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Association between maternal periapical lesions and brain inflammation in rat pups

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ABSTRACT

Objective: The objective of this study was to determine whether the presence of maternal tooth periapical lesions was associated with foetal brain inflammation in a pregnant rat model.

Methods: Sprague–Dawley rats were divided into two groups: pregnant rats with induced periapical abscesses (E, $n = 8$) and sham-operated control pregnant rats (S, $n = 8$). The pulps of the first and second maxillary right molars had been exposed and the tooth left open to the oral environment for two weeks prior to initiation of the pregnancy. Following delivery of the pups (E, $n = 99$; S, $n = 101$), each pup was decapitated and the brain was removed and immediately frozen in liquid nitrogen. The tissues were solubilized in PBS containing a protease inhibitor, and norepinephrine (NE), IL-6, IL-1-β, TNF-α, and myelin basic protein (MBP) were determined by ELISA. Group means were compared by factorial analysis of variance, a post hoc Tukey test, and Pearson's correlation test. $p < 0.05$ was used to reject the null hypothesis.

Results: E pups were significantly heavier than S pups. Brain tissue concentrations of IL-6, IL-1-β, and TNF-α were significantly higher and MBP and norepinephrine concentrations significantly lower in E pups than S pups. Concentrations of IL-6, TNF-α and IL-1-β were significantly correlated between E serum, pup birthweight, and E pup brain tissue. MBP, NE and IL-6 were significantly correlated within the brain tissues of E pups.

Conclusion: The data suggest that brain inflammation may be associated with maternal periapical inflammation. This association identifies a modifiable risk factor for adverse pregnancy outcomes.

1. Introduction

Tumour necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 are commonly associated with oral infections involving both periodontal[1–9] and periapical[10,11] tissues. There is increasing evidence for a relationship between maternal infection and foetal brain inflammation.[12–25] Infections in pregnant females have been reported to increase the concentration of their serum inflammatory cytokines, which may be transferred to the foetus through the blood–brain barrier.[5,12–14,20,21,26–28] These cytokines include IL-1-β, IL-6 and TNF-α which produce CNS damage by activation of microglial cells and astrocytes. Then these cells produce additional proinflammatory cytokines and chemokines within the brain tissue.[14,15,20,21,25,29,30] Inflammation and infection within the developing brain are associated with a white-matter lesion, commonly termed "periventricular leucomalacia (PVL)".[31–33] PVL results from

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abnormal development of myelin within the foetal brain [white matter damage, WMD], which has been associated with postnatal signs of cerebral palsy,[16] cognitive limitations,[16] behavioural problems,[34] and visuo-spatial difficulties.[35]

WMD is often associated with low-birth weight, pre-term infants.[7,8,19,23,24,27,36–40] Previous studies have used various methods to produce WMD in animals using one of the following techniques: (1) intraperitoneal injection of lipopolysaccharides (LPS) and endotoxin; (2) injection of LPS into the cervix of pregnant animals; and (3) *Escherichia coli* initiated sepsis in a pregnant animal. In addition, neuroinfection, initiated by injection of LPS into the brain of the newborn rat, has been used to elevate their brain cytokine concentrations.[41] Low birthweight has also been associated with the synthesis of norepinephrine and development of the frontal cortex in the rat.[42]

Numerous studies suggest that the cytokines associated with maternal inflammation are also important in the pathogenesis of WMD.[19,23,30,36–39] These maternal cytokines include interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α,[17] produced by activated leucocytes. The cytokines pass through the blood–brain barrier into the foetal brain, which produces cell and axonal damage.[28,43] TNF-α increases vascular permeability, crosses the blood–brain barrier, and probably facilitates maternal transfer of additional cytokines to the foetus.[26,44] Foetal WMD is usually associated with apoptosis of oligodendrocyte progenitors.[18,21] Since oligodendrocytes synthesize myelin during brain development,[45] damage to these progenitor cells could result in either an inadequate amount of myelin and/or abnormal myelin.[13] Within the developing rat brain, the maturation of the cerebral white-matter is incomplete prior to birth, making it vulnerable to injury in utero.[22] Immature oligodendrocytes appear to be particularly vulnerable to oxidative stress and free radical production.[28] Previous studies have reported decreased myelin basic protein (MBP) and elevated TNF-α, IL-1 and IL-6 concentrations within the foetal brain coincident to maternal inflammation, suggesting a mechanism for the adverse effects of proinflammatory cytokines on myelin synthesis.[15,30]

There is little information concerning the association between maternal periapical inflammation and birth outcomes, although there is substantial information concerning the association between maternal periodontal disease and those outcomes.[46] There had been no evidence that periapical lesions were associated with adverse pregnancy outcomes, until a recent study by us.[11] In that study, pregnant rats with periapical lesions had significantly longer pregnancies and delivered larger pups, compared to control rats. These data are opposite to those reported for pregnant females with periodontal inflammation (that is, they deliver preterm, low-birthweight babies).[2,4,5,8,46] Since the data from our experiments suggested that periapical lesions affected birth outcomes, it seemed worthwhile to study the brains of the pups born from to the mothers from our previous study. There is no information concerning brain development in the offspring of animals with periapical inflammation, so study of these animals would indicate whether a pre-existing maternal periapical infection could significantly affect development of the brain of their offspring.

2. Materials and methods

This study was approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

2.1. Analysis of pup brain cytokines and biomarkers

Pups born from ''Experimental'' and pregnant (EP, $n = 8$, 99 pups), and ''Sham-operated'' control and pregnant (SCP, $n = 8$, 101 pups) animals were studied.[11] To create these groups, in 16 female rats, following anaesthesia with ketamine (60 mg/kg body weight) and xylazine (7.5 mg/kg) (IP), the roof of the pulp chamber of the right first and second maxillary molar teeth was removed using a 1/4 round bur in a high-speed handpiece (''Experimental'' group). 16 female rats were sham-operated (''Sham-operated control'' group). Sham-operated control animals were anaesthetized as previously described and then experienced the touch of a rotating bur on the occlusal surface of the right M1 and M2 teeth, which removed a minimal amount of enamel. The left side was untreated in all animals. The pulp chambers were left open to the oral environment to minimize post-operative pain.[11]

Each pup was weighed within 12 h of delivery, using a microbalance. Then, each pup was decapitated, the brain removed, divided into halves, and the left half immediately frozen in liquid nitrogen. These tissues were used for assay of the brain tissue. For analysis of the biomarkers, the brain samples were thawed and ground in PBS containing a protease inhibitor (10 mg tissue/ml PBS + protease inhibitor). The total protein concentration of each tissue homogenate sample (average of triplicate aliquots) was determined by a bicinchoinic acid method using a commercial kit (Pierce Chemical Company, Rockford, IL). The absorbance of each well was determined using a microplate spectrophotometer at 590 nm and the protein concentration was calculated from a standard curve using standards supplied with the kits. The concentrations of norepinephrine (ALPCO Diagnostics, Salem, NH), MBP, TNF-α, IL-6, and IL-1-β (R&D Systems, Minneapolis, MN) were determined by ELISA techniques. The absorbance of each well was determined using a microplate spectrophotometer at 450 nm, and the biomarker concentrations were calculated from a standard curve using standards supplied with the kits. The concentration of each biomarker was normalized for total protein and was recorded as biomarker concentration/mg protein for each biomarker.

2.2. Statistical analysis

Outcome data included brain tissue concentrations of TNF-α, IL-6, IL-1-β, MBP, and norepinephrine. Group means (EP and SCP) were compared by factorial analysis of variance and a post hoc Tukey test using SPSS v17 (SPSS, Chicago, IL). Correlations between the group variables were determined by a Pearson's test for correlation. $p < 0.05$ was used to reject the null hypothesis.