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Microglia Activation and Schizophrenia: Lessons From the Effects of Minocycline on Postnatal Neurogenesis, Neuronal Survival and Synaptic Pruning

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The implication of neuroinflammation in schizophrenia, sustained by recent genetic evidence, represents one of the most exciting topics in schizophrenia research. Drugs which inhibit microglia activation, especially the classical tetracycline antibiotic minocycline are currently under investigation as alternative antipsychotics. However, recent studies demonstrated that microglia activation is not only a hallmark of neuroinflammation, but plays important roles during brain development. Inhibition of microglia activation by minocycline was shown to induce extensive neuronal cell death and to impair subventricular zone (SVZ) neurogenesis and synaptic pruning in the early postnatal and adolescent rodent brain, respectively. These deleterious effects contrast with the neuroprotective actions of minocycline at adult stages. They are of potential importance for schizophrenia, since minocycline triggers similar pro-apoptotic effects in the developing brain as NMDA receptor (NMDAR) antagonists, known to induce long-term schizophrenia-like abnormalities. Moreover, altered postnatal neurogenesis, recently described in the human striatum, was proposed to induce striatal dopamine dysregulation associated with schizophrenia. Finally, the effect of minocycline on synapse remodeling is of interest considering the recently reported strong genetic association of the pruning-regulating complement factor gene *C4A* with schizophrenia. This raises the exciting possibility that in conditions of hyperactive synaptic pruning, as supposed in schizophrenia, the inhibitory action of minocycline turns into a beneficial effect, with relevance for early therapeutic interventions. Altogether, these data support a differential view on microglia activation and its inhibition. Further studies are needed to clarify the relevance of these results for the pathogenesis of schizophrenia and the use of minocycline as antipsychotic drug.

Key words: minocycline/apoptosis/postnatal neurogenesis/subventricular zone/synaptic pruning/early therapy

More than 30 years ago signs of low-grade inflammation had been found post-mortem in brains of patients with schizophrenia.[1] Since then, accumulating evidence indicates a role of neuroinflammatory processes in schizophrenia. One of the earliest reported genetic associations found in schizophrenia was the major histocompatibility complex (MHC).[2] Meanwhile, this was confirmed by subsequent studies: in the largest genome-wide analysis of schizophrenia performed so far the most significant association was a locus on chromosome 6 that includes the MHC.[3] An important hallmark of neuroinflammation is the activation of microglia, which are resident macrophages and the main form of immune defense in the brain. Further support for increased activation of microglia in schizophrenia comes from recent translocator-protein [^{11}C]PBR28 positron emission tomography (PET) brain imaging data that demonstrated elevated microglial activity both in patients with schizophrenia and in persons at ultra-high risk of psychosis.[4] Altogether these results prompted high interest in understanding the pathophysiological mechanisms of neuroinflammation in schizophrenia and the identification of novel antipsychotics targeting inflammatory processes. In this respect, the antibiotic minocycline that effectively inhibits the activation of microglia[5] represents one alternative therapeutic option.

Minocycline is a semi-synthetic tetracycline marketed since 1966 that shows good diffusion into the brain. The therapeutic potential of minocycline in schizophrenia was serendipitously discovered in Japan in 2007 in a schizophrenic patient suffering from intercurrent pneumonia.[6] Subsequent clinical studies reported efficacy of minocycline especially in alleviating negative symptoms of schizophrenia when used as add-on therapy in combination with classical antipsychotics.[7–9] These findings are in line with antipsychotic-like effects of minocycline in animal models of schizophrenia, demonstrating, similar

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to haloperidol, improvement of the cognitive deficits induced by the NMDA receptor (NMDAR) antagonist MK-801.[10] Alleviation of protracted schizophrenia-like abnormalities (hyperlocomotion, sensorimotor gating, and social interaction deficits) by minocycline was described also in a mouse model of maternal immunization.[11] Moreover, minocycline may induce significant neuroprotective effects, as shown in mouse models of Huntington disease[12] and amyotrophic lateral sclerosis (ALS).[13] Hereby, minocycline acts not only by inhibiting microglia activation, but also by other mechanisms like the inhibition of pro-apoptotic caspases or cytochrome c release inhibition.[12,13] These beneficial effects raised hope in the use of minocycline in the treatment of neuropsychiatric disorders.[14]

In contrast to the beneficial action of minocycline in adulthood, several experimental studies demonstrated deleterious effects at early postnatal stages in rodents. Minocycline worsened hypoxic-ischemic brain injury in a perinatal mouse model.[15] Inactivation of microglia by minocycline treatment at the age of postnatal day 3 (P3) to P4 led to increased apoptosis, specifically in layer V of the perinatal cortex.[16] Moreover, minocycline-induced neuronal cell death, as assessed by caspase-3 expression, was found throughout all cortical layers[17] and also in other regions of the perinatal brain (hippocampus, nucleus accumbens).[18] Antagonists of NMDAR represent classical examples of potent inducers of cortical apoptosis during this developmental period.[19] Interestingly, the caspase-3 expression pattern triggered by minocycline was similar, but not identical to that exhibited by MK-801 being even more accentuated in some areas like the subiculum of the hippocampus.[18] Moreover, minocycline aggravated MK-801-induced cell death, an effect which is unusual for a substance with potential antipsychotic effects, considering that current neuroleptics like olanzapine significantly reduced apoptosis and long-term behavioral deficits triggered by early postnatal NMDAR blockade.[20] Up to date it is not known if the widespread perinatal neuronal apoptosis triggered by minocycline is followed by long-term effects with relevance for psychiatric disorders. This aspect would be of certain interest since early life MK-801 administration induces protracted behavioral abnormalities representing a classical developmental rodent model mimicking several features of schizophrenia.[21]

Minocycline affects not only neuronal survival, but also the generation of new neurons and glial cells in the early postnatal subventricular zone (SVZ) as shown in rats.[22] Interestingly, this effect appears to be not restricted to perinatal stages, since abundant accumulation of activated microglia occurs in the SVZ not only at early postnatal (P1–P10), but also at later, post-weaning stages (P30).[22] Minocycline decreased both neurogenesis/gliogenesis as well as the levels of several proinflammatory cytokines (ie, IL-1β, IL-6, TNF-α, IFN-γ) as measured in SVZ tissue lysates from P4 rats.[22] The most unexpected result of this study is that each cytokine induced differential effects on neurogenesis vs gliogenesis: in an in vitro model of coculture of neurospheres derived from embryonic neurons with microglia prepared from the cerebral cortex of P1 rats IL-1β and IFN-γ stimulated neurogenesis, whereas IL-1β and IL-6 enhanced oligodendrogenesis.[22] Moreover, some cytokines induced dose-dependent opposite effects: in cultured SVZ neural stem/progenitor cells derived from the neonatal (P1–P3) C57BL/6 mice exposure to 1 ng/ml TNF-α induced cell proliferation, whereas 10 and 100 ng/ml TNF-α induced apoptotic cell death.[23] As with the pro-apoptotic action, the long-term consequences of the deleterious effect of minocycline on postnatal SVZ neurogenesis/gliogenesis are not known. Recent data have demonstrated that the perinatal SVZ is an important reservoir of new neurons not only for the olfactory bulb, but also for the prefrontal cortex and striatum in rodents[24] and in humans.[25,26] Importantly, neurogenesis even persists throughout life in the striatum in humans.[26] One subpopulation postnatally-generated striatal neurons, the D3 dopamine receptor expressing granule neurons forming the Islands of Calleja, was proposed to play an important role in the generation of the striatal dopamine alterations associated with schizophrenia.[27,28] Therefore, factors that impair postnatal SVZ neurogenesis may affect the function of 2 brain regions tightly associated with schizophrenia (the prefrontal cortex and striatum).

Finally, a third effect of minocycline with potential implications for schizophrenia refers to the influence on the normal process of synaptic reorganization, the so-called synaptic pruning. One surprising finding in the last years was that the complement system modulates not only the immune response, but also developmental synaptic remodeling.[29] Microglia are the main cells in the brain expressing receptors for the complement system and drive synaptic pruning by engulfing and eliminating extranumerary synapses.[29] Minocycline significantly alters microglia-mediated engulfment and synaptic pruning in the retinogeniculate system at early postnatal stages.[30] However, this effect can be extrapolated also to adolescent stages, when most synaptic reorganization takes place in the cerebral cortex.[31] This appears very interesting, since abnormal synaptic pruning that was proposed in 1982 by Feinberg as main pathophysiological mechanism in schizophrenia.[32] Moreover, it raises the question, how these data can be reconciled with the proposed antipsychotic action of minocycline. Hereby one very interesting possibility is that in schizophrenia the effect of minocycline on synaptic pruning may not be deleterious, but could be on contrary even beneficial. This could be the case if one considers the recently described genetic variants in the complement system leading to overexpression of the *C4A* complement factor gene, as strong correlate of increased risk of schizophrenia.[33] Minocycline would soften/counteracted the hyperactive synaptic

pruning thought to occur in schizophrenia. However, the existence of such an effect is at present just hypothetical and needs to be verified in future, since it may have immediate relevance for early therapeutic interventions. From the perspective on a direct effect on the supposed hyperactive synaptic pruning in schizophrenia, minocycline would be effective in early phases of schizophrenia (onset of the disease, transition from high risk status to clinical manifestation), counteracting the putative causative pathological mechanism. However, we cannot exclude that minocycline could be effective also in later stages of schizophrenia and also by other mechanisms, as proposed previously, eg, acting on AMPA receptors.[34]

In sum, increasing evidence indicates that the activation of microglia and the so-called proinflammatory cytokines regulate not only neuroinflammation, but also important physiological processes during brain development.[35] These data are of potential importance for schizophrenia considering for example the implication of microglia in regulating several developmental processes like synaptic pruning in the juvenile brain. Hereby, we have to better understand the role of microglia not only in the normal brain, but also in conditions of a hyperactive complement system, as recently associated with schizophrenia.[33] Future studies may analyze also the contribution of other factors, like brain-derived neurotrophic factor (BDNF) that was proposed as link between microglial activation and neuroplasticity.[36]

On the other hand, a more differentiated view on microglia activation as potential therapeutic target ie, on the beneficial vs deleterious effects of minocycline is needed. We have to determine also the translational value of results obtained in the rodent model, before postulating cautiousness regarding the clinical use of tetracyclines. Doxycycline, which belongs to the same group as minocycline, do not to display any visible neurodevelopmental disturbances following its use in pregnancy and early childhood.[37] Hereby it is important to mention that possible differences between animal model experiments and human studies may be influenced by several factors, like possible differences in dosing, routes of administration, and the generation of species-specific metabolites. Therefore, we think that at the present time-point it is premature to make definitive statements regarding the safety of the use of these drugs in children or the monitoring of their use. Hereby, more investigations, including clinical studies and analysis in non-human primates are needed to establish possible deleterious effects with relevance for their pediatric use. Additionally, an important research goal would be to develop novel drugs with less side-effects; eg, since the immune modulatory and pro-apoptotic effects of minocycline may have different mechanisms, polycyclic compounds which have immune modulatory activity in the absence of pro-apoptotic effects may represent a suitable alternative.

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