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Effects of medroxyprogesterone acetate on cerebral oedema and spatial learning performance after traumatic brain injury in rats

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Abstract

Background: Given after brain injury (TBI), progesterone reduces cerebral oedema and facilitates functional recovery. Progesterone analogues have been synthesized for use in many medical conditions and exhibit different chemical and biological properties. Medroxyprogesterone acetate (MPA) is widely used in clinical practice, but oestrogen/MPA combinations may increase the risk of stroke and cardiovascular disease rather than preventing them. In some conditions, MPA can exhibit pharmacological actions that are different from those of natural progesterone.

Primary objective and hypothesis: Using laboratory rats, this study assessed the efficacy of MPA to determine whether this progestin and natural progesterone exert similar effects as a treatment after bilateral injury to the frontal cortex.

Main outcomes and results: MPA produced a dose-related reduction of cerebral oedema at 48 hours post-TBI but neither 4 nor 16 mg/kg doses of MPA enhanced behavioural recovery.

Conclusion: These findings help to clarify the divergent results from prior positive progesterone studies and the negative MPA clinical trials for hormone replacement therapy. The results can be taken to suggest that the control of cerebral oedema, while clearly desirable, is not the only contributor to progesterone-induced behavioural recovery.

Keywords: *Traumatic brain injury, psychopharmacology, trauma, intervention*

Introduction

Statement of the problem

Progesterone, in its natural form, is a potent neuroprotective agent. Progesterone reduces cerebral oedema and facilitates recovery of function when it is administered after brain injury (see [1–5] for recent reviews).

Over the last four decades, a variety of progesterone analogues have been synthesized for use in many medical conditions (birth control, testosterone reduction, vaginal bleeding, etc.). As Schumacher et al. [2] have noted, the synthetic compounds often exhibit chemical and biological properties that differ from those of natural progesterone. One of these agents, medroxyprogesterone acetate (MPA), is a synthetic progestin that has been widely used in clinical practice.

MPA is a component of one of the most common birth control pills. It has also been the predominant progestin used in clinical trials examining the effects of the hormone alone or oestrogen/progestin combination formulations on cardiovascular and neurovascular health in women. The Women's Health Initiative (WHI) international study, launched in 1991 with more than 16 000 women with intact uteri, was stopped earlier than expected because it showed that conjugated equine oestrogen combined with MPA increased the risk of breast cancer, stroke and cardiovascular disease as opposed to preventing them [6–8], suggesting that the overall risks from HT outweighed any potential benefits. These negative

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findings have added confusion to the notion that progesterone is neuroprotective after stroke or traumatic brain injury. However, not all progestagens are alike with respect to their molecular and morphological actions [9]. MPA has recently been shown to exhibit pharmacological actions and side effects [10] that are different from those of natural progesterone [11–13]. The goal in the present study was to determine whether MPA and natural progesterone would exert similar beneficial effects on cerebral oedema and functional outcome after bilateral injury to the frontal cortex in laboratory rats.

Materials and methods

Controlled cortical impact model

Rats were anaesthetized with 2.5% isoflurane prior to and during surgery. Body temperature was monitored and maintained by homeothermic heating blanket system (Harvard Apparatus, Holliston, MA). Pulse oximetry was used to maintain heart rate at ~350 beats per minute and blood oxygen saturation levels >95%. Animals were placed in a stereotaxic frame and their heads held horizontally in place by bars inserted in the ears. A midline incision was made to expose the skull and a 6-mm craniotomy was made over the medial frontal cortex, just rostral to bregma. The cortical impact injury was created by a computer-controlled, pneumatically driven 5-mm diameter steel impactor at a velocity of 2.25 m/s. The impactor penetrated to a depth of 2 mm and was in contact with the brain for 50 ms. Sham-operated rats underwent the same surgery but without the impact.

Hormone administration

MPA (16 and 4 mg/kg) and vehicle (sesame seed oil) were administered intraperitoneally at 1 hour and subcutaneously at 6 hours followed by one injection every 24 hours or until the animals were killed and their brains harvested for cerebral oedema or histological analyses. Sham-operated rats received only vehicle solution.

Experiment 1

Subjects. Fifteen male Sprague-Dawley rats weighing 275–300 g were used as subjects. Five rats were assigned to each of three treatment groups (MPA 4 mg/kg, MPA 16 mg/kg and vehicle). Two rats were lost due to surgical complications (one each from MPA 4 mg/kg and vehicle). This study was conducted in a facility approved by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) in accordance with NIH guidelines. All experimental animal procedures were approved by the Emory University Institutional Animal Care and Use Committee (IACUC), Protocol #146-2005.

Cerebral oedema measurement. Subjects were killed 24 hours post-injury and brains were then extracted and sectioned on ice-chilled glass as previously described [14]. The brain sections were placed in pre-weighed snap-top Eppendorf tubes, immediately closed, and re-weighed. The tubes were then reopened, placed in a vacuum drying oven at 40°C at 0.3 atm for 48 hours, then immediately closed and reweighed.

Percentage oedema was then calculated using the following formulae:

$$\frac{((\text{wet brain weight} - \text{vial weight}) - (\text{dry brain weight} - \text{vial weight}))}{(\text{wet brain weight} - \text{vial weight})} \times 100\% = \%\ \text{water}$$

$$\frac{(\text{injured tissue \% water} - \text{distal tissue \% water})}{\text{distal tissue \% water}} \times 100\% = \%\ \text{difference}$$

Experiment 2

Subjects. Thirty-five male Sprague-Dawley rats, ~90 days of age (300–400 g), were subjects. The animals were assigned to one of four groups: sham + vehicle, injury + vehicle, injury + 4 mg/kg MPA or injury + 16 mg/kg MPA. Treatments were administered by intraperitoneal injection at 1 hour post-injury. The remaining doses (6 hours post and then every 24 hours for 5 days) were administered subcutaneously.

Spatial learning in the Morris water maze (MWM). Testing began 7 days post-injury and 1 hour after the last injection of drug treatment or vehicle solution. The testing apparatus consisted of a circular tank with a diameter of 133 cm filled with opaque water (Artista^{TM} non-toxic white tempera paint) to a depth of 64 cm (23 cm from the top of the tank). An 11 cm × 11 cm platform was submerged to a depth of 2 cm and placed ~28 cm from the wall of the pool in the northeast quadrant. The position of the platform remained constant throughout the experiment. All rats were marked on

their heads with a black, non-toxic marker before testing to enable the computer system to track and record their swim path in the tank.

Each animal was tested for 10 days (two 5-day blocks) with two trials per day for a total of 20 trials. A trial consisted of placing the rats into the pool facing the wall at one of two starting points (north or west), the two farthest from the platform. Each rat was allowed to swim in the pool until it reached the platform or until 90 seconds had passed. If the rats did not reach the platform on their own, they were guided to it. Once they located the platform, each rat was allowed to remain on it for 20 seconds and was then removed from the pool and placed in a holding cage for a 20 second interval until the start of the second trial. Each rat was again placed in the water as in trial one, but from a starting point (south or east) directly across from the starting point of trial one.

Histological analysis. After completion of behavioural testing (day 35), the rats were given an overdose of Nembutal^{TM} (50 mg/kg) and perfused intracardially with phosphate buffered saline followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffered saline (pH = 7.4). The brains were removed and cryoprotected in buffered sucrose solutions of increasing concentration (10% for 1 day, then 20% from day 2). Coronal sections (16 μm) were cut on a cryostat. Every other section was collected and stored at −80°C. Every twelfth section was stained with thionin for lesion construction. If the necrotic cavity invaded the olfactory bulbs, the caudate-putamen or the septum, the rat was removed from further analysis.

Retrospective comparison. To compare the effectiveness of MPA and natural progesterone on cerebral oedema, progesterone data obtained in several recently completed studies was combined in the laboratory using the identical study design [15,16]. All data points from these previous studies were used to decrease the potential bias. The 48 hour post-injury cerebral oedema data for MPA and progesterone were converted to *z*-scores for comparison and analysis. *Z*-scores, sometimes called 'standard scores', are especially useful for comparing the relative standings of items from distributions with different means and/or different standard deviations, allowing for more direct comparison of means. In this analysis, positive scores indicate increased cerebral oedema and negative scores indicate less cerebral oedema compared to sham-operates. An analysis of variance (ANOVA) was performed on these *z*-scores.

To compare the neuroprotective effects of MPA to those of progesterone, the overall mean latencies to reach the platform in the MWM were converted to *z*-scores. All the converted scores were from rats that had the same anaesthetic (isoflurane), injury device and behavioural testing room. This allowed one to compare the performance of MPA-treated rats to previously tested progesterone-treated rats.

Statistical analysis. All results are expressed as mean ± SEM. The data were tested for normality and homoscedasticity before being analysed by either parametric one-way ANOVA or parametric repeated measures ANOVA. Following the use of ANOVAs, Fisher PLSD post-hoc tests were performed. The criterion for statistical significance was set at $p < 0.05$. For the comparative analyses, *z*-scores were calculated according to the following formula [17]:

$$Z = \frac{x - \mu}{\sigma}$$

Results

Experiment 1

A one-way ANOVA at 48 hours post-injury indicated differences among the groups ($F_{2,11} = 6.011$; $p < 0.05$). Post-hoc analysis indicated that MPA16 group had significantly less water content in the injured brain region than either the MPA4 group or the injured group given vehicle alone. The amount of cerebral oedema in the MPA4 group was intermediate to that of the MPA16 and vehicle groups (Figure 1).

The ANOVA performed on the *z*-scores of MPA vs 'progesterone historical oedema data' revealed significant differences among the groups ($F_{3,14} = 13.469$; $p < 0.05$). Subsequent post-hoc analyses showed that when brain-injured rats were treated with either 4 mg/kg of progesterone or MPA16 they had less cerebral oedema compared to rats receiving only vehicle. As shown in Figure 2, the *z*-scores of the progesterone-treated rats and the MPA16 group were not significantly different.

Experiment 2

A repeated measures ANOVA on latency to reach the platform in the MWM found differences among the groups ($F_{3,22} = 4.156$; $p < 0.05$). Post-hoc analysis over 10 days of testing indicated that neither dose of MPA (4 or 16 mg/kg) improved performance compared to vehicle-treated controls. All three injury groups were significantly worse than the sham-operates (Figure 3).

The ANOVA performed on the *z*-scores of MPA vs 'progesterone historical functional

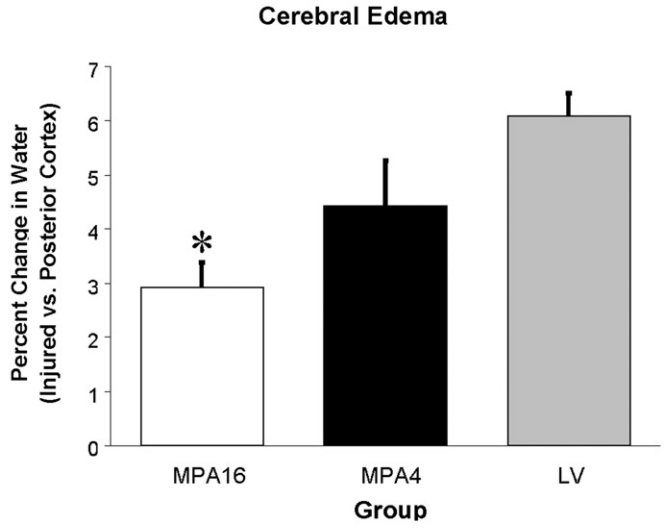

Figure 1. At 48 hours post-injury, treatment with MPA16 significantly reduced the level of cerebral oedema at the site of the cortical contusion compared to injured rats given vehicle. * = different from LV ($p < 0.05$).

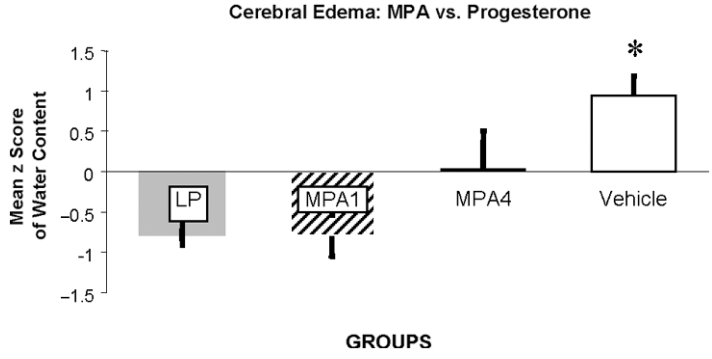

Figure 2. To compare the effects of MPA to progesterone on post-TBI cerebral oedema, the above oedema data were converted to *z*-scores and compared to previous progesterone treatment data from the same post-injury time point. Positive scores indicate greater oedema and negative scores indicate less oedema. * = different from LP and MPA16 ($p < 0.05$).

outcome data' revealed significant differences among the groups ($F_{6,45} = 4.428$; $p < 0.05$). All injured groups except the progesterone group were significantly worse than the sham-operates (Figure 4). Further analysis showed that the group treated with progesterone performed significantly better than rats given 4 mg/kg of MPA, 32 mg/kg of progesterone or vehicle. Necrotic cavity volume analysis found no differences between the three injury groups ($p > 0.05$).

Discussion

MPA (Provera^{TM}) is often used interchangeably with progesterone in human treatments and clinical trials. It is important to determine whether it has similar neuroprotective properties following a brain injury [9]. In this study, it was found that the acute administration of MPA to brain-injured male rats resulted in a dose-related reduction of cerebral oedema at 48 hours post-TBI but did not improve performance on a spatial learning task.

MPA's reduction of cerebral oedema was not surprising considering the anti-inflammatory properties of some synthetic steroid-like compounds [18]. In the retrospective comparison of MPA and progesterone, MPA was as effective as progesterone in reducing cerebral oedema at 48 hours after cortical impact injury, but it did not improve functional outcome on the MWM at either dose tested when compared with natural progesterone treatment. Indeed, MPA-treated rats performed as poorly as the injured rats given only the vehicle.

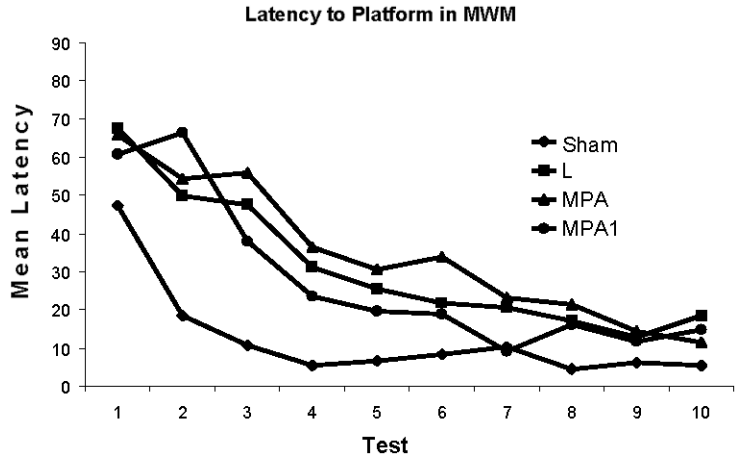

Figure 3. Mean latency (seconds) to reach platform in the Morris water maze. Analysis over 10 days of testing indicates that MPA at either dose did not improve performance over the injured animals that received vehicle, with all three injured groups performing significantly worse than the sham-operates.

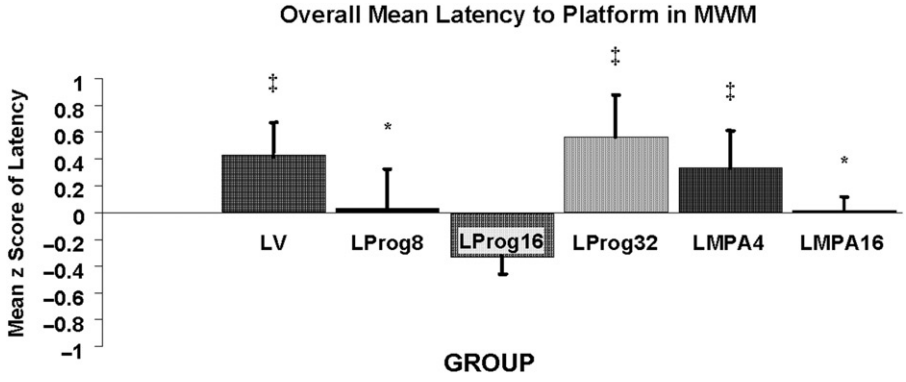

Figure 4. To compare the neuroprotective effects of MPA to progesterone, the overall mean latencies to reach the platform in the MWM were converted to *z*-scores. This allowed one to compare the performance of MPA-treated rats to progesterone-treated rats. Positive scores indicate poorer performance and negative scores indicate better performance. ‡ = significantly different from LProg16 and sham-operates. * = significantly different from sham-operates.

Other studies have shown that MPA exhibits different biological properties from those of natural progesterone. In one recent report, addition of MPA to conjugated equine oestrogens caused an increased insulin resistance in adipose tissue whereas oestrogen alone did not [19]. Bernardi et al. [20] found that natural progesterone and MPA do not have the same effects on the expression of central and peripheral allopregnanolone and beta-endorphin levels, suggesting somewhat different receptor mechanisms of action. An *in vitro* study, Nilsen et al. [21] added MPA to a cell culture medium and found increased cytotoxicity and neuronal death after exposure to glutamate. MPA is widely used in the US, while in Europe synthetic 19-nortestosterone-derived progestins are more typically prescribed, but both these agents show more affinity for androgen and glucocorticoid receptors than does natural progesterone [22, 23].

The reasons for MPA's different effects on outcome compared to natural progesterone are not completely understood. It is not always correct to assume that synthetic analogues will exhibit all the positive properties of the natural drug [9]. The mechanisms behind progesterone's neuroprotective effects are now known to be pleiotropic and to involve extra- and intra-cellular events [24, 25]. Acute progesterone administration after injury not only reduces cerebral oedema but also decreases the production of pro-inflammatory cytokines, reduces apoptosis, glutamate toxicity and lipid peroxidation, increases the expression of trophic factors, stimulates myelin synthesis, repairs the blood–brain barrier and improves behavioural outcomes that are

additive to the benefits of reducing oedema [1–5, 26–28]. These effects are probably attributable in part to progesterone's ability to interact with multiple receptors—membrane, intracellular and trans-genomic [2, 29–32]. These claims cannot yet be made for synthetic derivatives. In fact, unlike progesterone, *progestins* such as MPA are not converted to allopregnanolone. MPA may actually inhibit the metabolism of allopregnanolone and at the same time block some of the beneficial interactions with oestradiol in the brain. In addition, MPA appears to inhibit the enzyme that converts pregnenolone (the precursor of progesterone) to progesterone, so it is possible that MPA could interfere with some of the beneficial effects that would normally be induced by progesterone given after brain injury (see the excellent and comprehensive review by Schumacher et al. [2] for more extensive discussion of these issues).

From the data gathered in the present experiment one has learned that reducing cerebral oedema alone may not improve functional outcome after TBI. In this study MPA reduced oedema in a dose-dependent fashion, but it did not improve the rate or extent of behavioural recovery. Thus, while some progestins can mimic some of the effects of natural progesterone, they may not confer all the benefits of the hormone itself. Given the range of synthetic agents currently available to physicians, it becomes particularly important to be aware that the different agents may not have the same mechanisms of action or confer the same salutary effects. Especially with respect to the treatment of brain injury, serious negative consequences could result from substituting synthetic versions of progesterone for the natural product.

Conclusion

MPA reduces cerebral oedema in a dose-dependent fashion, but fails to improve neurological outcome. Treatments for neurological injuries or studies designed to test the hypothesis that progesterone is neuroprotective should avoid interchanging MPA with natural progesterone.

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