporal and spatial development of ischaemic lesion

As early as 30 min post-insult significant hyperintensity was apparent on T_2-weighted images at the place where the light guide was positioned. In control and treated rats the hyperintense lesion progressively expanded up to 24 h. The maximum size of the lesion, observed after 24 h, was much larger than the cross section of the optic fibre. After one week the extent of the oedema declined by 80%. One month later, the lesion was still visible (Figures 2 and 3).

The brains were then removed and the scars in the ipsilateral cortex were inspected histologically. All scars showed gliosis replacing neurones, highly dilated vessels, leptomeningeal fibrosis, and occasionally cystic cavities near the corpus callosum. The appearance of the scars was the same for both the BM27 and the control group. In general, hyperintense regions are apparent on T_2-weighted images under any conditions in which the water content is increased, as in infarction but also in gliosis and cystic cavities. This explains why the scar is still visible after 1 month although the oedema has disappeared.

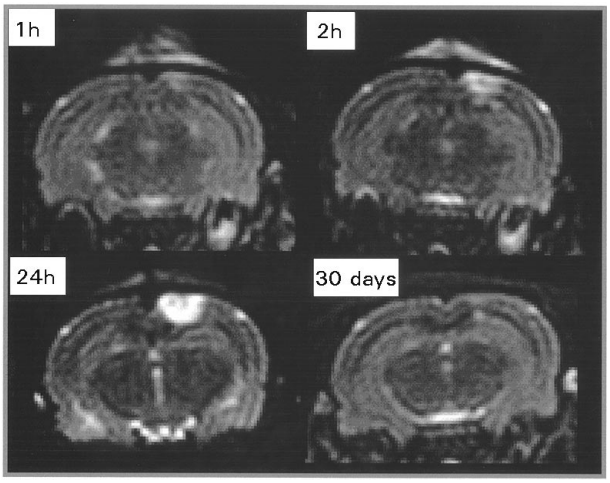

Figure 2. Coronal T_2-weighted spin-echo images showing lesion development in one rat, 1, 2 and 24 h, and 1 month post-insult, by use of the rose bengal model of focal cerebral infarction.

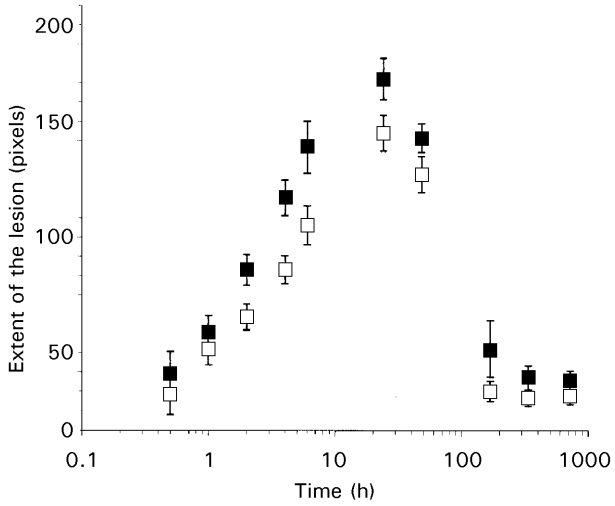

Figure 3. Effect of intraperitoneal BM27 (□; 5 mg kg^{-1}, 30 min pre-insult) in reducing infarction-associated cerebral oedema compared with the vehicle DMSO (■). Values are means ± s.e.m. At all times treatment with BM27 resulted in smaller lesions compared with control. The main effect of the treatment is highly significant ($P < 0.01$; analysis of variance).

Effect of BM27—statistical analysis

At all times lesions were smaller in rats treated with BM27 than in control rats (Figure 3); oedemas were typically 30% smaller between 2 and 6 h. The data obtained were analysed for statistical differences by repeated analysis of variance with treatment, subject and time as explanatory variables. The main effect of the treatment is highly significant ($P < 0.01$). The interaction between treatment and time was not significant. Differences at individual time points were greatest between 2 and 6 h but were not statistically significant individually (Student's *t*-test).

Discussion

Therapeutic intervention for acute stroke implies thrombolytic or neuroprotective therapy or both. Many types of neuroprotective drug are at different stages of development: competitive and non-competitive *N*-methyl-D-aspartate antagonists, glycine antagonists, presynaptic modulators of excitatory amino acid release, polypeptide growth factors, calcium and 5-hydroxytryptamine antagonists, antioxidants, and anti-adhesion molecules (Seega & Elger 1993; Muir & Lees 1995; Silver et al 1996).

This study has assessed the potential neuroprotective effect of an original lipophilic analogue of torasemide, BM27 (Masereel et al 1992), in an experimental stroke model evoked by rose bengal in rats. Cerebral oedema development was monitored by T_2-weighted magnetic resonance imaging. Temporal progression of oedema formation was consistent with previous findings (van Bruggen et al 1992; Norris et al 1994; Lee et al 1996). On T_2-weighted images the increase in water content of the lesion results in increased signal intensity. Histologically, hyperintense regions correspond to vasogenic and cytotoxic oedema (Allegrini & Sauer 1992).

We showed that BM27 at 5 mg kg^{-1}, administered intraperitoneally 30 min before photochemically induced focal cerebral ischaemia, reduced the extent of the lesion ($P < 0.01$). These results suggest BM27 has significant but modest neuroprotective effect. The mechanism by which BM27 protects cerebral tissue is not clear. It might be partly attributed to its capacity to inhibit the astrocytic $Na^+/2Cl^-/K^+$ co-transporter (Masereel et al 1994). This transporter is implicated in the massive entry of Na^+ and Cl^- from the extracellular space into the astrocyte as a result of abnormal glial volume regulation in cytotoxic brain oedema (Walz 1992). Thus inhibition of astroglial swelling could preserve microvasculature, the compression of which would otherwise contribute to the evolution of ischaemic injury (Kempski et al 1987; Fisher 1997). In the stroke model used (photochemically induced ischaemic lesion) vasogenic oedema occurs immediately as a result of acute breakdown of the blood–brain barrier (van Bruggen et al 1992; Lee et al 1996) and other mechanisms are probably involved. BM27 has no affinity for the central receptors A_1, A_2, α_1, α_2, β_1, β_2, D_1, D_2, $GABA_A$, $GABA_B$, $5\text{-}HT_{1A}$, sigma, kainic, AMPA, K-ATP dependant (sulphonylurea site), voltage-dependent Ca^{2+} channel (dihydropyridine and verapamil site), or Na^+ channel (BTX site). Interestingly, BM27 binds to the thromboxane A_2 receptor of platelets in man (unpublished results), and thus could have anti-aggregant properties. Torasemide, its parent, has been shown to antagonize the activity of thromboxane A_2 (Uchida et al 1992).

Both mechanisms, anti-aggregant properties and inhibition of the $Na^+/2Cl^-/K^+$ co-transporter could explain the neuroprotective effect we observed. Further investigations should be performed to determine whether BM27 or its analogues are of clinical interest.

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