Pharmacological Reports 69 (2017) 176–184

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Comparison of different osmotic therapies in a mouse model of traumatic brain injury

Vinoth K.M. Khandelwal[a,c], Prabhakar Singh[b], Tatiana Ravingerova[c], Marslin Gregory[d], Hardik Gandhi[a], Manu Chaudhary[a,*]

[a] Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan, Baddi, India
[b] Veterans Affairs Medical Centre, Kansas City, MO, USA
[c] Institute for Heart Research, Slovak Academy of Sciences and Centre of Excellence of SAS, Bratislava, Slovakia
[d] Centre for the Research and Technology of Agro-Environment and Biological Sciences, University of Minho, Portugal

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form 5 October 2016
Accepted 7 October 2016
Available online 11 October 2016

Keywords:
Glycerol
Mannitol
Osmotic therapy
Trauma

ABSTRACT

Background: Inflammation in the affected region, increased intracranial pressure, consequent oedema and congestion contribute to the negative outcome of traumatic brain injury. Osmotic therapies are recommended for improvement in cognitive and motor functions. Aim of the present study was to evaluate the effect of osmotic therapies in a mice model of traumatic brain injury.
Methods: Experimental closed head injury was performed in adult Swiss albino mice by the weight-drop method. Different group of animals were treated with normal saline (G1), mannitol (G2), mannitol + glycerin (G3) and Neurotol (G4). Neurological Severity Score (NSS) was recorded at different time-points upto a period of six days. Effect of treatments on cerebral oedema, learning and memory function, motor function and co-ordination were evaluated by gravimetry, Morris water maze and beam walk test respectively. Histopathology was performed to evaluate the treatment effects on microscopic complications arising from primary closed head injury (CHI).
Results: All the treatments showed a marked improvement in the evaluated parameters as compared with the vehicle control group. It was evident that G3 and G4 had a distinct advantage over mannitol therapy. Based on the NSS score, Neurotol proved to be comparatively safe and more efficacious than either mannitol or a combination of mannitol + glycerol. The effect of Neurotol could have been enhanced by the presence of VRP011 (a Mg^{+2} salt).
Conclusions: Neurotol is safe and exhibits better efficacy as compared with other treatments for the management of traumatic brain injury.

Introduction

Traumatic brain injury (TBI) and consequences of TBI represent the leading cause of death among people aged upto 45 years [1,2]. The World Health Organization (WHO) estimated that by 2020, traumatic brain injury due to road traffic accidents would be within the top three serious causes of global burden of deaths [3]. Secondary brain damage following severe head injury is considered to be a major cause for poor outcome. Pathophysiology of TBI involves a number of mechanisms leading to neuronal injury, including excitotoxicity, oedema, free radical damage, inflammation, necrosis, apoptosis, etc. Brain's injury also triggers auto-protective mechanisms, including up-regulation of anti-inflammatory cytokines and endogenous antioxidants [4,5].

The weight-drop model of TBI uses a guided, free-falling weight to produce focal brain injury. The severity of brain injury can be controlled by adjusting the falling height and mass of the weight used to induce injury. This model reproduces a wide range of effects associated with TBI like concussion, contusion and mild-to-severe closed head injury (CHI) [5,6]. Cerebral oedema, blood–brain barrier dysfunction and apoptotic cells are also detected to a significant extent in the injured hemisphere. The severity of the injury, can be assessed by various evaluation methods like the neurological severity score (NSS), Morris water maze test and cerebral oedema obtained starting from 1 h post trauma [6].

Substances such as glycerol and mannitol have been shown to decrease oedema formation in the brain [7,8]. Addition of glycerol to mannitol avoids rebound oedema likely to be observed with the intravenous administration of mannitol alone [9,10]. As a

* Corresponding author.
E-mail address: pc@vmrcindia.com (M. Chaudhary).

http://dx.doi.org/10.1016/j.pharep.2016.10.007
1734-1140/

result, studies have been carried out by combining glycerol and mannitol for the management of cerebral oedema or raised intracranial pressure [10]. This combination strategy can enhance the diffusion of water from cerebrospinal fluid back into plasma by elevating the osmolality of the plasma [11]. It favorably affects the recovery from trauma in two ways: one is by redistribution of cerebral blood flow and consequent reperfusion in ischemic region as a result of reduction in focal cerebral oedema [12,13]. Second is by utilization of glycerol as a source of energy, directly through metabolism in the brain or indirectly through enhanced lipogenesis [13].

Neurotol is a hyperosmolar solution of mannitol, glycerol and a salt of Mg^{2+} (VRP011). Magnesium (Mg^{2+}) plays an essential role as a messenger and modulator of enzymatic activity in the nervous system. Mg^{2+} is essential for the activity of several enzymes, including ATP synthase within mitochondria [14,15]. In the setting of TBI, Mg^{2+} therapy protects against mitochondrial respiratory dysfunction and improves cytosolic phosphorylation potential [16–18]. Based on this information, the present study aimed to determine the safety and efficacy of Neurotol in comparison to a combination of mannitol + glycerol and mannitol alone in a mice model of TBI.

Materials and methods

Materials

The injections for treatment were procured locally whereas all other chemicals and solvents used for histopathology were purchased from HiMedia Labs (Mumbai, India) and were of analytical or higher grade.

Animals

Healthy adult male Swiss albino mice weighing 30–35 g were used for the experiment. Animals were acclimatized in standard animal house environmental conditions for seven days before the start of the experiment. The study was approved by the institutional animal ethics committee (IAEC) of Venus Medicine Research Center. Mice were maintained under 12 h light: dark cycle in a temperature (23 ± 4 °C) and humidity (30–70% RH) controlled room. Pelleted chow (Ashirwad Industries, Chandigarh, India) and drinking water was provided *ad libitum*. All experimental procedures were performed in accordance with the CPCSEA guidelines, Ministry of Environment, Forests and Climate Change (MoEFCC), Govt. of India, New Delhi.

Experimental brain injury (Trauma model)

Experimental TBI was performed in mice using the method described by Chen et al. [6]. Briefly, the animals were anesthetized and a logitudinal incision was performed through the scalp skin to expose the cranium. Animals were placed on a platform directly under a weight drop device. Focal brain injury was induced by allowing a rod of 333 g (with a blunt tip of 2 mm diameter) to drop from a height of 2 cm in a free fall directly on the exposed crania (leading to a final impact of 0.065 J). The foci of injury was arbitrarily decided to be on the left hemisphere, 2 mm lateral to the midline in the mid-coronal plane. After the injury, the incision was closed by silk sutures and mice were allowed to recover (Fig. 1).

Treatment

Animals were divided into following groups: G1: Vehicle control (normal saline treatment); G2: mannitol—treated; G3: mannitol + glycerol—treated and G4: Neurotol—treated. Mannitol (20% v/v), mannitol + glycerol (10% v/v each), or Neurotol (10% v/v of mannitol and glycerol each, along with Mg^{+2} salt) was administered intravenously three times a day at a dose of 1 g/kg over a period of five minutes (Table 1). The treatment began immediately after induction of TBI and continued for three days.

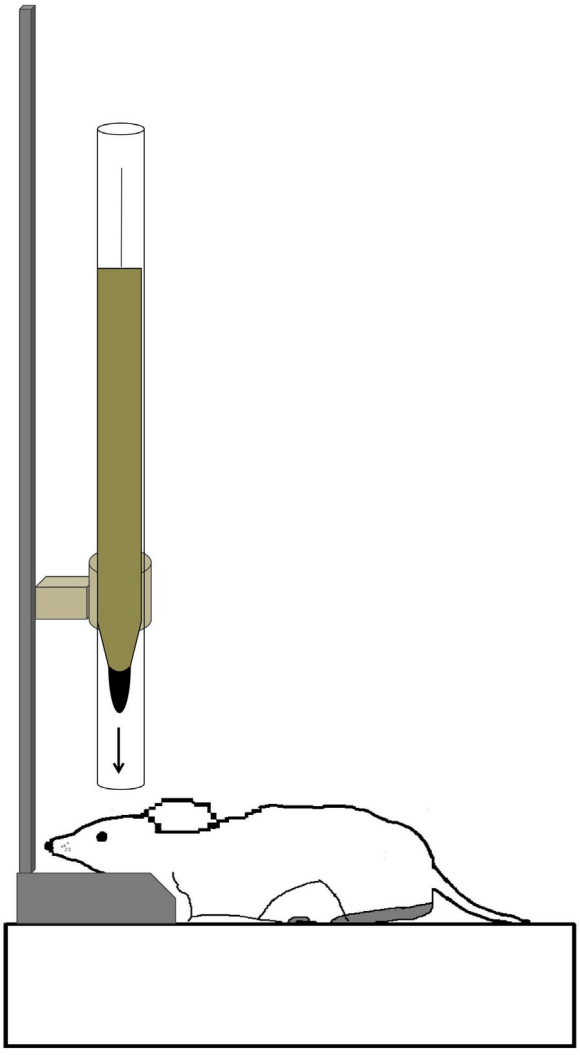

Fig. 1. Schematic representation of the weight-drop model for inducing experimental traumatic brain injury.

Table 1
Grouping and treatment allocation.

Group	No. of animals	Treatment	Dose	Frequency
G1	24	Normal saline	1 g/kg	*t.i.d*
G2	24	Mannitol		
G3	24	Mannitol + Glycerol		
G4	24	Neurotol		

Neurological severity score (NSS)

The paradigm for assessing NSS is given in Table 2. NSS was recorded at 1 h, 4 h, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h post injury (*i.e.* upto day 6). Motor functions and reflexes of the injured mice were evaluated at the scheduled time points after TBI by evaluating NSS [6]. A description on the measurement on each parameter is provided in detail in the supplementary section.