Beta-2 Receptor Antagonists for Traumatic Brain Injury: A Systematic Review of Controlled Trials in Animal Models

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A systematic review and meta-analysis of controlled trials was undertaken to assess the effects of beta-2 receptor antagonists in animal models of traumatic brain injury (TBI). Database and reference list searches were performed to identify eligible studies. Outcome data were extracted on functional status, as measured by the grip test or neurological severity score (NSS), and cerebral edema, as measured by brain water content (BWC). Data were pooled using the random-effects model. Seventeen controlled trials involving 817 animals were identified. Overall methodological quality was poor. Results from the grip test suggest that the treatment group maintained grip for a longer period than the control group; pooled weighted mean difference (WMD) = 8.28 (95% CI 5.78–10.78). The treatment group was found to have a lower NSS (i.e., better neurological function); pooled WMD = -3.28 (95% CI -4.72 to -1.85). Analysis of the cerebral edema data showed that the treatment group had a lower BWC than the control; pooled WMD = -0.42 (95% CI -0.59 to -0.26). There was evidence of statistical heterogeneity between comparisons for all outcomes. Evidence for small study effects was found for the grip test and BWC outcomes. The evidence from animal models of TBI suggests that beta-2 receptor antagonists can improve functional outcome and lessen cerebral edema. However, the poor methodological quality of the included studies and presence of small study effects may have influenced these findings.

Introduction

Traumatic brain injury (TBI), defined as a blow or jolt to the head or a penetrating head injury that disrupts the function of the brain [1], is a leading cause of global death and disability.

Primary brain injury describes the irreversible, direct injury sustained at the moment of impact. Pathophysiological studies indicate that this primary injury initiates a sequence of mechanisms that cause further secondary brain damage. The impact of secondary injury has been demonstrated by research in which it was found that approximately 40% of TBI patients who were able to speak or follow commands shortly after the event, later died from their injury [2].

Cerebral edema, resulting from the opening of the blood brain barrier (BBB), is one of the physiological assaults contributing to secondary brain injury [3,4]. Cerebral edema is thought to be, in part, caused by a sequence of events beginning with the release of the potent vasodilator, bradykinin (BK), a product of the Kinin-Kallikrein system [3,5,6]. BK is believed to contribute to the breakdown of the endothelial junctions that comprise the BBB, which enables the movement of substances into the brain leading to an accumulation of fluid [3,7]. This is thought to further increase intracranial pressure and thus exacerbate neural damage [4].

In consideration of the role of BK in cerebral edema and its contribution to secondary brain injury, the use of beta-2 receptor antagonists has been proposed [5,8].

They act to block the activity of the Kinin–Kallikrein system, thus preventing the production of BK and its impact on the BBB. In this way, it is hypothesized that the administration of beta-2 antagonists can prevent elevation of intracranial pressure.

Beta-2 receptor antagonists are not currently licensed for clinical use and research into their effects is in its early stages. There is currently no reliable evidence that beta-2 receptor antagonists are effective in reducing mortality and disability following TBI in humans. A recent Cochrane systematic review [9] identified three completed phase-II randomized controlled trials of beta-2 receptor antagonists in TBI patients, the pooled effect estimates indicate nonsignificant reductions in mortality and severe disability with beta-2 receptor antagonists. However, these findings are consistent with the play of chance and further well-conducted randomized controlled trials are required.

The results of animal experiments are used to inform decisions regarding the design and conduct of subsequent clinical trials. Systematic reviews of animal studies should be available to allow such decisions to be based on the entirety of the existing evidence synthesized in an unbiased manner. The case for the potential value of systematic reviews of animal experiments has been discussed elsewhere [10], yet they are relatively uncommon in the medical literature [11]. The objective of this study was to conduct a systematic review to assess the effects of beta-2 receptor antagonists on death and neurological damage in animal models of TBI.

Materials and methods

We undertook systematic searches for all controlled trials involving animal models of TBI investigating the effects of beta-2 receptor antagonists versus placebo. The primary outcomes of interest were death at the end of the observation period and functional status. Data on brain water content (BWC) as a proxy for cerebral edema were collected as a secondary outcome.

We searched MEDLINE, EMBASE, PubMed, and Current Controlled Trials to April 2008, the details of the search strategy are presented in table 1. We checked reference lists of relevant reports and contacted the pharmaceutical company Xytis (Nyon, Switzerland), to identify further trials. The searches were not restricted by language or publication status.

Two authors screened the records to assess for eligibility based on title, abstracts, and keywords. The full texts of all potentially relevant records were obtained and the two authors independently assessed whether each met the predefined inclusion criteria. One author extracted

data from each eligible study. Adequacy of allocation concealment and presence of blinded outcome assessment were used to assess methodological quality of the included studies.

We calculated weighted mean difference (WMD) and 95% confidence intervals (CIs) and data were pooled using the random-effects model. For studies comparing different doses and/or timings of drug administration with the same control group, we pooled data from all the intervention arms and compared these with the control group. However, for studies that only reported means and standard deviations (SD) such an approach was not possible. In these cases, each intervention arm was analyzed separately, compared to the control group; to avoid double counting, we used the control mean and SD, and divided the number of control group animals equally between the intervention arms.

We examined trial characteristics in terms of participants, interventions, and outcomes for evidence of biological heterogeneity. Statistical heterogeneity was examined by both the I^2 and chi-squared tests. Substantial heterogeneity is considered to exist when $I^2 > 50\%$ and a P -value of < 0.10 for the chi-squared statistic was used to indicate the presence of statistically significant heterogeneity [12].

The presence of small study effects was investigated with funnel plots and the Egger's test. For the Egger's test, a P -value of < 0.10 was used to indicate the presence of small study effects [13].

Results

Description of Studies

We identified 14 reports [14–27] describing 17 controlled trials, incorporating 42 comparisons that assessed the effects of beta-2 receptor antagonists on neurological outcome and/or brain edema in animal TBI models. The length of time of outcome measurement after injury ranged from 1 hour to 1 week. Twelve studies examined effects in rats and five examined effects in mice. A range of doses and timings of drug administration were investigated. All control groups received saline. Twenty-three trials measured functional status; 15 used the grip test method and eight measured the neurological severity score (NSS). The grip test involves measuring the length of time (up to 30 seconds) that an animal can remain on a taut string (for mice) or a 3-mm wide steel bar (for rats) 60 cm long, suspended 40 cm above a table between two metal bars. The grip score corresponds to the length of time that the animal can grip on to the string or bar in some manner (the higher the score, the better the functional status). For the NSS, points are assigned

Table 1 The databases searches were based on the following MEDLINE strategy, adapted as appropriate to the specifications of each database

1. exp CRANIOCEREBRAL TRAUMA/
2. exp Cerebrovascular Trauma/
3. exp BRAIN EDEMA/
4. ((brain or cerebral or intracranial) adj3 (oedema or edema or swell*)).ab,ti.
5. exp GLASGOW COMA SCALE/
6. exp GLASGOW OUTCOME SCALE/
7. exp UNCONSCIOUSNESS/
8. (Glasgow adj3 (coma or outcome) adj3 (scale* or score*)).ab,ti.
9. (Unconscious* or coma* or concuss* or 'persistent vegetative state').ab,ti.
10. "Rancho Los Amigos Scale".ab,ti.
11. ((head or crani* or cerebr* or capitis or brain* or forebrain* or skull* or hemisphere* or intra-cran* or inter-cran*) adj3 (injur* or trauma* or damag* or wound* or fracture* or contusion*)).ab,ti.
12. Diffuse axonal injur*.ab,ti.
13. ((head or crani* or cerebr* or brain* or intra-cran* or inter-cran*) adj3 (haematoma* or hematoma* or haemorrhag* or hemorrhag* or bleed* or pressure)).ab,ti.
14. or/1–13
15. exp Receptors, Bradykinin/
16. exp Bradykinin/
17. bradykinin*.ab,ti.
18. exp Receptors, Adrenergic, beta-2/
19. ((adrenergic* or antagonist*) adj3 (B?2 or Beta?2)).ab,ti.
20. (Anatibant or LF16–0687 or XY2405).ab,ti.
21. (Bradycor or CP-0127 or icatibant or cereport or CP-0597).ab,ti.
22. or/15–21
23. 14 and 22

for alterations in motor functions and behavior. The maximum score of 25 indicates severe neurological disability and score of zero indicates intact neurological condition. BWC, as a proxy for brain edema, was measured by 39 comparisons. For this measure, the animals were killed 24 hours after injury, the brains removed, and samples taken. The samples were weighed to obtain the wet weight (WW), after which the samples were dried at 105–110°C for 24 hours. The dried samples were weighed to obtain the dry weight (DW). The BWC is calculated as $[(WW-DW) \times 100]/WW$ (a lower value represents less brain edema). For studies in which BWC data for both brain hemispheres were presented, data from the injured hemisphere (the left in all such cases) were used for the analysis.

Methodological Quality of Included Studies

Seven trials reported using random allocation, none of which presented details of the method used to generate the randomization sequence. Allocation concealment was unclear for all of the included trials. Four studies reported blinded outcome assessment. None of the studies reported a sample size calculation.

Further details of the included trials are presented in table 2.

Grip Test

Fifteen comparisons, involving 311 animals, measured the length of time that each animal could maintain a grip (maximum 30 seconds). The pooled analysis (Fig. 1) indicates that animals in the treatment group maintained grip for a statistically significant longer period than animals in the control group (WMD = 8.28; 95% CI 5.78–10.78, $P < 0.00001$). There is evidence of statistical heterogeneity (chi-square = 156.44, $df = 14$ ($P < 0.00001$), $I^2 = 91.1\%$).

Neurological Severity Score

Eight comparisons, involving 155 animals, measured functional outcome using the NSS (Fig. 2). The pooled analysis indicates that the animals in the treatment group had statistically significant lower NSS (i.e., better neurological function) than the animals in the control group (WMD = -3.28 ; 95% CI -4.72 to -1.85 , $P < 0.00001$). There is evidence of statistical heterogeneity between comparisons (chi-square = 15.99, $df = 7$ ($P = 0.03$), $I^2 = 56.2\%$).

BWC

Thirty-nine comparisons, involving 690 animals, measured BWC as a proxy for brain edema (Fig. 3). The

Table 2 Characteristics of included studies

Study	Study design	Adequate allocation concealment?	Number of animals included	Animal/type of model	Intervention	Control Group	Outcome	Time of measurement of outcome (after injury)	Blinded outcome assessment?	Notes
Hellal 2003 [14]	Nonrandomized.	Unknown.	29	Mice: closed head trauma induced by a 50-g weight dropped along stainless steel string.	LF 16–0687 Ms administered at dose of 1, 3, or 10 mg/kg 30 minutes after CHT. (n = 15)	Saline (n = 14)	Grip test BWC	4 hours	NR	Grip test data presented in a format not suitable for inclusion in this analysis.
Ivashkova et al. 2006 [15]	Nonrandomized.	Unknown.	56	Rats: closed head trauma—cranial impact of 0.5 J delivered by a silicone-coated rod that protruded from the center of a free-falling plate.	LF 18–1505T, five different doses (n = 8 in all dose groups except for 3 µg/kg/min (n = 16)). One hour after CHT. (n = 48).	Saline (n = 8)	NSS BWC	24 hours	Yes	NSS data presented in a format not suitable for inclusion in this analysis.
Kaplanski et al. 2002 [16] (time-window study)	Nonrandomized.	Unknown.	Unclear—“eight groups containing 8–11 rats.”	Rats: closed head trauma—cranial impact of 0.5 J delivered by a silicone-coated rod that protruded from the center of a free-falling plate.	LF 16–0687 Ms Four groups; 2.25 mg/kg at 1, 2, 4, or 10 hours after injury. (n = unclear)	Saline (four control groups, one for each intervention arm). (n = unclear)	NSS BWC	24 hours	NR	Number of mice in each study arm for grip test is unclear, therefore not included in meta-analysis.

Table 2 Continued.

Study	Study design	Adequate allocation concealment?	Number of animals included	Animal/type of model	Intervention	Control Group	Outcome	Time of measurement of outcome (after injury)	Blinded outcome assessment?	Notes
Kaplanski et al. 2002 [16] (1-, 2-, and 3-day treatment study)	Nonrandomized.	Unknown.	48	Rats: closed head trauma—cranial impact of 0.5 J delivered by a silicone-coated rod that protruded from the center of a free-falling plate.	LF 16-0687 Ms. Three groups: 0.75 mg/kg twice on the first day; at 1 and 8 hours after CHT and then at corresponding times on the second day; at 1 and 8 hours after CHT on the first day and then at corresponding times on the second and third days. (n = 24)	Saline. Three groups: saline twice on the first day; saline at 1 and 8 hours after CHT and then at corresponding times on the second day; saline at 1 and 8 hours after CHT on the first day and then at corresponding times on the second and third days. (n = 24)	NSS	To 14 days	NR	NSS data presented in a format not suitable for inclusion in this analysis.
Kaplanski et al. 2002 [16] (dose-effect study)	Nonrandomized	Unknown.	40	Rats: closed head trauma—cranial impact of 0.5 J delivered by a silicone-coated rod that protruded from the center of a free-falling plate.	LF 16-0687Ms Four groups: 0.75 at 1 hour after CHT; 2.25 mg/kg at 1 hour after CHT; 0.75 twice at 1 and 8 hours after CHT; 2.25 mg/kg twice at 1 and 8 hours after CHT. (n = 40)	Saline (n = 8)	NSS BWC	24 hours	NR	NSS data presented in a format not suitable for inclusion in this analysis.

Table 2 Continued.

Study	Study design	Adequate allocation concealment?	Number of animals included	Animal/type of model	Intervention	Control Group	Outcome	Time of measurement of outcome (after injury)	Blinded outcome assessment?	Notes
Plotkine 2001 [18]	Nonrandomized.	Unknown	75	Mice: Injury administered by dropping a 21-g weight from a height of 25 cm.	LF16-0687 at 0.3, 1, 10, and 30 mg/kg 30 minutes after injury. (n = 60)	Saline (n = 15)	Grip test BWC	4 hours	NR	Same mice used to assess both outcomes.
Plotkine 2001 [18]	Nonrandomized	Unknown.	90	Mice: Injury inflicted by dropping 50-g weight from a height of 25 cm on to the skull.	LF16-0687Ms administered at 0.5, 1, or 2 hours after injury. (n = 45)	Saline (n = 45)	Grip test BWC	4 hours	NR	
Plotkine 2001 [18]	Nonrandomized	Unknown	104	Mice: Injury inflicted by dropping 50-g weight from a height of 25 cm on to the skull.	LF16-0687MS at doses 0.3, 1, 3, or 10 mg/kg or 2.5 or 25 mg/kg Bradycor 30 minutes after injury. (n = 90)	Saline (n = 14)	Grip test BWC	4 hours	NR	
Plotkine 2001 [17]	Nonrandomized	Unknown	44	Mice: Injury inflicted by dropping 50-g weight from a height of 25 cm on to the skull.	LF16-0687Ms at 3 mg/kg (n = 14) or 30 mg/kg (n = 15) 30 minutes after injury. (n = 29)	Saline (n = 15)	Grip test BWC	4 hours	NR	
Pruneau 1999 [19]	Randomized controlled trial	Unknown.	64	Rats: Cranial impact delivered by a silicone-coated rod that protruded from the center of a free-falling plate.	LF 16-0687MS administered 1 hour after injury. Three groups; 10, 30, or 100 µg/kg/min. (n = 48)	Saline (n = 16)	NSS BWC	1 week (measurements taken daily) 24 hours	NR	NSS data presented in a format not suitable for inclusion in this analysis.

Table 2 Continued.

Study	Study design	Adequate allocation concealment?	Number of animals included	Animal/type of model	Intervention	Control Group	Outcome	Time of measurement of outcome (after injury)	Blinded outcome assessment?	Notes
Shapira 11/2001 [20]	Randomized controlled trial	Unclear	40	Rats: Impact delivered by free falling plate from the center of which protruded a silicone-tipped rod that impacted the skull.	LF16-0687 at 0.75 mg/kg, (treated twice at 1 and 8 hour after injury), and at 2.25 mg/kg (treated twice at 1 and 8 hours after injury). (n = 32).	Saline (n = 8)	NSS BWC	24 hours	Yes	
Shapira 2002 [22]	Randomized controlled trial	Unknown	48	Rats: Impact delivered by free falling plate from the center of which protruded a silicone-tipped rod that impacted the skull.	LF16-0687Ms 0.75 mg/kg administered twice daily over 1-, 2- or 3-days	Saline administered twice daily over 1-, 2- or 3-days	NSS	Up to 14 days (24 hour data used for meta-analysis)	Yes	Intervention arms data pooled for meta-analysis.
Shapira 4/2001 [21]	Randomized controlled trial	Unknown	67	Rats: Injury delivered by a weight drop device.	LF16-0687Ms 2.25 mg/kg 1, 2, 4, or 10 hours after injury. (n = 35).	Saline administered at 1, 2, 4, or 10 hours after injury (n = 8 in each group). (n = 32)	NSS BWC	24 hours 24 hours	Yes	Same mice used to assess both outcomes.
Stover 5/2001 [26]	Randomized controlled trial	Unclear	20	Rats: Controlled cortical impact injury device.	LF16-0687Ms at 2.5, 5, or 10 mg/kg. (n = 15)	Saline (n = 5)	BWC	24 hours	NR	
Stover 5/2001 [27]	Randomized controlled trial.	Unclear	16	Rats: Controlled cortical impact injury device using a 5-mm bolt that was accelerated to a velocity of 7 m/sec, with a penetration depth of 1 mm and contact time of 0.3 sec.	LF16-0687Ms at 2.5 mg/kg (n = 8).	Saline (n = 8*)	BWC	24 hours	NR	*Data only presented for six control rats.

Table 2 Continued.

Study	Study design	Adequate allocation concealment?	Number of animals included	Animal/type of model	Intervention	Control Group	Outcome	Time of measurement of outcome (after injury)	Blinded outcome assessment?	Notes
Stover 5/2001 [25]	Randomized controlled trial	Unclear	38	Rats: Controlled cortical impact injury device using an 8-mm bolt that was accelerated to a velocity of 7 m/sec, with a penetration depth of 2 mm and contact time of 0.3 sec.	LF 16-0687Ms at 3 or 30 mg/kg. (n = 26)	Saline (n = 12)	BWC	24 hours	NR	
Stover 2000 [23, 24]	Nonrandomized.	Unknown	38	Rats: Trauma administered by a controlled cortical impact injury device using a 8-mm bolt that was pneumatically driven at a velocity of 7 m/sec, a depth of 2 mm and a control time of 0.3 sec.	LF 16-0687Ms – a low dose (3 mg/kg body weight, n = 12) and a high dose (30 mg/kg body weight = 14). Administered subcutaneously 52 minutes after trauma.	Saline (n = 12)	BWC	24 hours	NR

Figure 1 Forest plot showing results from meta-analysis of controlled studies reporting grip test data.

Figure 2 Forest plot showing results from meta-analysis of controlled studies reporting NSS data.

pooled analysis indicates that animals in the treatment groups had a lower BWC than the animals in the control groups (WMD = -0.42 ; 95% CI -0.59 to -0.26 , $P < 0.00001$). There is evidence of statistical heterogeneity

between the trials ($\chi^2 = 303.47$, $df = 38$ ($P < 0.00001$), $I^2 = 87.5\%$).

Funnel plot asymmetry was detected on visual inspection of the funnel plots for the grip test and BWC

Figure 3 Forest plot showing results from meta-analysis of controlled studies reporting data on BWC.

outcomes (Figs. 4 and 5). There was evidence of small study effects for both outcomes; grip test (Egger's test bias coefficient = -6.40 (90% CI -10.89 to -1.91) $P = 0.025$); BWC (Egger's test bias coefficient = -1.80 (90% CI -2.78 to 0.81) $P = 0.004$). A funnel plot was not generated for the NSS outcome due to the lack of comparisons.

Discussion

The results of this systematic review of controlled trials in animal models of TBI suggest that administration of beta-2 receptor antagonists can improve neurological outcomes and lessen brain edema in animal models.

The quality of reporting of trials was generally poor. A small number of trials failed to accurately report some basic information such as sample size and thus such deficiencies prohibited their inclusion in the meta-analysis. The overall methodological quality was also judged to be poor—of the 17 included trials, just seven of these reported using random allocation, none of which provided any information about allocation concealment. Additionally, just four studies reported using blinded outcome assessment.

The objective of this systematic review was to assess the overall effect of beta-2 antagonists; we did not undertake analyses to investigate the presence of any dose-response or timing of drug administration relationships.

Figure 4 Begg's funnel plot (with pseudo 90% confidence intervals) of controlled trials reporting grip test data.

Figure 5 Begg's funnel plot (with pseudo 90% confidence intervals) of controlled trials reporting BWC data.

It should be noted that our pooled analysis of all doses would lead to an under-estimate of efficacy, assuming a true dose–response relationship exists.

Publication bias is a threat to the validity of all systematic reviews. The searches for this review were extensive in an effort to identify all potentially relevant published and unpublished studies. We were also able to obtain a number of unpublished internal reports of eligible trials from Xytis Pharmaceuticals. Despite these efforts, the funnel plots and Egger's test suggest the possibility of publication bias or other small study biases affecting the grip test and BWC outcomes. Publication bias has been observed in previous systematic reviews of animal studies [28]. However, as found in this review, the existence of true heterogeneity should be considered as a potential explanation for the funnel plot asymmetry.

Our recommendations for the conduct of future trials conducted in animal models, echo those made by Macleod et al. [29]. The methodological quality of such trials needs to improve, with randomization, adequately

concealed allocation, and blinded outcome assessment being routinely employed. Furthermore, attention should be given to the quality of reporting of the trials' methods and results on publication which, based on the included studies in this review, remains poor. We welcome the forthcoming publication of a consensus statement regarding quality in animal models of stroke, which will be of relevance to all trials in animal models, including TBI.

Conclusions

This systematic review and meta-analysis shows that beta-2 receptor antagonists can reduce brain edema and improve neurological impairment in animal models of TBI. However, the methodological quality of the included studies was poor. Whilst these results do contribute to the evidence base for the effects of beta-2 receptor antagonists, the extent to which the results of animal experiments can be generalized to clinical trials in patient populations remains open to question [28].

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Conflict of Interest

The authors declare no conflict of interest.

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