UDC 616.894–092:615.3 doi: http://dx.doi.org/10.15407/ubj88.01.109

EFFECT OF β-AMYLOID PEPTIDE 42 ON THE DYNAMICS OF EXPRESSION AND FORMATION OF $Aβ_{40}$, IL-1β, TNFα, IL-6, IL-10 BY PERIPHERAL BLOOD MONONUCLEAR CELLS *IN VITRO* AND ITS CORRECTION BY CURCUMIN

V. V. SOKOLIK[1], O. K. KOLIADA[2], S. M. SHULGA[3]

[1]SI Institute of Neurology, Psychiatry and Narcology, National Academy of Medical Sciences of Ukraine, Kharkiv;
[2]SI D. F. Chebotarev Institute of Gerontology, National Academy of Medical Sciences of Ukraine, Kyiv;
[3]SI Institute for Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kyiv;
e-mail: sokolik67@rambler.ru

The toxic effect of Aβ-oligomers accompanies chronic inflammation, with cytokines as main mediators. Therefore, the cytokine link of inflammation becomes a new target on the way to restrain amyloidosis. The aim of the study was the effect of aggregated $Aβ_{42}$ on the dynamics of expression and formation of endogenous $Aβ_{40}$ and cytokines (IL-1β, TNFα, IL-6, IL-10) by peripheral blood mononuclear cells in vitro and its correction by curcumin. A suspension of mononuclear cells isolated ex tempore using ficoll-urografin gradient from venous blood samples of healthy volunteers were used to study the effects of $Aβ_{42}$ (15 nM), curcumin (54 pM), and their combined action (at similar concentrations) in time dynamics: 0, 1, 3, 6 and 24 h incubation at 37 °C. Polymerase chain reaction with appropriate primers was used to determine the relative expression of mRNA for AβPP, TNFα, IL-1β, IL-6, IL-10 and enzyme-linked immunosorbent assay – to determine the content of $Aβ_{40}$ and cytokines in mononuclear suspension during all periods of incubation. The individual dynamics AβPP and cytokine expression was shown under the action of the $Aβ_{42}$, which had influence on the content of $Aβ_{40}$, TNFα, IL-1β, IL-6 and IL-10 in mononuclear suspension. Curcumin displayed the inhibitory effect on gene expression of AβPP, TNFα and IL6, which resulted in the decrease of the level of these two cytokines and $Aβ_{40}$. Thus, the dynamics of anti-inflammatory effect of curcumin in vitro for transcriptional and translational levels of cytokine's formation by mononuclear cells was shown in the work. Direct inhibitory effect of curcumin on the concentration of endogenous $Aβ_{40}$ during the 24 h incubation in conditions of toxic action of $Aβ_{42}$ aggregates was established.

K e y w o r d s: curcumin, β-amyloid peptides 40 and 42, cytokines, mRNA, human peripheral blood mononuclear cells.

The family of β-amyloid peptides (Aβ) includes peptides with 38-43 amino acid residues (a. r.) being a product of amyloidogenic processing of the precursor protein of β-amyloid peptide (AβPP). Soluble $Aβ_{40}$ in standard conditions performs a trophic function in neurons [1] and only with manifestation of protein conformation disease (amyloidosis) its aggregates acquire toxic properties [2]. The Aβ isoform with 2 additional a. r. at C-end of this peptide (Aβ42) is more aggregation aggressive; the above acid residues increase its hydrophobicity and thus determine high bias towards formation of insoluble fibrils [3]. β-Amyloid peptides acquire toxic properties owing to their own oligomerization. Rather soluble Aβ oligomers than their monomers or insoluble amyloid fibrils are responsible for neurotoxicity and synaptic dysfunction under amyloidosis [4].

The toxic effect of Aβ-system which produces the excessive number of inflammation cytokines – interleukins-1β, -6, -8 (IL-1β, -6, -8), tumor necrosis factor α (TNFα), etc. [5], accompanies the course of chronic inflammatory process, cytokines being its main mediators. Hyperactivity of mononuclear-phagocytic link of the immune system appears as independent motive force of amyloidosis process. It has

been found out that cytokines affect metabolism of AβPP, activating its synthesis and processing in amyloidogenic way with formation of Aβ. IL-1 and IL-6 can regulate AβPP synthesis both transcriptionally and translationally, depending on the cell type [6-7]. Certain combinations of cytokines display additive effect on AβPP metabolism: combinations of TNFα and interferon γ (IFNγ) or IL-1β + IFNγ increase essentially Aβ secretion, while used separately they have no effect [8]. Thus, the cytokine link of inflammatory process becomes a new target on the way of amyloidosis suppression.

Polyphenols draw attention among natural substances with anti-inflammatory properties [9]. Curcumin [1,7-bis(hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dion] is one of the most studied representatives of this class compounds. The system bioavailability of curcumin is very low that is explained by its insolubility in water and quick metabolism in the liver, kidneys and gastric wall. Despite that, curcumin is considered one of the most promising candidates of natural origin having anti-inflammatory influence without side effects [10]. It has been established that the mechanism of anti-inflammatory activity of curcumin includes:

• activation inhibition of the transcription factor NFκB which regulates expression of pro-inflammatory gene products [11];

• down-regulation of expression of cyclooxygenase-2 (COX-2) [12];

• expression inhibition of inflammatory cytokines, including TNFα, IL-1β, IL-6, IL-8, chemokines [13].

The inhibiting effect of curcumin on the nuclear factor κB (NFκB)-signal path occupies a central place in ensuring its anti-inflammatory properties. Curcumin blocks IκB-mediated phosphorylation and degradation of inhibitory protein IκBα, as a result NFκB remains in the complex with IκBα in cytoplasm and cannot enter a nucleus to activate the transcription. Investigations of the suppression of NFκB activity have demonstrated further down-regulation of COX-2 and inducible NO-synthase, as well as a decrease of synthesis of inflammatory markers [14]. In correspondence with its effect on NFκB curcumin inhibits production of inflammatory cytokines, including IL-1, IL-2, IL-6, IL-8, IL-12 and others, by mononuclears or macrophages of peripheral circulation [15].

Microglia is a derivative of bone marrow monocytes. It consists of a network of immunoprotective cells which respond to impulse activity of neurons and mediate neuro-immune interaction [16]. That is why the modeling of the effects of β-amyloid peptide 42 and curcumin on monocyte suspension *in vitro* corresponds to the effect of these factors in the brain microglia *in vivo*.

The work aim was to investigate the effect of aggregated $A\beta_{42}$ on the expression dynamics and formation of endogenous $A\beta_{40}$ and cytokines (IL-1β, TNFα, IL-6, IL-10) by mononuclears of the peripheral blood *in vitro* and correction of such effect by curcumin.

Materials and Methods

The work has been made in accordance with *Universal Declaration on Bioethics and Human Rights* (UN, 1997). Mononuclear cells of human peripheral blood were isolated ex tempera with the help of ficoll-urografin gradient from the samples of venous blood of three healthy volunteers, separately. After triple washing of cell suspension with sterile physiologic solution of room temperature the mononuclears were resuspended in RPMI medium to concentration of 2×10^6 cells/ml. The RNAse inhibitor was added to RPMI to prevent mRNA degradation. Thus the obtained samples of suspensions of mononuclear cells were used to investigate the effect of β-amyloid peptide 42 (in a dose of 15 nM), curcumin (in a dose of 54 nM) separately and their united action (at analogous concentrations). The results were standardized by the corresponding indices of cell suspension which was added only 0.9% NaCl. The ratio of volumes of mononuclear suspension and corresponding impurities (curcumin, $A\beta_{42}$, 0.9% NaCl) was 100:1.

Aβ42_Human (Human Amyloid β Protein Fragment 1-42, Sigma-Aldrich), dissolved in bidistilled water, was aggregated during 24 h at 37 °C. Big coarse conglomerates of Aβ42_Human were dispersed with ultrasound and sterilized directly before adding. Since curcumin is low-soluble in water, the concentrated output curcumin solution was first prepared in 96% ethanol, diluted by bidistilled water about 0.7 g/l (directly before adding) to mononuclear suspension.

The dynamics of $A\beta_{42}$ and 0.9% NaCl effect on mononuclears was studied at the 0, 1, 3, 6 and 24th hours of their incubation at 37 °C and stirring rate 600 rpm. The dynamics of curcumin effect and joint action of $A\beta_{42}$ + curcumin was studied at the 3, 6 and 24th hours of incubation, thereat curcumin was

added after 1 h incubation of mononuclear suspension with $A\beta_{42}$ or with 0.9% NaCl. In corresponding terms of incubation the aliquots of suspensions were taken and cells were ruined under three-minute action of ultrasound with frequency 2.64 MHz and intensity 0.25 W/cm^3 in the device MUSSON-1. After that the samples were centrifuged at 6 000 rpm during 20 min, and supernatant was used for further measurements.

Relative expression at mRNA level for *AβPP*, *TNFα*, *IL-1β*, *IL-6* and *IL-10* genes was determined in mononuclears by the method of polymerase chain reaction (PCR) with the help of corresponding primers in all terms of incubation. RNA was isolated from cells using a set "RIBO-sorb" "AmpliSens" (RF). Gene expression analysis was performed by PCR method in real time. Complementary DNA (cDNA), obtained in the course of the reverse transcription reaction using a set "AmpliSens" (RF), were used as a matrix. Rotor-Gene Q (QIAGEN) (Germany) and specific primers produced by Sintol (RF) were used for amplification:

AβPP (NM_000484):
forward 3′-AACCAGTGACCATCCAGAAC-5′,
reverse 3′-ACTTGTCAGGAACG AGAAGG-5′,

IL-1β (NM_000576.2):
forward 3′-ACAGATGAAGTGCTCCTTCCA-5′,
reverse 3′-GTCGGAGATTCGTAGCTGGAT-5′,

TNFα (NM_000594.2):
forward 3′-CCCAGGGACCTCTCTCTAATC-5′,
reverse 3′-ATGGGCTACAGGCTTGTCACT-5′,

IL-6 (NM_000600):
forward 3′-GGTCTTTGCTGCTTTCACAC-5′,
reverse 3′-GGTACATCCTCGACGGCATC-5′,

IL-10 (NM_007527.3):
forward 3′-CATCGATTTCTTCCCTGTGAA-5′,
revese 3′-TCTTGGAGCTTATTAAAGGCATTC-5′,

ACTB (NM_001101.3):
forward 3′-GGATGCAGAAGGAGATCACTG-5′,
reverse 3′-CGATCCACACGGAGTACTTG-5′.

The amplification reactions were conducted as triplets for each gene in the following conditions: 60 s at 95 °C, 40 cycles: 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C using SYBR Green mix (Amplisence, RF). To the reaction mixtures were added the following concentrations of $MgCl_2$ (AmpliSence, RF): 1 mM for amplification of gene *AβPP*, 2 mM for amplification of genes *IL-1β*, *IL-6*, *IL-10* and *ACTB*, 4 mM for amplification of gene *TNFα*. The expression level was determined by Ct delta method. The data obtained were standardized by the expression of referent gene *ACTB* (for β-actin) in the form of the relation of the number of cDNA copies of the determined factor to the number of cDNA copies of *ACTB* [17] and were expressed in conventional expression units (c.e.u.).

ELISA determined the concentration of cytokines in supernatants of mononuclear suspension in correspondence to the protocol of Vector-Brest, RF for IL-1β, IL-6, IL-10 and TNFα, and also $A\beta_{40}$ by the set ELISA Kit Human Aβ40, Invitrogen Corporation. Optical density was read by microplate analyzer GBG Stat FAX 2100 (USA) at 450 nm with wavelength correction at 630 nm. ELISA data were computed for total protein (ng/g of protein). Results were presented on Figures in percentage of the indices of mononuclear suspensions, which had been incubated with 0.9% NaCl. Total protein concentration was determined by the Lowry method [18].

The results were processed statistically, mean values and standard deviations for mononuclear suspension indices were calculated. Statistical analysis of differences was carried out using Student's *t*-test. The value $P < 0.05$ was considered significant.

Results and Discussion

Table 1 data illustrate the absence of dynamics of the basal level of mRNA expression of *AβPP* and cytokines (*IL-1β*, *TNFα*, *IL-6*, *IL-10*) in mononuclears under the effect of 0.9% NaCl, except for inconsiderable, in view of the research objective, variation of $mRNA^{TNF\alpha}$, since the latter is connected with nonspecific circadian fluctuations of gene *TNFα* expression in mononuclear suspension *in vitro* [19, 20].

In contrast to basal expression level of mRNA under study, concentrations of $A\beta_{40}$ and cytokines (IL-1β, TNFα, IL-6, IL-10) in the mononuclear suspension under the effect of 0.9% NaCl changed in different directions during 24 h (Table 2).

The increase of content of endogenous $A\beta_{40}$ for the 6th hour of incubation with the absence of AβPP expression activation may be explained by intensification of amyloidogenic processing of already existing molecules of the precursor protein of β-amyloid peptide. As to dynamics of the basal level of cytokines (spontaneous production) in mononuclear suspension (Table 2), a probable decrease of IL-1β concentrations (for the 1 and 24th h), IL-6 (1-3th h) and IL-10 (1st h) explains the degradation of those peptides with a short half-life (<0.5 h) [21],

Table 1. Basal level of mRNA expression of AβPP and cytokines (IL-1β, TNFα, IL-6, IL-10) in mononuclears under the effect of 0.9% NaCl during 24 h

mRNA for	Incubation time (h)				
	0	1	3	6	24
AβPP	0.30 ± 0.06	0.44 ± 0.17	0.40 ± 0.06	0.39 ± 0.07	0.30 ± 0.15
IL-1β	2.41 ± 0.22	2.35 ± 0.23	2.75 ± 0.34	2.76 ± 0.26	2.76 ± 0.27
TNFα	3.39 ± 0.61	2.74 ± 0.78	3.02 ± 0.13	2.37 ± 0.18*	3.14 ± 0.31
IL-6	2.10 ± 0.48	1.98 ± 0.17	2.66 ± 0.48	1.19 ± 0.47	1.41 ± 0.49
IL-10	4.39 ± 0.29	4.18 ± 0.43	3.66 ± 0.48	4.11 ± 0.31	5.03 ± 0.58

* $P < 0.05$ compared to preincubation (0 h). Conventional expression unit

Table 2. Basal level of concentration of $A\beta_{40}$ and cytokines (IL-1β, TNFα, IL-6, IL-10) in mononuclear suspension under the effect of 0.9% NaCl during 24 h

Index	Incubation time (h)				
	0	1	3	6	24
$A\beta_{40}$	67 ± 6	75 ± 8	78 ± 9	128 ± 10*	57 ± 6
IL-1β	859 ± 87	610 ± 60*	745 ± 75	903 ± 90	608 ± 62*
TNFα	42 ± 4	96 ± 10*	178 ± 19*	286 ± 29*	133 ± 15*
IL-6	755 ± 80	302 ± 29*	493 ± 57*	662 ± 72	875 ± 88
IL-10	63 ± 7	36 ± 4*	53 ± 6	57 ± 6	56 ± 5

* $P < 0.05$ compared to preincubation (0 h). Nanograms per 1 g of protein.

while the increase of TNFα content on the background of the corresponding inhibition of expression (Table 1) probably owes to enzymatic activity of TNFα-converting enzyme (ADAM17) [22].

β-Amyloid peptide 42 in aggregated form did no effect on the expression of $mRNA^{TNF\alpha}$ in the suspension of mononuclears *in vitro* and caused an increase of concentration of the tumor necrosis factor α itself for the 3rd hour of incubation (Fig. 1).

Curcumin solution added to the suspension of mononuclear cells of human peripheral blood after 1 hour of toxic effect of $A\beta_{42}$ inhibited the expression of $mRNA^{TNF\alpha}$ (for the 3rd hour of incubation) and decreased considerably TNFα concentration. However, the inhibiting effect of curcumin proved short-term and, already for the 6-24th hour of incubation the expression level of $mRNA^{TNF\alpha}$ was renewed (Fig. 1, *A*), while TNFα content was doubled (Fig. 1, *B*). The modulating effect of curcumin itself on the dynamics of $mRNA^{TNF\alpha}$ and TNFα content in the mononuclear suspension coincided with this polyphenol effect on the background of toxic effect of $A\beta_{42}$ (Fig. 1).

In our research the changes of $mRNA^{IL\text{-}1\beta}$ expression by mononuclears were not clarified for any factor under study (Fig. 2, *A*), but the content of this pro-inflammatory interleukin varied essentially with adding curcumin (Fig. 2, *B*). Such non-apparent result may be explained only by curcumin ability to accelerate creation of the active form of IL-1β from its precursor under the catalytic action of caspase-1 [23].

The induction in the interval of $A\beta_{42}$ effect of 3-6 h and simultaneous essential expression inhibition with curcumin were established for gene IL-6 (Fig. 3, *A*). The inhibiting effect of curcumin at the transcription level in mononuclear suspension was preserved for the day of incubation as well. At the translation level the increase of $mRNA^{IL\text{-}6}$ under the effect of $A\beta_{42}$ resulted in the corresponding enrichment of the mononuclear suspension with IL-6 for the 3th h and further decrease of this interleukin concentration at time interval of 6-24 h (Fig. 3, *B*). The content of IL-6 proved to be lower in 6-24 h of curcumin effect itself compared to $A\beta_{42}$ effect in that period. Thus the effect of curcumin on the course of inflammatory process in mononuclears proved to be direct and dominating, though short-term, compared to $A\beta_{42}$ effect.

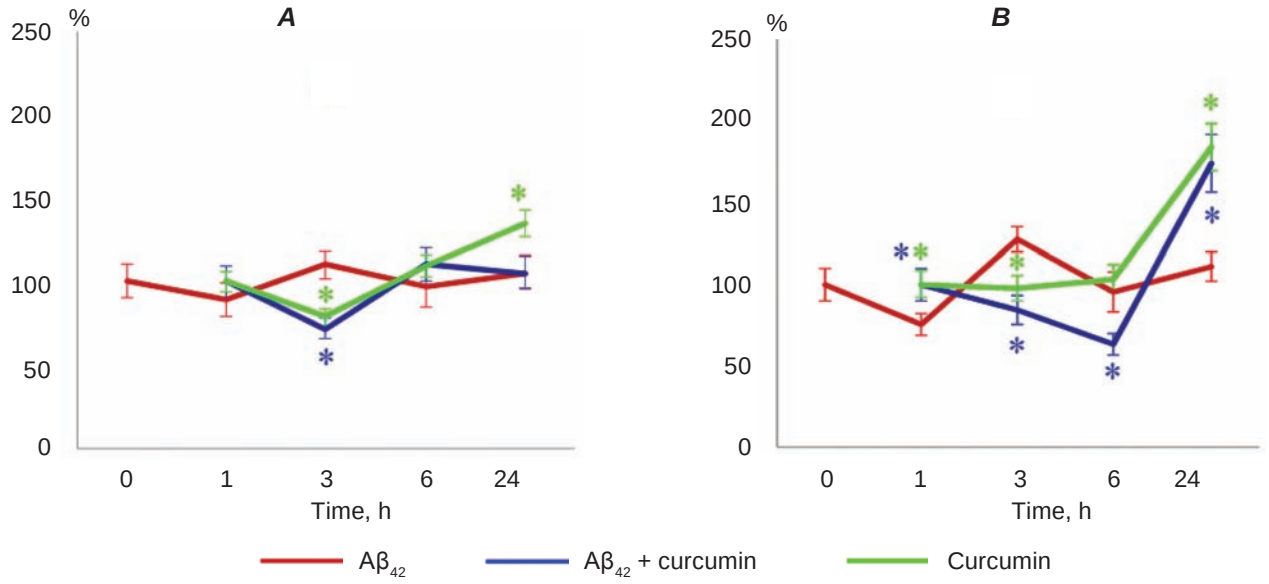

*Fig. 1. Dynamics of mRNA^{TNFα} expression (A) and TNFα content (B) in the suspension of mononuclears under the effect of $Aβ_{42}$, curcumin and their joint action (in % of the basal level with 0.9% NaCl, taken as 100%). Here and in Fig. 2-5 * $P < 0.05$ compared with $Aβ_{42}$ effect*

The results obtained conform to recently published data on dose-dependent inhibiting effect of curcumin on the content of IL-1β, TNFα, IL-6 and their mRNA in $Aβ_{42}$-activated microglia [24]. The work authors associate curcumin effect with phosphorylation of ERK1/2 and p38 and thus blocking of just these intracellular signal paths. Our data also partially coincide with results of the work by Jian Jiao with co-authors [25], where they show the increase of TNFα and IL-1β concentrations in microglia culture when adding monomers, oligomers or fibrils of $Aβ_{42}$ in concentration range of 0.625-2.5 μM with maximum for the 12th h of incubation. The authors suppose the NFκB-dependent induction of pro-inflammatory cytokines by various forms of $Aβ_{42}$.

The effect of $Aβ_{42}$ on the expression of anti-inflammatory IL-10 was not established (Fig. 4, *A*), but curcumin raised mRNA^{IL-10} content for the 6th h,

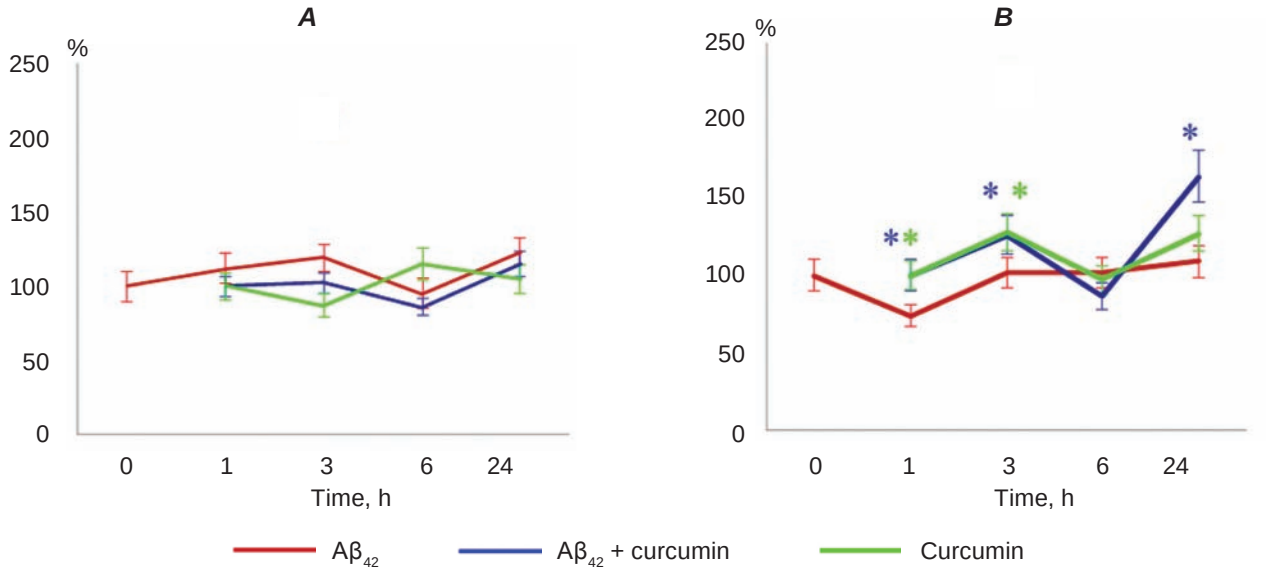

Fig. 2. Dynamics of mRNA$^{IL-1β}$ expression (A) and IL-1β content (B) in mononuclear suspension under the effect of $Aβ_{42}$, curcumin and their joint action (in % of the basal level with 0.9% NaCl, taken as 100%)

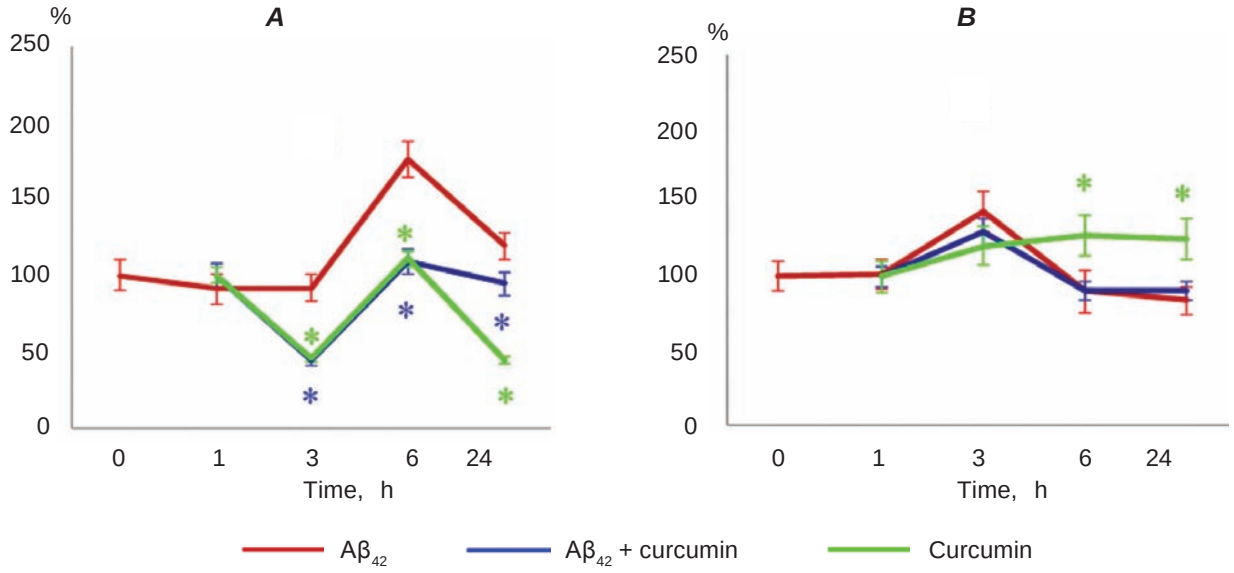

Fig. 3. Dynamics of mRNA$^{IL\text{-}6}$ expression (A) and IL-6 content (B) in mononuclear suspension under the effect of $A\beta_{42}$, curcumin and their joint action (in % of the basal level with 0.9% NaCl, taken as 100%)

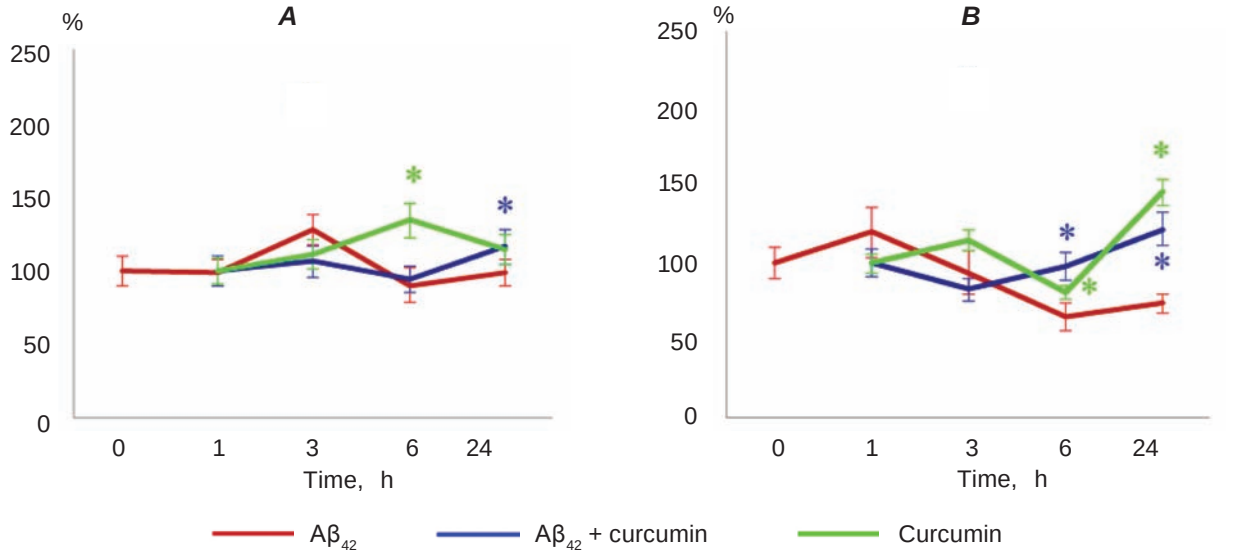

Fig. 4. Dynamics of mRNA$^{IL\text{-}10}$ expression (A) and IL-10 content (B) in mononuclear suspension under the effect of $A\beta_{42}$, curcumin and their joint action (in % of the basal level with 0.9% NaCl, taken as 100%)

while simultaneously with $A\beta_{42}$ – only for the 24th h of incubation. Beginning from the 1st h of incubation with $A\beta_{42}$ the authors observed a sharp drop of IL-10 level in mononuclear suspension, which could be stopped by curcumin from the 6th h only (Fig. 4, *B*). Some authors supposed that high level of IL-10 might be a good prediction sign in the dynamics of inflammatory process in general and under amyloidosis in particular [26, 27]. But there appeared researches which results evidence for the effect of IL-10 on the level of apolipoprotein E and clearance of β-amyloid peptide that led to formation of amyloid platelets [28].

We have studied the dynamics of concentration of endogenous $A\beta_{40}$ in mononuclear suspension under the effect of exogenous $A\beta_{42}$ (Fig. 5, *B*). We can see two maxima of this peptide formation: for the 1st and 24th h of incubation. In view of the absence of *AβPP* expression activation for the 1st h (Fig. 5, *A*) such a quick and intensive response to the action of

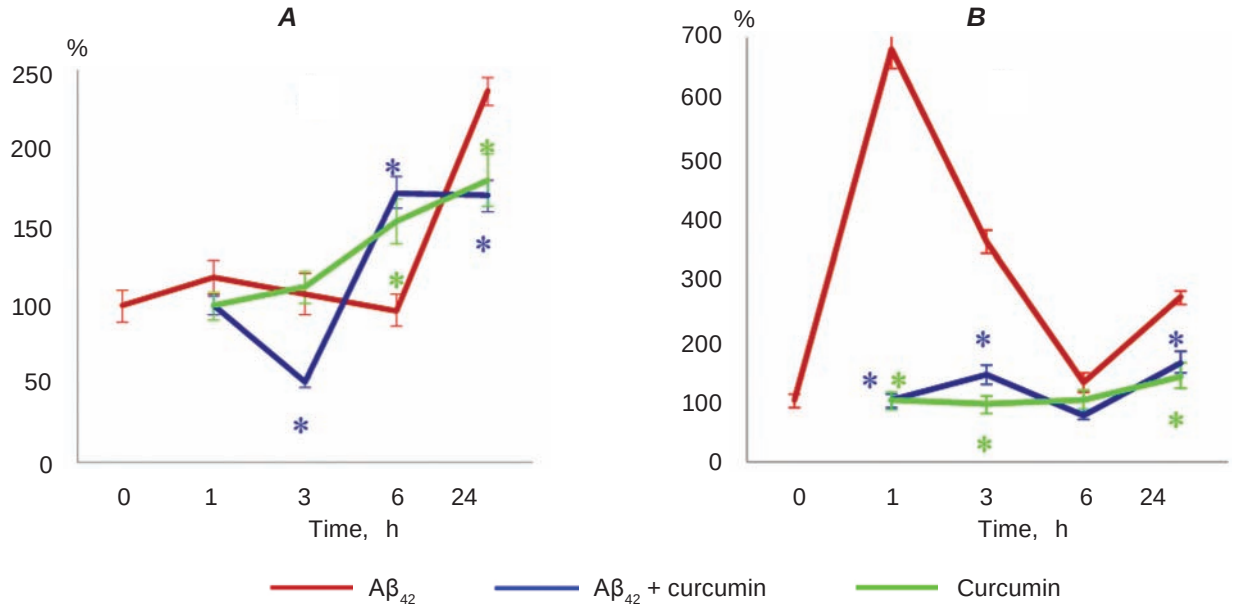

Fig. 5. Dynamics of mRNA^{AβPP} expression (A) and $Aβ_{40}$ content (B) in mononuclear suspension under the effect of $Aβ_{42}$, curcumin and their joint action (in % of the basal level with 0.9% NaCl, taken as 100%)

$Aβ_{42}$, compared with the cytokine system, owes to activation of processing of β-amyloid peptide precursor following the amyloidogenous scenery with formation of endogenous $Aβ_{40}$. The second peak of $Aβ_{40}$ concentration (for the 24th h) is the result of induction of AβPP expression and increase of its mRNA^{AβPP} in this time interval (Fig. 5, *A, B*).

The inhibiting effect of curcumin has been shown both on the expression and synthesis of AβPP and formation of $Aβ_{40}$. The mRNA^{AβPP} expression minimum was noted for the 3rd h of incubation, and the level of $Aβ_{40}$ in the presence of curcumin did not essentially differ from the initial one (Fig. 5, *A*). The data obtained may evidence for curcumin efficiency in normalization of β-amyloid peptide metabolism even in pro-inflammatory condition, determined by the local excess of $Aβ_{42}$ aggregates. Curcumin properties in inhibiting the formation and destabilization of Aβ-oligomers as well as in ruining a senile platelet are well known now [29-30].

Thus the dynamics of anti-inflammatory effect of curcumin *in vitro* at transcriptional and translational levels of cytokines formation by mononuclears has been cleared, as well as its direct inhibiting effect on the level of endogenous $Aβ_{40}$ during the 24-h incubation under the toxic effect of $Aβ_{42}$ aggregates has been established in this work.

ВПЛИВ β-АМІЛОЇДНОГО ПЕПТИДУ 42 НА ДИНАМІКУ ЕКСПРЕСІЇ І УТВОРЕННЯ $Aβ_{40}$, IL-1β, TNFα, IL-6, IL-10 МОНОНУКЛЕАРАМИ ПЕРИФЕРІЙНОЇ КРОВІ *IN VITRO* ТА ЙОГО КОРЕКЦІЯ КУРКУМІНОМ

В. В. Соколік[1], О. К. Коляда[2], С. М. Шульга[3]

[1]ДУ «Інститут неврології, психіатрії і наркології НАМН України», Харків;
[2]ДУ «Інститут геронтології ім. Д. Ф. Чеботарьова НАМН України», Київ;
[3]ДУ «Інститут харчової біотехнології і геноміки НАН України», Київ;
e-mail: sokolik67@rambler.ru

Токсичний ефект Аβ-олігомерів супроводжує перебіг хронічного запального процесу, основними медіаторами якого є цитокіни. Тому цитокінова ланка запального процесу постає новою мішенню на шляху приборкання амілоїдозу. Метою дослідження був ефект агрегованого $Aβ_{42}$ на динаміку експресії і утворення ендогенного $Aβ_{40}$ і цитокінів (IL-1β, TNFα, IL-6, IL-10) мононуклеарами периферійної крові *in vitro* та його корекція куркуміном. Суспензію мононуклеарних клітин, ізольованих *ex tempore* за допомогою фікол-урографінового градієнта зі

зразків венозної крові здорових добровольців, використовували для дослідження впливу $A\beta_{42}$ (15 нМ), куркуміну (54 пМ) та їх поєднаної дії (за аналогічних концентрацій) в динаміці часу: 0, 1, 3, 6 і 24 год інкубації при температурі 37 °С. Методом полімеразної ланцюгової реакції визначали експресію генів *АβPP*, *TNFα*, *IL-1β*, *IL-6* і *IL-10* та імуноензимним аналізом встановлювали вміст $A\beta_{40}$ і цитокінів у мононуклеарній суспензії в динаміці інкубації. Показали індивідуальну динаміку експресії АβРР і цитокінів за дії $A\beta_{42}$, який впливав на вміст $A\beta_{40}$, TNFα, IL-1β, IL-6 і IL-10 у мононуклеарній суспензії. Куркумін виявив інгібування експресії генів *АβPP*, *TNFα* і *IL-6*, що позначилося на зниженні рівня цих двох цитокінів і $A\beta_{40}$. У роботі показано динаміку антизапального впливу куркуміну *in vitro* на транскрипційному і трансляційному рівнях утворення цитокінів мононуклеарами. Встановлено безпосередній пригнічувальний ефект куркуміну на концентрацію ендогенного $A\beta_{40}$ протягом добової інкубації за умов токсичної дії агрегатів $A\beta_{42}$.

Ключові слова: куркумін, β-амілоїдні пептиди 40 і 42, цитокіни, мРНК, мононуклеари периферійної крові людини.

ВЛИЯНИЕ β-АМИЛОИДНОГО ПЕПТИДА 42 НА ДИНАМИКУ ЭКСПРЕССИИ И ОБРАЗОВАНИЯ $A\beta_{40}$, IL-1β, TNFα, IL-6, IL-10 МОНОНУКЛЕАРАМИ ПЕРИФЕРИЧЕСКОЙ КРОВИ *IN VITRO* И ЕГО КОРРЕКЦИЯ КУРКУМИНОМ

В. В. Соколик[1], А. К. Коляда[2], С. М. Шульга[3]

[1]ГУ «Институт неврологии, психиатрии и наркологии НАМН Украины», Харьков;
[2]ГУ «Институт геронтологии им. Д. Ф. Чеботарёва НАМН Украины», Киев;
[3]ГУ «Институт пищевой биотехнологии и геномики НАН Украины», Киев;
e-maiL: sokolik67@rambler.ru

Токсический эффект Аβ-олигомеров сопровождает течение хронического воспалительного процесса, основными медиаторами которого являются цитокины. Поэтому цитокиновое звено воспалительного процесса становится новой мишенью на пути обуздания амилоидоза. Целью исследования был эффект агрегированного $A\beta_{42}$ на динамику экспрессии и образования эндогенного $A\beta_{40}$ и цитокинов (IL-1β, TNFα, IL-6, IL-10) мононуклеарами периферической крови *in vitro* и его коррекция куркумином. Суспензию мононуклеарных клеток, изолированных *ex tempore* с помощью фикол-урографинового градиента из образцов венозной крови здоровых добровольцев, использовали для изучения влияния $A\beta_{42}$ (15 нМ), куркумина (54 пМ) и их сочетанного действия (при аналогичных концентрациях) в динамике времени: 0, 1, 3, 6 и 24 ч инкубации при температуре 37 °С. Методом полимеразной цепной реакции с помощью соответствующих праймеров определяли относительную экспрессию мРНК для *АβPP*, *TNFα*, *IL-1β*, *IL-6*, *IL-10* и иммуноэнзимным анализом устанавливали содержание $A\beta_{40}$. Показали индивидуальную динамику экспрессии АβРР и изучаемых цитокинов под действием $A\beta_{42}$, которая влияла на содержание $A\beta_{40}$, TNFα, IL-1β, IL-6 и IL-10 в мононуклеарной суспензии. Куркумин выявил ингибирующий эффект на экспрессию генов *АβPP*, *TNFα* и *IL6*, что сказалось на снижении уровня этих двух цитокинов и $A\beta_{40}$. В работе показана динамика антивоспалительного влияния куркумина *in vitro* на транскрипционном и трансляционном уровнях образования цитокинов мононуклеарами. Установлен непосредственный угнетающий эффект куркумина на концентрацию эндогенного $A\beta_{40}$ в течение суточной инкубации в условиях токсического действия агрегатов $A\beta_{42}$.

Ключевые слова: куркумин, β-амилоидные пептиды 40 и 42, цитокины, мРНК, мононуклеары периферической крови человека.

References

1. Koudinova N. V., Berezov T. T., Koudinov A. R. Beta-amyloid: Alzheimer's disease and brain beta-amyloidoses. *Biochemistry* (Mosc). 1999; 64(7): 752-757.
2. Hardy J., Selkoe D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science.* 2002; 297(5580): 353-356.
3. Jarrett J. T., Berger E. P., Lansbury P. T. Jr. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry.* 1993; 32(18): 4693-4697.
4. Walsh D. M., Klyubin I., Fadeeva J. V., Cullen W. K., Anwyl R., Wolfe M. S.,

Rowan M. J., Selkoe D. J. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature*. 2002; 416(6880): 535-539.
5. Rasin S. M., Podkoritov V. S., Rasin M. The role of chronic system inflammation and insulin resistance in the pathway of senile dementia. *Psychiatry*. 2011; 2(50): 46-54. (In Russian).
6. Forloni G., Demicheli F., Giorgi S., Bendotti C., Angeretti N. Expression of amyloid precursor protein mRNAs in endothelial, neuronal and glial cells: modulation by interleukin-1. *Brain Res. Mol. Brain Res.* 1992; 16(1-2): 128-134.
7. Yang Y., Quitschke W. W., Brewer G. J. Upregulation of amyloid precursor protein gene promoter in rat primary hippocampal neurons by phorbol ester, IL-1 and retinoic acid, but not by reactive oxygen species. *Brain Res. Mol. Brain Res.* 1998; 60(1): 40-49.
8. Blasko I., Veerhuis R., Stampfer-Kountchev M., Saurwein-Teissl M., Eikelenboom P., Grubeck-Loebenstein B. Costimulatory effects of interferon-gamma and interleukin-1beta or tumor necrosis factor alpha on the synthesis of Abeta1-40 and Abeta1-42 by human astrocytes. *Neurobiol. Dis.* 2000; 7(6 Pt B): 682-689.
9. Nawaz A., Khan G. M., Hussain A., Ahmad A., Khan A., Safdar M. Curcumin: a natural product of biological importance. *Gomal. Univ. J. Res.* 2011; 27(1): 7-14.
10. Basnet P., Skalko-Basnet N. Curcumin: an anti-inflammatory molecule from a curry spice on the path to cancer treatment. *Molecules.* 2011; 16(6): 4567-4598.
11. Perrone D., Ardito F., Giannatempo G., Dioguardi M., Troiano G., Lo Russo L., De Lillo A., Laino L., Lo Muzio L. Biological and therapeutic activities, and anticancer properties of curcumin. *Exp. Ther. Med.* 2015; 10(5): 1615-1623.
12. Plummer S. M., Holloway K. A., Manson M. M., Munks R. J., Kaptein A., Farrow S., Howells L. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex. *Oncogene*. 1999; 18(44): 6013-6020.
13. Jobin C., Bradham C. A., Russo M. P., Juma B., Narula A. S., Brenner D. A., Sartor R. B. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. *J. Immunol.* 1999; 163(6): 3474-3483.
14. Han S. S., Keum Y. S., Seo H. J., Surh Y. J. Curcumin suppresses activation of NF-kappaB and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells. *J. Biochem. Mol. Biol.* 2002; 35(3): 337-342.
15. Lee W. H., Loo C. Y., Bebawy M., Luk F., Mason R. S., Rohanizadeh R. Curcumin and its derivatives: their application in neuropharmacology and neuroscience in the 21st century. *Curr. Neuropharmacol.* 2013; 11(4): 338-378.
16. Villoslada P., Moreno B., Melero I., Pablos J. L., Martino G., Uccelli A., Montalban X., Avila J., Rivest S,. Acarin L., Appel S., Khoury S. J., McGeer P., Ferrer I., Delgado M., Obeso J., Schwartz M. Immunotherapy for neurological diseases. *Clin Immunol.* 2008; 128(3): 294-305.
17. Vignini A., Morganti S., Salvolini E., Sartini D., Luzzi S., Fiorini R., Provinciali L., Di Primio R., Mazzanti L., Emanuelli M. Amyloid precursor protein expression is enhanced in human platelets from subjects with Alzheimer's disease and frontotemporal lobar degeneration: a real-time PCR study. *Exp. Gerontol.* 2013; 48(12): 1505-1508.
18. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951; 193(1): 265-275.
19. Hashiramoto A., Yamane T., Tsumiyama K., Yoshida K., Komai K., Yamada H., Yamazaki F., Doi M., Okamura H., Shiozawa S. Mammalian clock gene Cryptochrome regulates arthritis via proinflammatory cytokine TNF-alpha. *J. Immunol.* 2010; 184(3): 1560-1565.
20. Keller M., Mazuch J., Abraham U., Eom G. D., Herzog E. D., Volk H. D., Kramer A., Maier B. A circadian clock in macrophages controls inflammatory immune responses. *Proc. Natl. Acad. Sci. USA.* 2009; 106(50): 21407-21412.
21. Surgery: Basic Science and Clinical Evidence. Ed. Norton J. A., Bollinger R. R., Chang A. E., Lowry S. F. New York: Springer-Verlag, 2001. 2095 p.
22. Schwarz J., Broder C., Helmstetter A., Schmidt S., Yan I., Müller M., Schmidt-Arras D., Becker-Pauly C., Koch-Nolte F., Mittrücker H. W., Rabe B., Rose-John S., Chalaris A. Short-term TNFα shedding is independent of cytoplasmic

phosphorylation or furin cleavage of ADAM17. *Biochim. Biophys. Acta*. 2013; 1833(12): 3355-3367.

23. Schönbeck U., Mach F., Libby P. Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing. *J. Immunol.* 1998; 161(7): 3340-3346.
24. Shi X., Zheng Z., Li J., Xiao Z., Qi W., Zhang A., Wu Q., Fang Y. Curcumin inhibits Aβ-induced microglial inflammatory responses *in vitro:* Involvement of ERK1/2 and p38 signaling pathways. *Neurosci. Lett.* 2015; 594: 105-110.
25. Jiao J., Xue B., Zhang L., Gong Y., Li K., Wang H., Jing L., Xie J., Wang X. Triptolide inhibits amyloid-beta1-42-induced TNF-alpha and IL-1beta production in cultured rat microglia. *J. Neuroimmunol.* 2008; 205(1-2): 32-36.
26. Apelt J., Schliebs R. Beta-amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 mice with Alzheimer plaque pathology. *Brain Res.* 2001; 894(1): 21-30.
27. Asselineau D., Benlhassan K., Arosio B., Mari D., Ferri E., Casati M., Gussago C., Tedone E., Annoni G., Mazzola P., Piette F., Belmin J., Pariel S., Bornand A., Beaudeux J. L., Doulazmi M, Mariani J., Bray D. H. Interleukin-10 Production in Response to Amyloid-β Differs between Slow and Fast Decliners in Patients with Alzheimer's Disease. *J. Alzheimers. Dis.* 2015; 46(4): 837-842.
28. Michaud J. P., Rivest S. Anti-inflammatory signaling in microglia exacerbates Alzheimer's disease-related pathology. *Neuron.* 2015; 85(3): 450-452.
29. Ono K., Hasegawa K., Naiki H., Yamada M. Curcumin has potent anti-amyloidogenic effects for Alzheimer's beta-amyloid fibrils *in vitro. J. Neurosci. Res.* 2004; 75(6): 742-750.
30. Garcia-Alloza M., Borrelli L.A., Rozkalne A., Hyman B. T., Bacskai B. J. Curcumin labels amyloid pathology *in vivo*, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. *J. Neurochem.* 2007; 102(4): 1095-1104.

Received 29.01.2016