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POLY (ADP-RIBOSE) POLYMERASE-1 INITIATED NEURONAL CELL DEATH PATHWAY—DO ANDROGENS MATTER?

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Abstract—Activation of poly (ADP-ribose) polymerases (PARP) contributes to ischemic damage by causing neuronal nicotinamide adenine dinucleotide (NAD^+) depletion, release of apoptosis-inducing factor and consequent caspase-independent cell death. PARP-mediated cell death is sexually dimorphic, participating in ischemic damage in the male brain, but not the female brain. We tested the hypothesis that androgen signaling is required for this male-specific neuronal cell death pathway. We observed smaller damage following focal cerebral ischemia (MCAO) in male PARP-1 knockout mice compared to wild type (WT) as well as decreased damage in male mice treated with the PARP inhibitor PJ34. Protection from ischemic damage provided by PJ-34 in WT mice is lost after removal of testicular androgens (CAST) and rescued by androgen replacement. CAST PARP-1 KO mice exhibit increased damage compared to intact male KO mice, an effect reversed by androgen replacement in an androgen receptor-dependent manner. Lastly, we observed that ischemia causes an increase in PARP-1 expression that is diminished in the absence of testicular androgens. Our data indicate that PARP-mediated neuronal cell death in the male brain requires intact androgen-androgen receptor signaling.

Key words: cerebral ischemia, stroke, poly-ADP ribose polymerase, androgen.

Poly (ADP-ribose) polymerases (PARP) are members of a family of enzymes that are particularly abundant in cell nuclei and can function as sensors of DNA damage. Poly-(ADP-ribosyl)ation of proteins is a post-translational modification catalyzed by PARP and a key step in the regulation of multiple physiological cellular functions such as DNA repair, gene transcription, gene expression, cell cycle progression, cell death, chromatin function, and genomic stability (Pacher and Szabo, 2008). PARP-1 is the most abundant isoform of the PARP enzyme family and is an important regulator of neuronal cell death and cellular responses to DNA damage resulting from physiological circumstances as well as from neuronal injury (Eliasson et al., 1997). Activation of PARP-1 after ischemia-induced DNA damage is well recognized as a key factor in neuronal NAD^+ depletion, mitochondrial release of apoptosis-inducing factor, and caspase-independent cell death (Jagtap and Szabo, 2005).

This PARP-1-initiated cell death pathway has been recently shown to be sexually dimorphic (McCullough et al., 2005; Lang and McCullough, 2008; Yuan et al., 2009). Genetic deletion or pharmacological inhibition of PARP improves brain outcomes from cerebral ischemia in males (Eliasson et al., 1997) but not in females regardless of ovarian hormone status (McCullough et al., 2005). Similar sex specificity has been reported in the neonatal brain treated with hypoxia-ischemia (Hagberg et al., 2004; Zhu et al., 2006). These initial studies suggested that PARP dependent, caspase independent, neuronal death pathway may be highly engaged in male ischemic brain, less so in the female. Subsequent work has confirmed and expanded our understanding that PARP signaling through apoptosis-inducing factor (AIF) is an important target in male cerebral ischemic pathology, while the intrinsic, caspase-dependent pathway is vital to female neuronal death (Lang and McCullough, 2008; Yuan et al., 2009).

The biological basis for PARP's sexually dimorphic death signaling in cerebral ischemia is unclear. We hypothesized that the surprising specificity of this molecular mechanism is enabled by androgen availability and androgen receptor (AR) signaling in the male. The role of male sex steroids in ischemic sensitivity is relatively understudied. Male sex is a recognized risk factor for cerebrovascular disease and stroke (Foulkes et al., 1988), and male animals consistently exhibit greater damage following experimental ischemia (For recent reviews, see Herson et al., 2009; Vagnerova et al., 2008; Hurn et al., 2005). Consistent with the notion that androgens increase damage following cerebral ischemia, removal of endogenous testosterone by castration results in decreased ischemic damage in male rodents (Yang et al., 2002; Cheng et al., 2007). Importantly, infarct volume following middle cerebral artery occlusion (MCAO) increases in castrated males when testosterone is replaced (Hawk et al., 1998; Toung et al., 1998; Yang et al., 2002; Cheng et al., 2007). In the present study, we explored the interaction of male sex hormones and PARP-1 following focal cerebral ischemia. Our study breaks new ground by focusing on the role of androgens

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Abbreviations: AIF, apoptosis-inducing factor; AR, androgen receptor; CAST, castrated; cDNA, complementary DNA; DHT, dihydrotestosterone; F, flutamide; KO, knock out; LDF, laser-Doppler flowmetry; MCAO, middle cerebral artery occlusion; mRNA, messenger RNA; NAD, nicotinamide adenine dinucleotide; PARP, poly(ADP-ribose) polymerase; PJ34, N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-(N,N-dimethylamino)acetamide hydrochloride; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; TTC, triphenyl tetrazolium chloride; WT, wild type.

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and AR in PARP-1 cell death signaling *in vivo*. Furthermore, since PARP inhibitors such as minocycline are currently in clinical trial, our study sheds new light on the effectiveness of these agents in male stroke patients and may serve as a prototype of sex-specific anti-ischemic treatments for brain.

EXPERIMENTAL PROCEDURES

Animals

The present study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research and under protocols approved by the Oregon Health and Science University Animal Care and Use Committee. We used PARP-1 gene deficient (KO) male mice raised in our laboratory (homozygous breeding with intermittent confirmation of genotype, as described previously (Eliasson et al., 1997); animals bred to confluence on background strain [129S1/SvImJ]). 129S1/SvImJ mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) to minimize strain and vendor variability and served as wild type (WT) controls. Male animals were randomized to groups of the same age and weight (22–30 g).

Ischemic model

Methods are as previously published in mouse (Vagnerova et al., 2006). Cerebral ischemia was induced by 90 min reversible MCAO via the intraluminal suture technique (6−0 monofilament nylon surgical suture with a heat-rounded tip) under isoflurane anesthesia. Adequacy of MCAO was confirmed by laser-Doppler flowmetry (LDF) measured over the ipsilateral parietal cortex and by neurological deficit scoring during continuous occlusion as follows: 0=no deficit, 1=failure to extend forelimb, 2=circling, 3=unilateral weakness, 4=no spontaneous motor activity (Hurn and Macrae, 2000; Hurn et al., 1995). Only mice with clear neurological deficits (neurological deficit scoring≥2) were included in the treatment groups. Physiological measurements were performed in separate cohorts (n=3 per group), as previously described (Goyagi et al., 2001). In each animal, a femoral arterial catheter was placed for arterial blood pressure and blood gas measurement.

Gonadectomy and hormones

Orchidectomy, as described previously, was performed under isoflurane anesthesia (Toung et al., 1998) 7 days prior to ischemia concurrent with administration of dihydrotestosterone (DHT) by s.c. implant technique (5 mg, continuous 21-day release implant, Innovative Research of America, Sarasota, FL, USA) (Hurn et al., 1995). AR antagonist flutamide (F) (5 mg, continuous 21-day release implant, Innovative Research) was implanted s.c. at the time of castration and DHT implantation. Sham castration was performed under anesthesia 7 days prior to MCAO.

Poly (ADP-ribose) polymerase inhibition with PJ-34

Immediately before MCAO, 129S1/SvImJ mice (Taconic, Hudson, NY, USA) were injected i.p. with the PARP-1 inhibitor PJ-34 (10 mg/kg) or saline control (Garcia et al., 2001).

Imaging and analysis

The brains were harvested at 24 h post MCAO and sliced into five 2-mm thick coronal sections for staining with 1.2% triphenyl tetrazolium chloride (TTC) in saline (Takahashi et al., 1997). Infarction volume was measured by a blinded investigator using digital imaging and image analysis software (Sigma Scan Pro, SPSS Inc., Chicago, IL, USA). The area of infarct was measured on the rostral and caudal surfaces of each slice and numerically integrated across the thickness of the slice to obtain an estimate of infarct volume in each slice. Infarct volume of the total hemisphere, striatum, and cortex were measured. Volumes from all five slices were summed to calculate total infarct volume expressed as a percentage of contralateral structure volume. Infarct volume was corrected for edema by comparing the volume of ischemic to nonischemic hemispheres (Goyagi et al., 2001).

TaqMan real-time qPCR

Total RNA was obtained using RNeasy Mini kit (Qiagen, Valencia, CA, USA) per manufacturer's instructions. RNA concentration was determined by UV measurement; cDNA was reverse transcribed from 500 ng total RNA using the high capacity cDNA archive kit (Applied Biosystems, Foster City, CA, USA); 50 ng cDNA was used for qPCR in a 96-well plate with a total volume of 50 μL. Each TaqMan reaction was performed in triplicate. Specific primer and probe sets for PARP were obtained from Applied Biosystems. 18S RNA levels were also determined to serve as internal control, and final results were expressed as the ratios of PARP-1 to 18S.

Statistical analysis

All data are expressed as mean±SEM. Infarction volume and all densitometry measures were analyzed with one-way analysis of variance (ANOVA), and post hoc comparisons were made by Tukey test. Physiological and LDF values were analyzed by two-way ANOVA and post hoc Newman–Keuls to determine differences among treatment groups. The criterion for statistical significance was set at $P<0.05$.

RESULTS

Infarct volumes of intact and castrated WT and PARP-1 KO male mice

Gonadally intact and castrated male 129S1/SvImJ mice (WT) and PARP-1 KO (KO) mice were subjected to 90 min MCAO and infarct volume of cortex, striatum, and total (hemisphere) was analyzed. Castrated WT mice had significantly smaller total infarct volumes compared to intact WT male mice, consistent with our recently published results (Cheng et al., 2007; Uchida et al., 2009), 16.9%±2.1% (n=10) in CAST vs. 37.3%±6.9% (n=10) in intact. Similar findings were observed in cortical and striatal infarct volumes (cortex: 12.6%±3.2% in CAST vs. 32.6%±6.7% in intact; striatum: 86.2%±6.8% in CAST vs. 96.6%±9.4% in intact). In agreement with previous reports, infarct volume was significantly smaller in gonadally intact male KO mice compared to WT males (Fig. 1). Surprisingly, the protection afforded by KO was lost following castration. In fact, CAST KO males exhibited significantly increased infarct volume compared to intact KO mice in cortex (23.4%±4.3% in CAST vs. 7.4%±3.2% in intact), striatum (85.1%±9.7% in CAST vs. 30.5%±5.0% in intact), and total infarction (24.1%±3.9% in CAST vs. 8.8%±2.2% in intact) (Fig. 1).

Effect of androgen/androgen receptor signaling on infarct volumes in WT and PARP-1 KO mice

In order to assess the role of androgen receptor signaling, castrated WT and KO mice were implanted with DHT, a