

PARP-1 Initiated Cell Death Pathway – Does Androgen Matter?

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Abstract

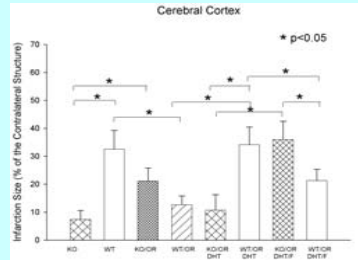
The nuclear enzyme poly ADP-ribose polymerase (PARP-1) is important regulator of neuronal cell death and cellular responses to DNA repair under physiological circumstances and in neuroinjury, ischemia, inflammation and sepsis (1). This cell death pathway is gender-specific (2, 3), deletion of PARP-1 isoform or pharmacological inhibition of the enzyme attenuates ischemic brain injury only in males but not females. We have shown previously using our focal ischemia middle cerebral artery occlusion (MCAO) model that neuroprotection enjoyed by PARP-1 gene deletion is lost in the absence of testicular androgens. We confirmed our data with PARP-1 enzyme inhibition in genetically matched 129 SvEv wild type mice and concluded that PARP-1 activation requires androgen background to be a key step in ischemic cell death and that androgen-PARP-1 interactions may be crucial for male ischemia sensitive phenotype. We now focused on hypothesis that androgens have a direct impact on PARP-1 either by increasing baseline or intra-ischemic PARP-1 gene transcription (PARP-1 mRNA levels) and/or that androgens stimulate the PARP-1 enzymatic activity (NAD⁺ levels). We also evaluated the effect of ischemia on gene transcription of another PARP isoform PARP-2 in PARP-1KO mice, to confirm that there is no compensatory up regulation of this isoform.

Methods

-140 male SvEv 129 male mice (20 to 25 g); Taconic
 -60 male PARP-1 deficient (PARPKO) mice, in-house colony
 -General inhalational anesthesia: Isoflurane (1.5-2%), O₂ (30%) enriched air
 -90 min of middle cerebral artery occlusion (MCAO), confirmed by laser-Doppler flowmetry (LDF) and neurological deficit, 24 hr reperfusion
 -Body temperature controlled at 36.5±1.0°C
 Criteria of inclusion: unilateral weakness and circling to the affected side at 80th minute of MCAO
 -Plasma total and free testosterone measured in each animal with commercial radioimmunoassay kits (Diagnostic Products Corp) with inter- and intra-assay variations of 4.4% and 6.8%, respectively
 -Real Time qPCR: TaqMan real-time qPCR is used for PARP-1 mRNA assay
Treatment
 -Orchiectomy 7days prior to MCAO;
 -Dihydrotestosterone (DHT) 5mg pellet (IRA) implantation subcutaneously 7 days prior to MCAO
 -PJ34 (PARP-1 inhibitor, Sigma), 10mg/kg, intraperitoneally prior MCAO
 -Flutamide (F)(IRA), androgen receptor blocker, 5 mg pellet implantation subcutaneously 7 days prior to MCAO
Treatment groups
 1)PARPKO, 2) WT, 3) orchiectomized PARPKO (KO/OR), 4) orchiectomized WT (WT/OR), 5) orchiectomized PARPKO + DHT (KO/OR/DHT), 6) orchiectomized WT + DHT (WT/OR/DHT) 7) orchiectomized PARPKO + DHT+Flu (KO/OR/DHT/F), 8) orchiectomized WT + DHT + Flu (WT/OR/DHT/F), 9) WT/Control, 10) WT + PJ34 (PJ 34), 11) orchiectomized WT (OR), 12) orchiectomized WT + PJ34 + (OR/PJ 34), 13) orchiectomized WT + DHT + PJ34 (OR/DHT/PJ 34)
Physiology groups
 -femoral artery catheter for physiologic monitoring of MAP, ABG
 -intra-ischemic physiological parameters carried out in separate treatment cohorts (n=3 for each treatment group): pH, pCO₂, pO₂, HCO₃, SBE, Sat O₂, Hgb, glu; laser-Doppler flowmetry, control of body temperature
Histology
 -brains harvested at 24hr of reperfusion,
 -infarction volume determined with 1.2% TTC (triphenyltetrazolium chloride)
 -percentage of infarcted volume calculated for cerebral cortex, caudate putamen, total hemisphere
 Statistics: one-way ANOVA (post-hoc: Tukey)
 PARP Activity (22). PARP activity is assessed using [adenylate 32P] NAD (NEN Life Sciences) in the presence or absence of PARP inhibitor benzamide or PJ34. Brains for qPCR and PARP activity measurements are harvested as described above, cerebral cortex is dissected and stored in -80° C.

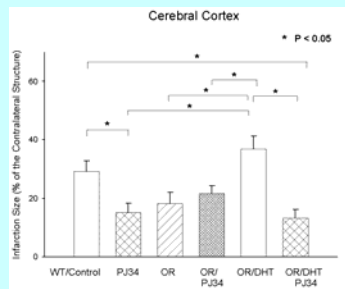
Results 1

Androgens and AR blockade in PARPKO and WT males



• In cerebral cortex PARP-1 genetic deletion attenuates ischemic injury in males only when androgens are present. Blockade of androgen receptor (AR) is neuroprotective only in WT males but not in PARPKOs.

PARP-1 pharmacological inhibition and levels of Androgen in WT males

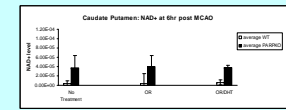
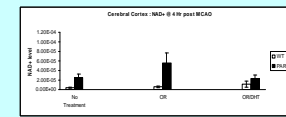
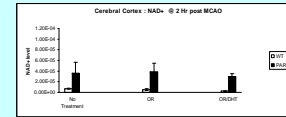


• PARP-1 pharmacological inhibition by PJ 34 attenuates ischemic injury in males only when androgens are present.

•Physiologic variables were not different among groups.

Results 2

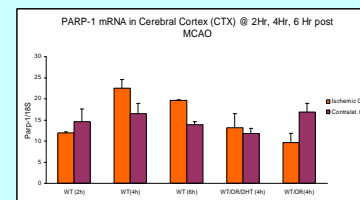
PARP-1 enzymatic activity at baseline and post ischemia



•PARP-1 enzymatic activity (NAD⁺ levels at 2hr, 4hr, and 6hr post ischemia) in cerebral cortex and striatum is significantly higher in WT males as compare to PARPKOs.
 •No differences amongst treatment groups of castrated males compare to DHT replaced and intact males both in WTs and PARPKOs.

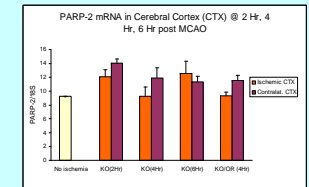
PARP-1 mRNA at baseline – castration +/- testosterone repletion has no effect on enzyme transcription in WT males.

PARP-1 mRNA levels post MCAO in PARP-1 deletion in ischemic and contralateral hemisphere



•PARP-1 mRNA post MCAO - castrated WT animals 4hr post ischemia have significantly lower levels of PARP-1 mRNA in cerebral cortex of ischemic hemisphere as compared to control non ischemic hemisphere

PARP-2 mRNA levels post MCAO in PARP-1 deletion



•PARP-2 mRNA - orchiectomy with or without testosterone repletion had no effect on enzyme transcription in PARPKO males.

Summary

-Neuroprotection afforded by either PARP-1 genetic deletion or pharmacological inhibition is dependent on the presence of DHT and DHT/AR interaction.
 -PARP-1 enzymatic activity increases after stroke (NAD⁺ levels at 2hr, 4hr, and 6hr post ischemia) in cerebral cortex and striatum in WT males as compared to PARPKOs but no differences amongst treatment groups of castrated compare to DHT repleted and intact males.
 -Castrated WTs 4hr post ischemia have significantly lower levels of PARP-1 mRNA in cerebral cortex of ischemic hemisphere compare to control non ischemic.
 -In intact and DHT repleted males PARP-1 mRNA levels were higher in ischemic cortex compare to non ischemic at different time points post MCAO (2hr, 4hr, and 6hr).
 - PARP-2 mRNA levels demonstrated no changes under ischemic conditions neither in intact nor castrated PARP-1 knock out males.

Conclusion

Our results might indicate that under ischemic conditions PARP-1 gene transcription is dependent on background androgens levels and it is likely that androgens enable PARP-1 mediated ischemic cell death mechanisms in vivo. Our data show that direct interaction between androgen and PARP-1 activity and transcription is not likely, so other protein – protein interactions, like AR-PARP interactions, should be investigated.

1. Eliason et al, Nature Medicine 3: 1089, 1997.
2. Yu et al, Science 297: 200, 2002
3. McCullough et al, J Cereb Blood Flow Metab 25: 502, 2005
4. Hagberg et al, J Neurochem 90: 1068, 2004

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 Supported by AHA-Bugher Foundation Award 0575058N