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Different influence of antipsychotics on the balance between pro- and anti-inflammatory cytokines depends on glia activation: An in vitro study

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ABSTRACT

The microglial hypothesis of schizophrenia suggests that its neuropathology is closely associated with neuroinflammation manifested, *inter alia*, by an increased expression of cytokines. However, clinical investigations imply that schizophrenia is a heterogeneous disease and in some groups of patients the activated inflammatory process does not contribute to the disease-associated impairment of brain function. Clinical studies revealed also an equivocal impact of antipsychotics on peripheral and CSF cytokines, whereas experimental research performed on the stimulated glia cultures showed their inhibitory effect on pro-inflammatory cytokine levels. In the present study, the effect of chlorpromazine, haloperidol and risperidone (0.5, 5 or 10 μM) on production of pro-inflammatory cytokines IL-1β and TNF-α and anti-inflammatory IL-10 was investigated in the unstimulated and lipopolysaccharide-stimulated primary rat mixed glial cell cultures. In the unstimulated cultures, haloperidol at all applied concentrations, risperidone at 5, 10 μM and chlorpromazine at 10 μM increased IL-10 levels in the culture supernatants without a significant influence on IL-1β or TNF-α levels, and all drugs applied at 10 μM induced a robust increase in IL-10 mRNA expression. Under strong inflammatory activation, haloperidol and risperidone at all concentrations reduced production of both pro-inflammatory cytokines, without adverse effects on IL-10 expression when used at 10 μM. Chlorpromazine at all concentrations diminished the production of three cytokines and did not induce anti-inflammatory effect. These results suggest that dependently on glia activation antipsychotics via different mechanisms may induce anti-inflammatory effect and that this activity is not common for all drugs under conditions of strong glia activation.

1. Introduction

Apart from the well-evidenced disturbances in cerebral dopaminergic and glutamatergic transmission, a role of immune dysfunction in the schizophrenia pathophysiology is also postulated [1]. It is known that nervous and immune systems interact with each other and that cytokines play a pivotal role in mediating the cross-talk between them. Cytokines include interleukins (ILs), interferons (IFNs), tumor necrosis factors (TNFs), transforming growth factors (TGFs) and chemokines. In the central nervous system (CNS), cytokines produced predominantly by the activated microglia influence neurotransmitter function, neurocircuitry, neurogenesis and neuroplasticity whereas cytokines synthesized peripherally communicate with the CNS mainly indirectly through different pathways [2]. However, clinical studies conducted in various models: post-mortem studies [3], positron emission tomography (PET) imaging of the 18 kDa translocator protein (TSPO), a marker of activated microglia [4–8] and analysis of cerebrospinal fluid (CSF) inflammatory markers [9] brought inconsistent results showing an increased or unchanged expression of microglial markers in some groups of patients. Moreover, findings from the studies concerning the effects of antipsychotic drugs on peripheral cytokines in humans indicated their pro- or anti-inflammatory activity [10]. In addition, experimental data obtained in investigations on the influence of antipsychotics on pro-inflammatory cytokine (IL-1β, TNF-α, IL-6, IL-2) or NO production by lipopolysaccharide (LPS)- or IFN-γ-stimulated microglia cell lines or the mixed primary glia cell cultures unequivocally suggested their immunosuppressive activity [11–16]. Taking into consideration all the above-mentioned data, in the present study, the effect of typical antipsychotics: haloperidol, chlorpromazine and atypical risperidone on the expression of two pro-inflammatory cytokines: IL-1β, TNF-α and anti-inflammatory IL-10 was investigated in unstimulated and LPS-stimulated primary rat mixed glial cell cultures.

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Determination of the simultaneous effects of antipsychotics on the production of pro- and anti-inflammatory cytokines seemed to be required to answer the question how they affect the balance between both groups of cytokines with opposite properties. Until now anti-inflammatory properties of these drugs have been the subject of a few experimental in vivo studies concerning their effects on peripheral production of IL-10 [17–19] or brain IL-10 and TGF-β production [20]. Furthermore, in the present study, for the first time, the effect of antipsychotics on cytokine production was estimated under conditions of strong glia activation induced by LPS and slight glia activation caused by cell preparation and culture procedure confirmed by microscopic observations. LPS is an exogenous immunostimulator, the so-called "golden standard of inflammagens" frequently used in in vitro studies to activate glial cells which respond strongly to the LPS challenge with the secretion of cytokines, among the others [21]. In our previous experiments performed on rat mixed glial cultures, LPS concentration and time of incubation required for the maximal stimulation of cytokine secretion were determined [22]. A better understanding of the capacity of antipsychotics to influence cytokine expression in a more complex experimental model seems to be important because the control of neuroinflammation has been postulated to be a target for treatment of schizophrenia and psychotic disorders [1].

2. Materials and methods

2.1. Materials

LPS (*Escherichia coli* serotype 0111:B4), haloperidol and chlorpromazine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Risperidone was kindly donated by LEK-AM (Poland). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin and fungizone were purchased from Gibco-BRL (USA) while TRIzol from Invitrogen (USA). Sterile cell strainers and 96-well culture plates were obtained from Becton-Dickinson (San Jose, CA, USA). Agglutinin-1 was purchased from Vector Laboratory (Burlingame, CA, USA). Anti-GFAP (anti-glial fibrillary acidic protein) antibodies were purchased from Sigma-Aldrich (St Louis, MO, USA). Anti-MAP-2 (anti-mitogen activated protein-2) antibodies were obtained from Promega (USA).

2.2. Primary mixed glial culture

Mixed glia were prepared from the cerebral hemispheres of one day old Wistar rats as described previously [23]. In brief, brains were removed aseptically, separated from the blood vessels and membranes and were disrupted by mechanical triturating in ice-cold DMEM with 20% heat inactivated FBS containing 100 UI/ml penicillin, 100 μg/ml streptomycin and 25 μg/ml fungizone. After filtration through sterile cell strainers (70 μm and 10 μm), 0.1 ml of cell suspension (1.4×10^6 cells/ml) was poured into wells of 96-well culture plate or was plated in 35-mm Petri dishes (1.4×10^6 cells/dish) and then cultured in the standard condition (37 °C; 95% air and 5% CO_2). The medium was replenished on day 1 after plating and then every 3rd day with medium supplemented with 10% FBS and the above-mentioned antibiotics. The experiments were conducted on 13-day cultures which contained about 60–65% microglia (*Ricinus communis* agglutinin-1 positive cells) and 30–35% astrocytes (GFAP-immunoreactive cells). No neurons were detected (MAP-2-positive cells). The 13-day mixed glial cultures contain the mature microglia cells being the most reactive to LPS and immature astrocytes which become mature on ca. 21st day of culture. Observation under an inverted fluorescence confocal microscope Olympus X70 showed that in the unstimulated cultures mainly ramified resting forms and some activated round-shaped forms of agglutinin-1-positive cells were present. After LPS stimulation (2 μg/ml for 48 h), the stained cells were round and enlarged without any visible processes (Fig. 1A and B).

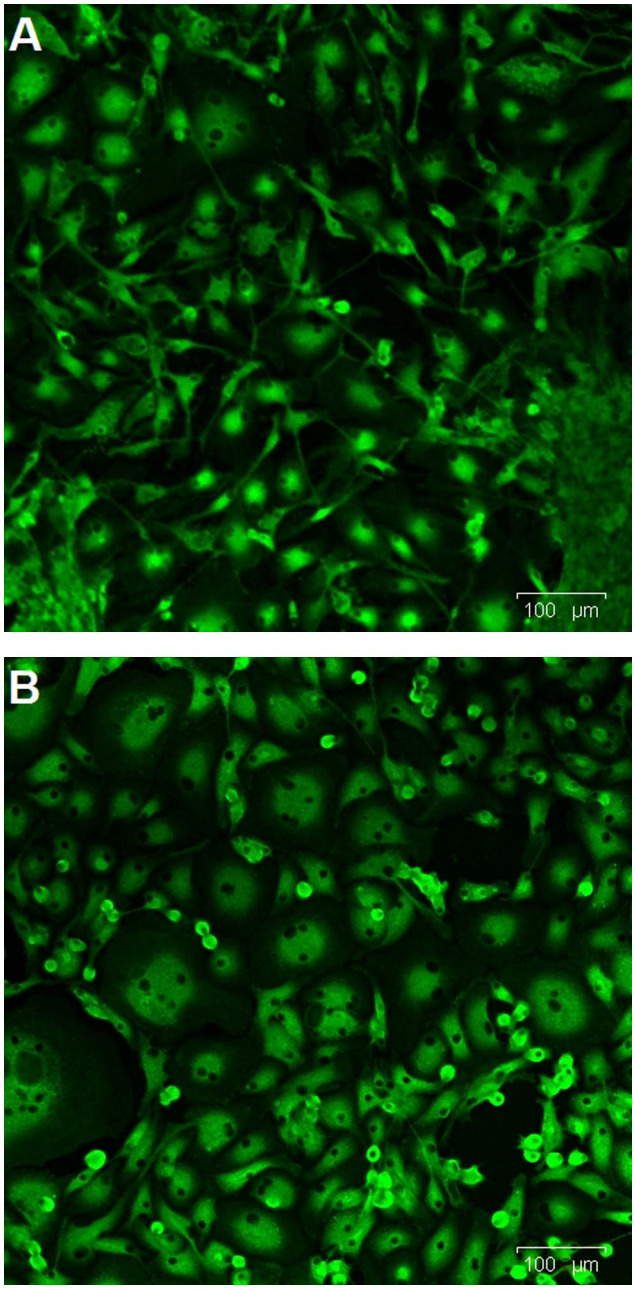

Fig. 1. The representative phase contrast image of mixed glial cell cultures. Microglia cells were stained with a specific marker *Riccinus communis* agglutinin-1 (green fluorescence) In unstimulated cultures mainly ramified forms with pseudopodia and some activated round-shaped cells are present (A). Cultures exposed to LPS (2 μg/ml for 48 h) are dominated by activated cells (B) (magnification, x200) scale bar, 100 μm (for interpretation of the reference to color, the reader is referred to the web version of the article).

2.3. Drug treatment

Chlorpromazine hydrochloride, haloperidol or risperidone were initially dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) to obtain a concentration of 2.4 mM and then were diluted with culture medium to achieve the final concentration of 0.5, 5, 10, 20 or 30 μM. At these concentrations, the effect of antipsychotics on cell viability was evaluated. The highest concentration of DMSO was 0.1%. In experiments concerning the influence of antipsychotics on cytokine release, the studied drugs were applied at a concentration of 0.5, 5 or 10 μM. On