

Association of hypothalamic inflammation and cerebrospinal fluid orexin levels with brain radiation-induced fatigue

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Objective

Fatigue, the most common acute and subacute toxicity of partial or whole brain radiation therapy (WBRT), significantly decreases quality of life (QOL) for patients, abrogating the benefit afforded by improved tumor control. Little is known about the mechanisms underlying brain-radiation induced fatigue, and there are currently no effective treatments. The objective of this study is to evaluate the role of diminished orexin neuron activity in the pathogenesis of fatigue induced by clinically significant WBRT fractionation.



Decreased Orexin release

Methods

- Adult male Sprague-Dawley rats received whole brain RT (WBRT) 4 Gy x 5 fractions
- · Fatigue Behavior
 - Home Cage Locomotor Activity
- Anthropometrics
 - Body weight
 - Food intake
- Gene expression
 - · Hypothalamic & Hippocampal mRNA (qRT-PCR)
 - Inflammation (IL-1β, Tnf, IL-6, Ccl2, IL-1ra, Hmgb1, Gfap, Iba-1, Itgam)
- Oxidative Stress (Nox4, Cyba, Ncf1, Sod1, Sod2)
- CSF collected from 11 pediatric primary brain cancer patients treated with proton radiation at 4 time points: pre-RT, then q 30 days post-RT (post-RT 2, 3, 4),
- CSF cytokine concentrations measured using multiplex magnetic bead immunoassav
- Physician-assessed CTCAE 4.0 fatigue prospectively collected during brain RT CSF Orexin-A concentration
 - · Collected by cisterna magna puncture (rats) or lumbar puncture (patients)
 - I¹²⁵ Radioimmunoassay
- Statistical Analyses
 - · 2-way ANOVA with Bonferroni corrected t-tests
- Student's t-test · Logistic Regression for association between fatigue and CSF cytokine levels
- Significance set at p<.05

Study Design





hypothalami isolated at fatigue onset, nadir, and recovery. *, p<.05, **, p<.01, ***, p<.001



Figure 4: WBRT activates hypothalamic microglia. Immunohistochemistry for Iba-1 (green) and DAPI (blue).



Figure 6: Heatmap of CSF cytokine levels in pediatric patients treated with RT. CSF collected prior to RT, then serially post-RT. Cytokine concentration measured using multiplex magnetic bead immunoassay. All values normalized to pre-RT levels. Fatigue associated with post-RT [sTNFR1] (p=0.04) and [sTNFR2] (p=.04). Statistical analysis using logistic regression.

Results

· LMA was decreased in WBRT-treated rats following the first fraction and continued to decrease until reaching a nadir following the final fraction. LMA slowly recovered over the next 7 days.

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lancer

- Food intake and weight gain were significantly reduced in WBRTtreated rats, recovering to match sham rats within 2 weeks of the first fraction.
- Hypothalamic mRNA demonstrated a significant increase in inflammatory gene expression (IL-1b, IL-6, TNF, CCL2, GFAP, Itgam) following 1- and 5-fractions, which largely resolved by fatigue recoverv.
- Immunohistochemistry showed increased Iba-1 positive cells with morphology consistent with activated microglia.
- WBRT-treated animals showed decreased CSF [orexin-A] at activity nadir. which recovered with restoration of LMA.
- · Orexin levels were decreased and inflammatory cytokine levels increased in CSF samples from pediatric patients for whom fatigue was recorded as a toxicity during treatment.



Figure 7: Relationship between changes CSF [Orexin-A] and fatigue. CSF collected prior to RT. then 30 d post-RT. Orexin-A concentration measured using radioimmunoassay. Statistical analysis using 2-way ANOVA.

Conclusions

- 1. WBRT-induced fatigue can be modeled in preclinical research using home cage LMA.
- 2. The acute fatigue phenotype is observed following the first fraction, with an activity nadir following the final fraction and recovery approximately one week later.
- 3. WBRT-induced inflammation follows the behavioral and anthropometric changes, suggesting a possible causative role.
- 4. Fatigue is associated with decreased CSF orexin levels and increased brain inflammation in rats and patients.
- 5. Further studies are needed to test the utility of orexin-replacement as a therapeutic strategy for radiation-induced fatigue.



Sham

D YPT

Day 12

the CSF. CSF [orexin-A] collected at the end of the

dark cycle at each time point. ***, p<.001

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