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Research Paper

Comparable impediment of cognitive function in female and male rats subsequent to daily administration of haloperidol after traumatic brain injury

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ABSTRACT

Antipsychotic drugs, such as haloperidol (HAL), are prescribed in the clinic to manage traumatic brain injury (TBI)-induced agitation. While preclinical studies have consistently shown that once-daily administration of HAL hinders functional recovery after TBI in male rats, its effects in females are unknown. Hence, the objective of this study was to directly compare neurobehavioral and histological outcomes in both sexes to determine whether the reported deleterious effects of HAL extend to females. Anesthetized adult female and male rats received either a controlled cortical impact (CCI) or sham injury and then were randomly assigned to a dosing regimen of HAL (0.5 mg/kg, i.p.) or vehicle (VEH; 1 mL/kg, i.p.) that was initiated 24 h after injury and continued once daily for 19 consecutive days. Motor function was tested using established beam-balance/walk protocols on post-operative days 1–5 and acquisition of spatial learning was assessed with a well-validated Morris water maze task on days 14–19. Cortical lesion volume was quantified at 21 days. No statistical differences were revealed between the HAL and VEH-treated sham groups and thus they were pooled for each sex. HAL only impaired motor recovery in males ($p < 0.05$), but significantly diminished spatial learning in both sexes ($p < 0.05$). Females, regardless of treatment, exhibited smaller cortical lesions vs VEH-treated males ($p < 0.05$). Taken together, the data show that daily HAL does not prohibit motor recovery in females, but does negatively impact cognition. These task-dependent differential effects of HAL in female vs male rats may have clinical significance as they can direct therapy.

1. Introduction

Traumatic brain injury (TBI) is often thought to be a disease of young males. However, females comprise approximately 40% of the TBI population (Guerrero et al., 2000). In the era of personalized medicine, it is critical that we examine questions related to sex and outcomes after TBI, not only from the perspective of spontaneous recovery, but also from the response to treatment. Many pharmacological agents are administered post-TBI, and given the biological differences in males and females, the beneficial or deleterious effects of these pharmacological agents may or may not be equivalent between the sexes.

In general, female patients are more likely to report side effects as a result of a medication, while males may be more sensitive as demonstrated by Morag and colleagues (2013) who compared growth inhibition sensitivities from immortalized human lymphoblastoid cell lines (LCLs) in unrelated females and males to examine sensitivity to

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common drugs. LCLs can be used to assess inter-individual variability in responses to various drugs, which is important for personalized medicine (Stark et al., 2010; Wheeler and Dolan, 2012). The data showed that males were more responsive and sensitive to the typical and atypical antipsychotic drugs (APDs), haloperidol (HAL) and risperidone, respectively. Females were more affected than males by the SSRI paroxetine (Stark et al., 2010; Wheeler and Dolan, 2012). These findings suggest that there may indeed be differences between how females and males respond to various clinically-relevant drugs, which may be related to the effect of hormones. Regarding the dopaminergic system, Wagner and colleagues reported that female rats exhibited smaller TBI-induced decreases in cortical and striatal dopamine transporter (DAT) expression relative to males (Wagner et al., 2005). Increased DAT density has also been shown in non-TBI female rats (Rivest et al., 1995) and in healthy human females (Lavalaye et al., 2000) relative to males. Thus, sex differences in dopamine transmission exist regardless of whether there is a brain injury or not, and provides a strong rationale for evaluating the effects of dopaminergic drugs, such as HAL in females and males.

APDs are often prescribed in the clinic to manage agitation and aggression, that may be, in part, a consequence of TBI. One of several APDs that are prescribed is HAL. However, HAL has consistently been shown to hinder functional recovery in male rats after experimental TBI when provided daily and compared to vehicle (VEH) controls (Wilson et al., 2003; Kline et al., 2008; Hoffman et al., 2008; Folweiler et al., 2017). Moreover, the deleterious effects of HAL persist for months after drug withdrawal (Phelps et al., 2015). Whether the detrimental effects of HAL extend to females after TBI is not established. The significance of knowing how APDs affect females is that both sexes exhibit comparable levels of agitation post injury (Kadyan et al., 2004; Magnotti et al., 2008) and based on current standard practice, both females and males would receive APDs, such as HAL, without consideration for possible differential effects. Hence, the objective of this study is to determine if differences exist between female and male rats in the response to chronic HAL treatment after a controlled cortical impact (CCI) injury of moderate severity.

2. Materials and methods

2.1. Subjects

Seventy-two aged-matched (3 months old) adult female ($n = 36$; 260–290 g) and male ($n = 36$; 300–325 g) Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed in standard steel-wire mesh cages and maintained in a temperature (21 ± 1 °C) and light (on 7:00 a.m. to 7:00 p.m.) controlled environment with ad libitum food and water. After one week of acclimatization all rats underwent a single day of beam-walk training, which consisted of 3–5 trials to traverse the entire beam under 5 s. Following training, the rats were randomly assigned to a the following groups: TBI + VEH (1.0 mL/kg; $n = 13$ female and $n = 13$ male), TBI + HAL (0.5 mg/kg; $n = 13$ female and $n = 13$ male; Sham + VEH (1.0 mL/kg; $n = 5$ female and $n = 5$ male), and Sham + HAL (0.5 mg/kg; $n = 5$ female and $n = 5$ male). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Every attempt was made to limit the number of rats used and to minimize suffering.

2.1.1. Determination of estrous stage

On the morning of surgery, normal cycling female rats were evaluated for estrous stage using classic cytology. Briefly, following a vaginal smear the epithelial cells were examined by light microscopy and based on traditional distinguishable characteristics of each stage (proestrous = predominantly nucleated epithelial cells with few cornified epithelial cells and leukocytes; estrous = predominantly cornified epithelial cells; diestrous = predominantly leukocytes with some nucleated epithelial cells) the rats were classified accordingly (Wagner et al., 2004; Monaco et al., 2013). Normal cycling females were used as this paradigm more closely mimics clinical TBI (3–4 rats from each group fit the criteria for each stage). Moreover, we have previously shown that estrous stage at the time of injury does not impact subsequent recovery (Wagner et al., 2004; Monaco et al., 2013).

2.1.2. Surgery

CCI injury was produced as previously described (Dixon et al., 1991; Kline et al., 2010; de Witt et al., 2011; Matter et al., 2011; Bondi et al., 2014). Briefly, surgical anesthesia was induced with 4% isoflurane in 2:1 N_2O:O_2 and the rats were intubated and secured in a stereotaxic frame. During surgery the rats were maintained at a surgical level with 2% isoflurane and carrier gases. Utilizing aseptic procedures a midline scalp incision was made, the skin and fascia were reflected to expose the skull, and a craniectomy (6-mm in diameter) was made in the right hemisphere (encompassing bregma and lambda and between the sagittal suture and the coronal ridge) with a high speed dental drill. The bone flap was removed and the craniectomy was enlarged further. Subsequently, the impacting rod was extended and the impact tip (6 mm, flat) was centered and lowered through the craniectomy until contact was made with the dura mater, then the rod was retracted and the impact tip was advanced 2.8 mm farther to produce a brain injury of moderate severity (2.8 mm tissue deformation at 4 m/s). Core body temperature was maintained at 37 ± 0.5 °C with a heating blanket. Immediately after the CCI, anesthesia was discontinued and the incision was promptly sutured. The rats were subsequently extubated and assessed for acute neurological outcome. Sham rats underwent similar surgical procedures, but were not subjected to the impact.

2.1.3. Acute neurological evaluation

Hind limb reflexive ability was assessed immediately upon terminating the anesthesia by gently squeezing the rats' paw with forceps every 5 s and recording the time to elicit a withdrawal response. Return of the righting reflex was determined by the time required to turn from the supine to prone position. These tests are sensitive indicators of injury severity and duration of anesthesia (Dixon et al., 1991; Kline et al., 2010; de Witt et al., 2011; Matter et al., 2011; Bondi et al., 2014).

2.1.4. Drug administration

HAL (Sigma, St. Louis, MO) was prepared daily by dissolving in 1:1 dimethyl sulfoxide (DMSO)/saline, which also served as the VEH. The dose of HAL was chosen because it has been reported to be comparable to that used clinically to control psychosis (Rosengarten and Quartermain, 2002) and has been used in several brain injury studies investigating functional outcome (Feeney et al., 1982; Hovda and Feeney, 1985; Wilson et al., 2003; Kline et al., 2007, 2008; Hoffman et al., 2008; Phelps et al., 2015; Folweiler et al., 2017). Treatments began 24 h after CCI or sham surgery and were provided intraperitoneally once daily for 19 days. The half-life of HAL using this dose and route is reported to be 2.6 h (Kapetanovic et al., 1982) and thus it was provided *after* the daily behavioral assessments to circumvent sedative effects, which may confound the results.

2.1.5. Motor performance

Well established beam-balance and beam-walk tasks were utilized to assess motor performance (Dixon et al., 1991; Kline et al., 2010; de Witt et al., 2011; Matter et al., 2011; Bondi et al., 2014). Briefly, the beam-balance task consists of placing the rat on an elevated (90 cm) narrow beam (1.5 cm wide) and recording the time it remained on for a maximum of 60 s. The beam-walk task, modified from Feeney and colleagues (Feeney et al., 1982), and used extensively in our laboratory (Kline et al., 2002, 2004, 2007, 2010; Sozda et al., 2010; Bondi et al., 2014), consists of training/assessing rats using a negative-reinforcement paradigm to escape bright light and white noise by traversing an elevated narrow beam (2.5 cm wide × 100 cm long) and entering a darkened goal box at the opposite end. Beam-walk performance was