Antibiotic Alters Inflammation in the Mouse Brain During Persistent Chlamydia pneumoniae Infection

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Summary
Using our mouse model, we investigated whether treatment with moxifloxacin hydrochloride had an effect on the inflammatory process and amyloid deposition in the mouse brain during persistent Chlamydia pneumoniae (Cpn) infection. We established a persistent Cpn-infection in BALB/c mice, treated with antibiotic at days 7-21 or up to 84-98 (p.i.). With early antibiotic treatment, astrocytic activation was more prominent at 3 months p.i.. Delayed treatment resulted in less cellular reactivity although labeling for Cpn and amyloid was still prominent. Thus, early antibiotic treatment may modulate the cellular inflammatory processes following infection and, ultimately, the extent of amyloid deposition in the mouse brain.

Introduction
Although the neuropathology characteristic of both familial- and late-onset sporadic Alzheimer’s disease (AD) is similar, the detailed initiating mechanisms remain to be elucidated for sporadic AD. A number of risk factors have been identified for sporadic AD, including age (Evans et al., 1989), APOEε4 expression (Roses, 1996), and head injury (Nicoll et al., 1995). This proposal addresses an environmental culprit, Chlamydophila (Chlamydia) pneumoniae, that we believe is involved in the pathogenesis of AD. We previously identified and localized Cpn in areas of neuropathology in sporadic Alzheimer’s brains (Balin et al., 1998). Subsequently, we developed a mouse model to determine how this intracellular respiratory pathogen could infect brain tis-
issues of a mouse after inoculation of the nasal passages and analyzed whether pathological changes occurred in the brains of these infected mice. Following intranasal infection with Cpn isolated from a human AD brain, our non-transgenic BALB/c mice developed amyloid-reactive plaques (Little et al., 2004). The amyloid deposits, similar to plaques found in sporadic Alzheimer’s disease, were identified with the density, size and number of plaques increasing with extended time of infection. These data suggested that infection with Cpn triggered the induction and formation of amyloid deposits. The current study addresses an intervention strategy using antibiotics to assess whether antibiotic treatment will eradicate or modulate outcomes of the infectious trigger in our animal model.

Materials and Methods

AR-39, a respiratory Cpn isolate, was propagated in HEp-2 cells, homogenized and passed through a 0.8-micron filter. Under manual constraint, 70 three month old, female BALB/c mice were inoculated into their nares with $1 \times 10^7$ inclusion forming units of AR-39 diluted in 20-30 μl HBSS. Thirty age and sex matched mice were mock infected with uninfected HEp2 cell lysate as controls for each time point. Animals were orally gavaged for 14 days with 0.25mg Moxifloxacin hydrochloride/HBSS [100mg/g wt per day] according to the treatment regime. At 3-month intervals, mice were intracardiac perfusion fixed with 4% paraformaldehyde. The brains were paraffin embedded and sectioned at 7-10 mm thickness. Sections were de-paraffinized, rehydrated, and endogenous peroxidase activity quenched. Following a PBS rinse they were processed for antigen retrieval and blocked in 2% Fetal Bovine Serum/PBS. The sections were incubated with primary antibodies (anti-chlamydia or anti-amyloid) for 1.5 hr at 37°C, rinsed with PBS, and incubated with secondary antibodies for 1 hr at 37°C. The sections were visualized using 3, 3'-Diaminobenzidine (DAB) or AP Red, rinsed, Hematoxylin counterstained, mounted, and viewed on a Nikon Eclipse microscope. Images were captured on a Spot RT camera.

Results

All BALB/c mice received either Cpn infected cell lysate or uninfected cell lysate. Following infection, groups of 5 mice were treated with antibiotic at days 7-21, 28-42, 56-70, or 84-98, or received no antibiotic administration (FIG 1F). Chlamydia-specific antibodies revealed the presence of Chlamydia at 6 months post infection in olfactory tissues indicative of persistent infection. This labeling was found in the nasal neuroepithelium (FIG 1A) and olfactory bulb (FIG1B).

At six months post-infection, the number and size of amyloid plaques was reduced by earliest antibiotic intervention following infection. This antibiotic administration (days 7-21) resulted in a total number of amyloid plaques similar to that observed in uninfected mice. Uninfected mice had an average of 16 plaques and infected-mice receiving early antibiotic administration had
an average of 25 plaques (2 fold increase). In contrast, there was a 8 to 9 fold increase in number of amyloid plaques in the brains of mice receiving late administration of antibiotic (after day 70). This was similar to mice that
received no antibiotic administration wherein a 6 to 12 fold increase in the average total number of plaques was observed relative to uninfected control mice (FIG 1E).

Glial cell activation was more prominent at 3 months post-infection independent of antibiotic administration. In mice that received no antibiotic administration, labeling of activated glial cells in proximity to the head of the dentate gyrus was much more prominent at 3 months post-infection (FIG 1C) than comparable regions analyzed at 6 months post infection (data not shown). When groups of mice that received early antibiotic administration were compared at 3 and 6 months, again glial cell activation was prominent at 3 months post-infection (data not shown) and greatly reduced at 6 months post infection (FIG 1D). Early antibiotic administration did not substantially alter the glial cell activation state at either 3 months or 6 months post-infection, whereas the time after initial infection (3 versus 6 months) seemed to be the determining factor in the activation state of glial cells.

Conclusions

Early antibiotic intervention post-infection with Chlamydia pneumoniae appears to be more effective than delayed intervention in limiting the development of amyloid plaques in the mouse brain. Evidence for Chlamydia pneumoniae in the mouse brains and olfactory tissues is apparent through 6 months post-infection even after antibiotic treatment has been initiated. Thus, complete eradication of the organism is not necessary in order for antibiotic intervention to mediate beneficial effects, as supported by a reduction in the number of amyloid plaques. Glial cell activation, as noted by morphologic appearance, appears more prominent in the 3-month post-infection mice than the 6-month post-infection mice.

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References


