Laboratory Study

Effect of pretreatment with a tyrosine kinase inhibitor (PP1) on brain oedema and neurological function in an automated cortical cryoinjury model in mice

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ABSTRACT

Cerebral oedema is a significant cause of morbidity in neurosurgical practice. To our knowledge, there is no ideal drug for prevention or treatment of brain oedema. Based on the current understanding of the pathogenesis of brain oedema, tyrosine kinase inhibitors could have a role in reducing brain oedema but preclinical studies are needed to assess their effectiveness. We evaluated the role of pretreatment with 4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP1), an Src tyrosine kinase inhibitor, in reducing cerebral oedema and preserving neurological function measured 24 hours after an automated cortical cryoinjury in mice. Sixteen adult male Swiss albino mice were subjected to an automated cortical cryoinjury using a dry ice–acetone mixture. The experimental group (n = 8) received an intraperitoneal injection of PP1 dissolved in dimethyl sulfoxide (DMSO) at a dose of 1.5 mg/kg body weight 45 minutes prior to the injury. The control group (n = 8) received an intraperitoneal injection of DMSO alone. A further eight mice underwent sham injury. The animals were evaluated using the neurological severity score (NSS) at 24 hours post-injury, after which the animals were sacrificed and their brains removed, weighed, dehydrated for 48 hours and weighed again. The percentage of brain water content was calculated as: [(wet weight – dry weight)/wet weight] × 100. The mean (standard deviation, SD) NSS was 11.7 (1.8) in the experimental group and 10.5 (1.3) in the control group (p = 0.15). The mean (SD) percentage water content of the brain was 78.6% (1.3%) in the experimental group and 77.2% (1.1%) in the control group (p = 0.03). The percentage water content in the experimental and control groups were both significantly higher than in the sham injury group. The immediate pre-injury administration of PP1 neither reduced cerebral oedema (water content %) nor preserved neurological function (NSS) when compared to a control group in this model of cortical cryoinjury.

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1. Introduction

Cerebral oedema, an inevitable consequence of any significant brain injury, is a common cause of morbidity and mortality in neurosurgical practice.1 Osmotic agents, diuretics and steroids, presently used to control cerebral oedema secondary to tumours, trauma, and stroke, or in the postoperative setting, have their limitations.2-5 Mannitol, glycerol and urea dehydrate both normal and injured brain tissue. Dexamethasone is not optimal in the management of vasogenic oedema from head trauma or cerebral infarction.4,5

Endothelial tight junctions of the blood–brain barrier are being investigated as the sites responsible for initiation of cerebral oedema.6-11 The extracellular signal-regulated kinase 1 and 2 (ERK 1/2) pathway, downstream of Src tyrosine kinase, has been implicated in brain injury caused by cerebral ischaemia, trauma or hemorrhage.12,13 In addition, vascular endothelial growth factor (VEGF), an angiogenic factor that is produced in response to ischaemic injury, promotes vascular permeability. VEGF-mediated vascular permeability in the brain has been demonstrated to be under Src tyrosine kinase regulation. There is a decrease in vascular permeability and cerebral oedema following a stroke, when Src activity is suppressed.14 Based on these results, it has been hypothesized that Src tyrosine kinase inhibitors could reduce cerebral oedema following trauma.

The Src tyrosine kinase inhibitor 4-amino-5-(4-methylphenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine (PP1) has reduced oedema in models of surgical injury as well as cryoinjury of the cerebral cortex.15,16 However, its role in preserving neurological function was not addressed in these studies. In this study we examined the effect of PP1 on cerebral oedema and neurological function in mice using an automated cortical cryoinjury model.
Cryoinjury-induced cerebral oedema is a well-established model for vasogenic oedema.\textsuperscript{17–19}

2. Materials and methods

The protocol was approved by the Institutional Review Board and the Animal Ethics Committee of Christian Medical College.

2.1. Cold injury model

Sixteen adult Swiss albino mice, 3 months to 6 months of age were divided into two groups. All mice were given a general anaesthetic (ketamine 100 mg/kg and xylazine 10 mg/kg injection) and immobilized on a stereotactic frame (TSE Systems, Bad Homburg, Germany) after ensuring loss of the toe-pinch withdrawal reflex. The respiratory rate and colour of the extremities were observed during the procedure. A midline incision was made over the scalp to expose the skull from the coronal suture to the lambdoid suture. Using an electric dental drill, a 5 mm craniotomy was performed to the right of the midline between the coronal and lambdoid sutures to expose the dura mater. A hollow copper cylinder with a 3 mm tip was cooled to between -50 °C and -55 °C using a mixture of dry ice and acetone. This pre-cooled hollow copper cylinder was placed over the intact dura and contact maintained for 3 minutes. This model of cryoinjury was a modification of previously reported protocols.\textsuperscript{20,21}

In order to standardise the amount of force (impact) and the duration of time the probe made contact, we designed and developed an automated device. Using a force transducer attached to the copper cylinder (Fig. 1A), the force used to place the probe on the dura and depress it by 1 mm was standardised using a weight of 10 g, and this was measured each time the injury was performed. Fig. 1B shows the sharp rise in the force applied at the exact time the cooled copper cylinder touches the intact dura: this force is constant for the entire period of contact of the cylinder with the dura. There is an abrupt fall in the force applied the moment the cylinder is lifted off the dural surface.

Fig. 1. (A) Cryoinjury apparatus with the pre-cooled hollow copper cylinder (C) connected to the force sensor (F) that transmits the signal to the amplifier (A), which is recorded on the computer screen. (B) A graphical representation of real-time administration of the automated cold injury over a period of 3 minutes.

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2.2. Experimental and control groups

The experimental group (n = 8) received an intraperitoneal injection of PP1 (Biomol-Plymouth Meeting, PA, USA) at 1.5 mg/kg dissolved in dimethyl sulfoxide (DMSO) 45 minutes prior to cryoinjury and the control group (n = 8) received intraperitoneal injection of DMSO alone. PP1 and DMSO were further diluted in phosphate buffered saline (PBS) and were freshly constituted just before the experiment. The dose of PP1 administered was determined from prior reports of neuroprotective properties of the drug in ischaemic stroke and surgically induced brain injury.14,15 In addition, eight mice underwent sham surgery: four undergoing craniotomy without injury to the cortex (Sham 1) and four undergoing craniotomy with the placement of the copper probe without the use of dry ice and acetone (Sham 2). The sham animals received neither DMSO nor PP1.

2.3. Evaluation of neurological function

The neurological severity score (NSS) consists of six components: spontaneous activity, asymmetry of limb movements, forepaw outstretching, climbing, body proprioception, and response to vibrissae touch.22 Each component was scored from 0 to 3 and scores were added to give a total score. A higher score indicated a better performance by the animal. The evaluation was performed 24 hours post-injury by an assessor who was blinded to the pretreatment received by the animals.

2.4. Assessment of brain water content

At 24 hours post-injury, after evaluation of the NSS, the animals were sacrificed and brains removed and weighed immediately (wet weight). The brains were dehydrated in a hot air oven at 105 °C for 48 hours before weighing again (dry weight). The percentage water content was assessed as (Equation 115):

\[ \text{Percentage water content} = \frac{\text{(wet weight} - \text{dry weight})}{\text{wet weight}} \times 100. \]  

2.5. Statistical analysis

Data were summarised using means and standard deviations (SD). The percentage water content and NSS were compared between the two study groups using the Mann–Whitney U-test. A p value < 0.05 was considered statistically significant. All statistical analysis was done using STATA version 10.0 (StataCorp, College Station, TX, USA).

3. Results

All animals recovered from anaesthesia following injury. However, one animal in the control group died 2 hours post anaesthesia. There were no deaths in the experimental group.

Table 1 shows the mean (SD) NSS for each group. The sham injury groups had significantly higher scores than the control group and the experimental group (both p < 0.05), suggesting significant injury with loss of neurological function in the experimental and control groups. The NSS did not show a significant difference between the experimental group and the control group (p = 0.15).

Table 1 lists the mean (SD) percentage water content for each group. The experimental and control groups had significantly higher percentage water content than the sham injury groups (all p < 0.05). The percentage water content of the brain in the experimental group was significantly higher than in the control group (p = 0.03).

4. Discussion

Contrary to published results,23–32 pretreatment with PP1 did not offer neuroprotection, nor did it prevent development of cerebral oedema in an automated cortical cryoinjury murine model.

4.1. PP1 and its mechanism of action

PP1, an inhibitor of Src tyrosine kinase, has shown promise as a neuroprotective agent when administered pre-operatively in preclinical studies.13–15,30–32 Its neuroprotective effect is via preservation of the blood–brain barrier by: (i) inhibition of the Src tyrosine ERK 1/2 cascade; (ii) inhibition of VEGF; and (iii) increase in the levels of zonula occludens-1 (ZO-1), a tight junction protein.15 This effect is mechanistically distinct from that of agents being used in current clinical practice, including osmotic agents, diuretics and steroids.

PP1 has also been shown to decrease glucose metabolism activation, cell death and behavioural abnormalities after intracerebral hemorrhage in the striatum of adult rats.31 Treatment with PP1 also reduced infarct size in a cerebral ischaemia model as well as reducing inflammation in a mild spinal cord compression model in rats.33 Early administration of PP1 seems to contribute to greater reduction in oedema than administration at 6 hours after injury.33

4.2. PP1 and cerebral oedema

In our model of automated cryoinjury of the cerebral cortex, pretreatment with PP1 failed to preserve neurological function or reduce the cerebral oedema as measured by percentage water content of the brain. This could be attributable to VEGF upregulation occurring between 3 hours and 72 hours following injury and persisting for up to 7 days.34–36 Although we did not measure the VEGF levels, Src tyrosine kinase expression may have been too high 24 hours after injury to be adequately suppressed by PP1. The temporal profile of VEGF induction in cortical and pial cells varies.35 This could contribute to the inability of a single pre-injury dose of PP1 to suppress the Src tyrosine kinase-induced elevation of VEGF levels. The effect of multiple doses of the drug at varying time intervals on improving neurological function and reducing oedema needs to be assessed. The percentage water content in the control group was significantly lower than in the experimental group, further indicating that the development of oedema is multifactorial.

Paul et al.16 showed that while cold-induced brain trauma was associated with increased cerebral VEGF formation, VEGF levels significantly declined during pneumococcal meningitis, thus demonstrating that there are different mechanisms of regulation of...
blood–brain barrier permeability in these two pathological states. Use of PP1 might be limited to a specific aetiology of vasogenic oedema.

Nag et al.37 showed that repair following brain injury is associated with differential expression of VEGF-A and VEGF-B at both the mRNA and protein level, and for expression at both basal and peak levels, possibly related to their specific roles during angiogenesis. It is still not known which of the two pathways PP1 acts on.

A better understanding of different types of oedema related to different pathological entities and their molecular mechanisms may open the way to targeted therapies. Since many molecules are involved in the pathogenesis of cerebral oedema, effective treatment may not be achieved by a single agent but is likely to require a variety of agents acting on different targets during the course of oedema development.

4.3. PP1 and neurological function

Although pre-injury administration of PP1 failed to reduce cerebral oedema, PP1-administered animals tended to show a higher NSS at 24 hours, although this was not statistically significant. PP1 may preserve neurological function by means other than reducing oedema that have not yet been elucidated. If a larger number of animals had been included in the study, the difference in NSS may have reached statistical significance.

4.4. Limitations of the study

A limitation of this study is the small sample size. Although the difference in percentage water content of the brain between the experimental and control group was small, cerebral oedema was significantly lower in the latter, thus proving that PP1 was not advantageous. However, other groups have reported beneficial effects of PP1 with a similar number of animals in a variety of experimental vasogenic edema surrounding brain tumors in humans. Ann Neurol 1986;19:592–5.


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