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NEUROPROTECTIVE EFFECT OF CURCUMIN ON LPS-ACTIVATED ASTROCYTES IS RELATED TO THE PREVENTION OF GFAP AND NF-KB UPREGULATION

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We examined the effects of 2.0 μM curcumin, a known antioxidant, on the cultures of normal rat astrocytes and of those subjected to the action of 0.01 μM lipopolysaccharide, LPS. As was found, 24-h-long exposure of astrocytes to LPS resulted in a relatively mild decrease in the cell viability of astrocytes (a decrement of 22%). The action of curcumin significantly ameliorated this decrease. LPS induced significant upregulation of GFAP, NF-κB, and poly(ADP-ribose)polymerase (PARP) in activated astrocytes. Curcumin significantly decreased these effects (to 60, 71, and 89% as compared to those in astrocytes subjected to isolated action of LPS). Thus, curcumin significantly prevents the decrease in cell viability and moderates the cytoskeleton rearrangements and disorders in NF-κB-dependent regulation in astrocytes activated by LPS.

Key words: activated astrocytes, GFAP, NF-κB, curcumin, neuroprotection.

INTRODUCTION

Neurodegenerative diseases are, in general, characterized by inflammation, growth of oxidative damages, and decreased cell viability resulting from disorders in the cell regulatory pathways. Pathogenesis of these diseases is closely related to dysfunctions of glial cells [1]. Astrocytes, the most numerous glial cell population, play a crucial role in the maintenance of homeostasis and neuronal survival in the CNS. Overactivation of astrocytes is a molecular and metabolic marker of a number of CNS pathologies; this is accompanied by the overproduction of glial fibrillary acidic protein (GFAP) and secretion of various cytokines. The cytokine secretion stimulates endothelial cells in the brain vessels, which disturbs signalization from and feedback relations with the surrounding astrocytes [2]. The astrocyte reactivity initiates an inflammatory response, which leads to intensification of neuronal death and is critical with respect to both neuroprotection and CNS cell damage [3]. Antioxidants are capable of modulating the cell reactivity via anti-inflammatory signaling pathways. Curcumin, an agent in widely used natural food product (spice) was found to be a potent antioxidant. Most studies on the effects curcumin have been focused on the respective protective influences on different normal and cancer cells, while the effects of curcumin on astrocytes were examined only in single works. The role of intermediate filaments of astrocytes in the astroglial reactivity remains practically unknown.

Considering the dual role of astrocytes in triggering the glial reactivity (from neuroprotection to increased cell damage), we investigated the effects of curcumin on the reactivity of primary rat astrocytes using lipopolysaccharide (LPS)-induced inflammatory events in an *in vitro* model.

METHODS

Primary astrocyte cell cultures were obtained using a most widely accepted technique, from newborn (1-day-old) rats [4]. Cultured cells were divided into four groups: (i) intact control (Contr), (ii) astrocytes stimulated with 0.01 μM LPS (LPS), (iii) intact astrocytes treated with 2.0 μM curcumin (Curc), and (iv) LPS-stimulated astrocytes treated with curcumin in the above concentration (LPS+Curc). Exposures to both LPS and curcumin were 24 h long. After this, cells of all four groups

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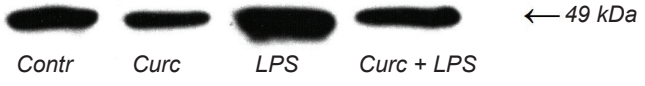
were collected by scratching from the medium without trypsinization. Activation of astrocytes was examined with Western blot as levels of expression of GFAP, transcriptional factor NF-κB, and poly(ADP-ribose)polymerase (PARP). The blot results were analyzed using densitometry (software TotalLab TL120, USA) and normalized with respect to the intensity of the bands obtained for β-actin. Statistical analysis was performed using Excel (Student's *t*-test) and SPSS (MANOVA).

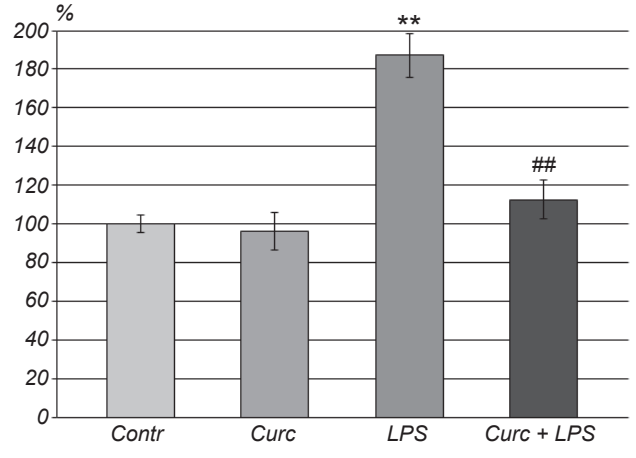
RESULTS AND DISCUSSION

Cultured primary astrocytes treated with 0.01 μM LPS demonstrated a mild but significant ($P < 0.05$) lowering of the cell viability index (22% as compared to the untreated Contr group). Astrocytes subjected to isolated action of 2.0 μM curcumin showed a small (statistically insignificant, $P > 0.05$) decrease in the viability (11% vs. Contr). At the same time, LPS-stimulated astrocytes treated with curcumin (LPS+Curc group) were characterized by a smaller decrement of the cell viability (also statistically insignificant, $P > 0.05$) compared to that in the LPS group.



F i g. 1. Western blot of GFAP in cultured control (Contr) and treated primary rat astrocytes. Curc is 2.0 μM curcumin; LPS is 0.01 μM LPS; LPS+Curc corresponds to combined treatment with LPS and curcumin.

Р и с. 1. Результати Вестерн-блотингу ГФКП у культивованих первинних астроцитах щура.



F i g. 2. Normalized content of GFAP in cultured rat astrocytes. Designations are similar to those in Fig. 1. $^{**}P<0.01$ in comparison with Contr; ## $P<0.01$ in comparison with LPS

Р и с. 2. Нормовані значення рівнів ГФКП у культивованих первинних астроцитах щура.

In primary astrocytes stimulated with LPS, we found dramatically increased GFAP expression (by 87%, on average, as compared to the Contr group; $P < 0.05$). Similar shifts were observed after 24-h exposure of astrocytes to LPS in the NF-κB and PARP contents (75 and 62%, respectively, as compared to the untreated control). The treatment of non stimulated rat astrocytes with 2.0 μM curcumin resulted in minor insignificant ($P > 0.05$) decreases in the contents of the three studied proteins. A maximal respective shift was observed for NF-κB (14%). At the same time, considerably more substantial effects of curcumin were found with respect to LPS-stimulated astrocytes. If to take increments in the contents of the above three proteins in the LPS group as 100%, the respective shifts in the LPS+Curc group were only 60, 71, and 89%. Therefore, curcumin considerably prevented LPS-induced upregulation of GFAP, NF-κB ($P < 0.05$), and, to a lesser extent, of PARP.

Our results are quite comparable with the recent data on the effects of different doses of curcumin on human astrocytes; there was no significant increase in the cell death index under the action of 5 μM curcumin [5].

NF-κB belongs to a family of transcription factors that is greatly responsible for the maintenance of cell survival and controls diverse biological processes, including inflammatory and apoptotic responses. Recent data demonstrated that curcumin can beneficially affect astrocyte populations in the CNS under conditions of a neuroinflammatory environment [6]. Pimentel-Gutierrez et al. found that curcumin downregulated the NF-κB in REH acute lymphoblastic leukemia cells measured using flow cytometry with anti-phospho-NF-κB p65 antibody [7].

The effect of curcumin on NF-κB expression observed in our study may be implemented due to a decrease in the ROS level and modulation of PARP activity. Our data comply with the results of Kauppinen et al., who showed that PARP-induced changes in the NAD^+ levels can modulate NF-κB transcriptional activity through the effects on p65 acetylation [8].

The PARP expression leads to increased consumption of metabolic energy and, as a result, is followed

by decreased cell viability. Precisely this situation was observed in our experiments in the LPS group; upregulation of PARP in LPS-stimulated astrocytes correlates with a noticeable drop in the cell viability. The treatment of reactive astrocytes with curcumin provided a clear neuroprotective effect; the decrement of the viability index in the LPS-Curc group was considerably smaller than that in the LPS group. Isolated action of 2.0 μM curcumin on nonactivated astrocytes provided nearly negligible shifts in the contents of GFAP, NF-κB, and PARP. At the same time, such treatment of LPS-activated astrocytes significantly ameliorated increases in the expression of these proteins. Therefore, the positive neuroprotective effect of curcumin with respect to LPS-affected astrocytes may be related to the above-mentioned amelioration of expression of the three regulatory proteins.

The observed protective effects of curcumin are probably based on the antioxidant and antiproliferative ability of this natural polyphenol. The increase of ROS production in LPS-activated astrocytes switches-in numerous mechanisms controlling transcriptional factors, including NF-κB, and, as a result, intensifies the cytokine secretion. Both these effects (ROS hyperproduction and facilitation of the cytokine release) determine excessive cell reactivity accompanied by a number of molecular damages and the development of cell dysfunctions. It should be taken into account that the action of 2 μM curcumin on normal astrocytes did not induce any antiproliferative effect. At the same time, treatment of LPS-stimulated astrocytes with the above dose of curcumin significantly decreases the NF-κB and PARP levels (potent regulators of cell reactivation). Thus, the antiproliferative proprieties of curcumin on reactive astrocytes are based on partial neutralization of NF-κB and PARP expressions.

It is obvious that LPS induces significant GFAP upregulation in cultured astrocytes, and this is the most obvious sign of reactivation of these cells. The abnormally increased GFAP expression is associated with reconstruction of the astrocyte cytoskeleton. Curcumin exerts a clear normalizing effect on the GFAP expression in LPS-stimulated astrocytes.

Taken together, our data demonstrated that a relatively low dose (2 μM) of curcumin exerts a complex positive influence; it ameliorates an abnormal reactivity of primary rat astrocytes subjected to the action of LPS. This complex action includes positive effects on the cell viability, rearrangements of the cytoskeleton, and transcriptional regulation of the cell response.

All stages of the study were in accordance with the provisions of the European Convention for the Protection of Animals used in Experimental Trials (86/609 / EEC, 1986, Strasbourg) and the norms of the Bioethics Committees in the both mentioned universities.

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НЕЙРОПРОТЕКТОРНИЙ ВПЛИВ КУРКУМІНУ НА АКТИВОВАНІ ЛІПОПОЛІСАХАРИДОМ АСТРОЦИТИ БАЗУЄТЬСЯ НА ПРОТИДІЇ ПОСИЛЕНІЙ ПРОДУКЦІЇ ГФКП ТА NF-κB

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Р е з ю м е

Ми досліджували впливи відомого антиоксиданта куркуміну (2.0 мкМ) на культивовані нормальні астроцити щура та астроцити, піддані дії 0.01 мкМ ліпополісахариду (LPS). Виявилося, що 24-годинна експозиція астроцитів із LPS призводить до відносно помірного зменшення життєздатності астроцитів (декремент у середньому 22 %). Дія куркуміну зумовлювала вірогідне зменшення такої втрати життєздатності. Аплікація LPS індукувала вірогідне збільшення рівнів ГФКП, NF-κB та полі(АДП-рибозо)полімерази (ПАРП) в активованих астроцитах. Куркумін значно зменшував такі ефекти (до 60, 71 та 89 % у порівнянні з відповідними значеннями в астроцитах, підданих ізольованій дії LPS). Отже, куркумін істотно протидіє зменшенню життєздатності клітин, перебудовам клітинного скелета та розладам NF-κB-залежної регуляції в астроцитах, активованих під дією LPS.

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