Anti-Cytokine Activity of Curcumin and Its Binding to a Fragment of AβPP

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Abstract: Since it has been shown that Alzheimer’s disease is accompanied by higher peripheral concentrations of cytokines, we focused on the correction of chronic inflammation causing Aβ excess and aggregation what should have a positive impact. Proinflammatory cytokines action is realized by NF-κB intracellular signals. Curcumin can inhibit activation of the proinflammatory transcription factor NF-κB and is able to be mounted in the amyloid-β fibrils and encourage their disaggregation. To study the influence of anti-inflammatory effect of curcumin and its ability to model complexes with a fragment of AβPP. Investigation was carried out on rats with model of Alzheimer’s disease using nasal therapy with curcumin. Concentration of cytokines in cerebral cortex, hippocampus and blood serum, and indicators of conditioned active escape reflex were determined in this study. Methods of molecular dynamics and docking were used to examine the interactions of curcumin with fragment of AβPP. Aβ42_Human in the hippocampus of rats provoked chronic neuroinflammation specifically and primarily at the injection site. Curcumin performance revealed specific depressing effect on cytokines in the cerebral cortex and in the hippocampus it appeared similar to the above, but of lower level. Anti-inflammatory activity of curcumin led to the recovery of memory parameters. Analysis of restriction sites of AβPP has shown that position of curcumin in Site II is energetically more favorable for binding. Our suggestion that curcumin is an effective anti-cytokine factor was confirmed by experimental results obtained in vivo and explain the mechanism describing its effect on NF-κB Curcumin doesn’t block the excision Aβ but binding with its hydrophobic region.

Keywords: Alzheimer's Disease, Curcumin, Amyloid-β, Cytokine, Rat, Docking

1. Introduction

Recently, aggregation of amyloid-β (Aβ) to form fibrils or depositions is considered to be one of the believable factors of the pathology of Alzheimer’s disease (AD), being the major pathogenetic event [1-4]. In particular, several studies have shown that Aβ accumulates abundantly in certain brain areas namely, hippocampus and cerebral cortex which subserve information acquisition and processing, and memory [5-7]. This peptide is formed within the amyloidogenic processing of the amyloid-β protein precursor (AβPP) [8-10].

Alternatively, processing within the non-amyloidogenic pathway involves full-length AβPP cleaved by α- and γ-secretases in the trans-Golgi network apparatus and plasma membrane, precluding amyloid-β formation (or amyloidogenesis). The inverse internalization of certain AβPP parts of the plasma membrane and their transport to late endosomes leads to Aβ isoforms excision, which have 38-43 amino acid residues in length, caused by β- and γ-secretases cleavage [11]. A variety of factors is believed to play a role of switch between non-amyloidogenic and amyloidogenic AβPP processing pathways, such as AβPP excess, degree of its phosphorylation, receptor SORL1 expression intensity, the presence of mutations in AβPP and
Endogenous Aβ is a critical player in the synaptic plasticity and memory in normal central nervous system. It has been proposed that at low (picomolar) Aβ concentrations could act as the trophic signal and modulator of synaptic activity, with implications in memory and learning. Furthermore, Aβ could work as antioxidant, due to its ability to capture redox metals, such as Cu, Fe and Zn, and, thus, prevent their participation in redox cycles with other ligands; hence Aβ has the ability to function as a chelator and antioxidant molecule. Amyloid-β may be vital for the development of neurons, their plasticity and memory in normal central nervous system.

The anti-inflammatory IL-10 is not proven, with exception of TNF, chemokines, cell surface adhesion molecules and cyclin D1; to inhibit the expression of growth factor receptors and the production of AβPP and its processing by amyloidogenic mechanism by which amyloid-β can cause neurons damage and death is reactive oxygen species generating during its self-aggregation process. At the same time lipid peroxidation is activated on neuron membranes and functions of ion-motive ATPases (glucose and glutamate transporters) are impaired.

At high concentrations of Aβ (nanomolar to micromolar), neurotoxic aggregates are formed: oligomers or fibrils, resulting in amyloidosis and cell death. The mechanism by which amyloid-β can cause neurons damage and death is reactive oxygen species generating during its self-aggregation process. At the same time lipid peroxidation is activated on neuron membranes and functions of ion-motive ATPases (glucose and glutamate transporters) are impaired.

Existing therapeutic approaches to the treatment of amyloidosis in Alzheimer's disease focused on reducing the production and aggregation of amyloid-β or symptomatic therapy are ineffective.

Since has previously been shown that AD is accompanied by an inflammatory response, particularly higher peripheral production and aggregation of amyloid-β or other ligands; hence Aβ has the ability to function as a chelator and antioxidant molecule. Amyloid-β may be vital for the development of neurons, their plasticity and memory in normal central nervous system.

It was found that CUA is able to be mounted in the fibrils amyloid-β and encourage their disaggregation by forming a salt bridge with amino acid residues of the polypeptide chain, namely, Asp23 and Lys28, competing thus with metal ions Zn²⁺ or Cu²⁺. However, the probability interaction of CUA with sites of AβPP cleavage by relevant secreta ses is of great interest.

The purpose of the research was to study the influence of anti-inflammatory effect of curcumin and modeling of its complexation with a fragment of AβPP (Fragment_63Rat) that containing Aβ.

2. Materials and Methods

2.1. Design of Research

Investigation was carried out on 30 white mature male Wistar rats six-month age weighting 200-250 g. All animals were maintained with a controlled 12-h light-dark cycle and standard rodent chow and tap water. The experimental protocols were performed in accordance with General ethical principles of animal experiments (Kiev, 2011), European Convention for the Protection of Vertebrate Animals used for experimental and other Scientific Purposes (Strasbourg, 1986) and the internationally recognized principles for the use and care of laboratory animals, asstated in the US guidelines.

The rats were divided into 5 groups at random (n = 6 in each group). The Control group included of intact animals; Main group 1 - rats with AD experimental model; Main group 2 - rats with AD experimental model, which obtained nasal therapy with aqueous CUA solution; Main group 3 - rats with AD experimental model, which obtained nasal therapy with aqueous CUA solution containing Aβ.

Curcumin (CUA) has been shown to regulate negatively transcription factors NF-kB, AP-1; to suppress the expression of cyclooxygenase-2, lipoxygenase, NO synthase, matrix metalloproteinase-9, urokinase-type plasminogen activator, TNF, chemokines, cell surface adhesion molecules and cyclin D1; to inhibit the expression of growth factor receptors and activity of JNK, protein tyrosine kinases, and several other protein serine/threonine kinases. This natural polyphenol is nowadays well described as an inhibitor of DNA methyltransferase so that it is considered as a DNA hypomethylating agent. It reestablishes the balance between histone acetyl transferase and histone deacetylase (HDAC 1, 3, 4, 5, 8) activity to selectively activate or inactivate the expression of genes. Finally, curcumin modulates the activity of miRNAs (miR-15a, miR-16, miR-21, miR-22, miR-26, miR-101, miR-146, miR-200, miR-203, and let-7) and their multiple target genes.

The purpose of the research was to study the influence of anti-inflammatory effect of curcumin and modeling of its complexation with a fragment of AβPP (Fragment_63Rat) that containing Aβ.
seconds. If the conditioned active escape reflex was conditional reflex escaper’s action absent the unconditional signal was performed to the animal (electric current on a metal grid floor of the cage with a voltage of 30-40 for 10 seconds). The conditional signal was performed to each rat five times a day with pauses 1-3 seconds every day. The positive result was considered conditional reflex answers to metronome sound without mistakes. In animals as quantitative indicators of cognitive ability and memory have registered of the part of positive reactions (in percent) and the duration of latency periods of conditional reflex answers in seconds [61]. The duration of latency period was determined with stopwatch beginning from the metronome start until the rat moved in the other part of the cage.

These indicators of conditioned active escape reflex tested animals of all groups after animal model of AD formation and curcumin therapy, respectively.

2.3. Alzheimer’s Disease Animal Model and Curcumin Therapy

Effects of Aβ42_Human in homoaggregate form was studied after 1 month after a single administration at a dose of 15 nM Aβ42_Human (65 µg) to hippocampus of rats. The volume of the solution was 10 µl per animal. Aβ42 was delivered to the hippocampus in stereotaxic installation using chromatographic syringe at a rate of 0.03 µl per second for 5 min.

Aβ42_Human (Human Amyloid β Protein Fragment 1-42, Sigma-Aldrich) dissolved in bidistilled water was aggregated for 24 hours at 37°C. Large, coarse conglomerates Aβ42_Human dispersed by ultrasound and sterilized immediately before introduction.

Stereotactic coordinates of the left hippocampus were determined according by the map of the brain by J. Bures [62], which corresponds to the distance from the point of intersection of the sagittal suture with bregma (zero point): distal - 2 mm, lateral - 2 mm and in depth - 3.5 mm. Stereotactic operation were carried out on rats under the general anesthesia by means of intraperitoneal injections of thiopental, 50 mg/kg body weight.

Since curcumin is poorly soluble in water, concentrated stock curcumin solution was originally prepared in 96% ethanol. CUA remained stable in ethanol at room temperature for several weeks, but degraded in water at neutral or mildly basic pH [63]. Therefore, CUA stock solution was freshly diluted daily to 0, 7 g/l in bidistiller water (the ratio alcohol: water in the working solution of curcumin was 1:100) immediately before every nasal administration to rats using a disposable pipette at a dose of 3, 5 µg per animal.

The animals were decapitated after treatment and their brain and blood were obtained quickly. Hemispheres were dissected and interesting regions (hippocampus and cerebral cortex) were removed, frozen and stored until ELISA analysis of cytokines. Blood was collected and centrifuged at 1000×g for 20 minutes. Serum was collected, frozen and stored until cytokine tested. The hippocampal and cerebral cortex tissues were homogenized in Tris buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.5), centrifuged at 14,000×g for 5 minutes and collected supernatant.

2.4. Cytokine Quantification Perfomed by Enzyme-Linked Immunosorbent Assays

Samples of hippocampal and cerebral cortex supernatant and blood serum were processed to quantitively measure the amount of cytokine ELISA method in accordance with the manufacturer’s instructions Rat ELISA Kits Invitrogen BCM DIAGNOSTICS, USA for interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor-α (TNFα). OD was read by microplate analyzer GBG Stat FAX 2100 (USA) at 450 nm with the wavelength correction at 630 nm [64]. ELISA assay data (µg/l cytokine) were normalized to g total protein or expressed at ng/l of blood serum. On figures, were the obtained indexes are presented as a percent from Control indexes. Total protein content was determined by Lowry [65].

2.5. Molecular Dynamics (MD) Simulations and Protein Ligand Docking

To simulate the interactions of curcumin with AβPP-fragment Fragment_63Rat - A4_RAT (P08592: Ile661-Leu720) of 63 amino acid residues, containing amyloid-β and the three restriction site for β-, α- and γ-secretase, was selected. Because of the lack of experimentally obtained protein structures, homology modeling was impossible. Therefore, we performed a preliminary parameterization and analysis of hypothetical spatial organization using protein secondary structure prediction servers: ProtParam (http://web.expasy.org/protparam/) and SOPMA (http://npsa-phil.ibcp.fr/) for modeling using several templates and de novo methods [66-67].

3D-models of Fragment_63Rat were built using I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) and SWISS-MODEL (http://swissmodel.expasy.org) profile modeling servers, QUARK de novo modeling tool (http://zhanglab.ccmb.med.umich.edu/QUARK/), and original algorithm of de novo mathematical modeling tool Molecular Constructor [68]. Optimization of built models geometry and verification of folding were performed using a full-atomic force field CHARMM [69] and software package GROMACS 4.5 [70]. Checking of models stability was based on root-mean-square deviation between C-atoms (RMSD) and total conformational energy - CE (the energies of van der Waals and Coulomb interactions) [71].

To evaluate curcumin as potential inhibitor of enzymatic restriction, we used flexible molecular docking in CCDC GOLD Suit 5.1. Parameterization of molecular target (Fragment_63Rat), ligand (curcumin) and conditions of docking were performed according to CCDC recommendations, with the maximum docking site: maximum radius from selected atom. Evaluation of the docking, potential affinity of ligand and binding site was performed basing on evaluation functions of CCDC GOLD (http://www.ccdc.cam.ac.uk): GoldScore and ASP [72].
Visualization and analysis of molecular modeling and docking results were performed in PyMOL [73] and BIOVIA DS Visualizer (http://accelrys.com/).

2.6. Statistical Analysis

The results were treated statistically; the average values and standard deviations were calculated. Statistical analysis of differences was performed using Student’s t-test for the corresponding Main and Comparison groups, and among Control indexes. The value of $p<0.05$ was considered significant.

3. Results

3.1. Cytokines Changes in Chronic Neuroinflammation Induced Homoaggregates of β-Amyloid Peptide 42_Human (AD Animal Model) in Rats

Expressive changes in the content of IL-1β, TNFα, IL-6 and IL-10 in blood serum of the animals with AD animal model (Main group 1) was not revealed comparing with the Control group. As shown in Table 1, only serum concentrations of TNFα increased by 20% and IL-10 declined by 54%, respectively. Similar trends were noted in the Comparison group 1 (false-acts animals). Thus, cytokine levels in blood serum doesn’t reflect the specific action of Aβ42_Human in the brain, but the result intracerebral intervention.

Table 1. Action of Aβ42_Human of homoaggregate and curcumin on IL-1β, TNFα, IL-6 and IL-10 in blood serum of examine rats.

| Cytokine | Control group (ng/l) | Main group 1 (ng/l) | Comparison group 1 (ng/l) | Main group 2 (ng/l) | Comparison group 2 (ng/l) |
|----------|----------------------|---------------------|--------------------------|---------------------|--------------------------|
| IL-1β    | 17.2±1.2             | 16.8±1.1            | 15.7±0.6                 | 20.9±2.3 *#&         | 16.2±1.2                 |
| TNFα     | 7.9±0.8              | 9.5±0.6 *           | 10.7±1.0 *               | 10.3±1.4 *#          | 13.0±1.2 *&              |
| IL-6     | 4.8±1.0              | 3.7±0.6             | 5.3±1.1                  | 1.8±0.3 *&          | 1.8±0.3 *&               |
| IL-10    | 3.9±0.4              | 1.8±0.2 *           | 1.3±0.2 *#              | 4.0±0.5 #&          | 1.6±0.2 *                |

The results given as the mean value ± S. D.

* - $p\leq0.05$ in comparison with Control; *# - $p\leq0.05$ among suitable Comparison group; * - $p\leq0.05$ in comparison with Main group 1 (AD animal model).

![Cerebral cortex](image)

**Figure 1.** Effect of Aβ42_Human and CUA therapy on levels of cytokines (IL-1β, TNFα, IL-6, IL-10) in the cerebral cortex of brain rats. The results are expressed in % of Control, and given as the mean value ± S. D. * - $p\leq0.05$ compared to Control; # - $p\leq0.05$ among suitable Comparison group; & - $p\leq0.05$ compared to Main group 1 (AD animal model).
The study of cytokines in the cerebral cortex of the rats with AD animal model (Main group 1) showed a low-grade inflammatory response, namely increase in level of IL-6 by 29% and reduce of IL-10 by 31%, comparing to the concentrations in false-acts animals (Fig. 1). Significant increase in level of IL-1β by 109% of the rats with AD animal model compared with those of Control group corresponds to level of this cytokine in Comparison group 1 (105%). This could come from injection itself. The content of TNFα in the cerebral cortex and hippocampus of rats of Main group 1 and Comparison group 1 did not differ from Control index and among themselves.

Hippocampal levels of IL-1β and IL-10 in rats with AD animal model increased significantly, compared with those in Control (by 221% and 111%) and Comparison 1 (by 110% and 78%) groups. In this brain part, concentration of IL-6 rats of the Main group 1 did not differ from values in intact animals, but was reduced by 44% against IL-6 concentration in false-acts rats (Fig. 2). These data suggest that homoaggregates of Aβ42_Human in the hippocampus of AD animal model rats provoked chronic neuroinflammation specifically and primarily at this region of the brain. However, in the cerebral cortex of these rats activation of inflammation reaction was also featured, albeit to a lesser extent. This result confirms our previous research, in which it was shown that homoaggregates of Aβ40_Human introduction into the cerebral cortex of rats causes more cytokine response in that part of the brain of rats in comparison with the situation in hippocampus [74].

Figure 2. Effect of Aβ42_Human and CUA therapy on levels of cytokines (IL-1β, TNFα, IL-6, IL-10) in the hippocampus of brain rats. The results are expressed in % of Control, and given as the mean value ± S. D. * - p≤0.05 compared to Control; # - p≤0.05 among suitable Comparison group; & - p≤0.05 compared to Main group 1 (AD animal model).
Intrahippocampal introduction of Aβ42_Human homoaggregate caused inhibition of conditional active escape reflex in rats of Main group 1. The study of cognitive capabilities and memory revealed reduction of the proportion of positive reactions and increase of latent period in these animals among rats of Control group (Fig. 3). However, the part of positive reactions with AD animal model rats did not differ from the index of false-acts animals, describing the effect of intracerebral interventions.

3.2. Effect of Curcumin on the Concentration of IL-1β, TNFα, IL-6, IL-10 in Rats with AD Animal Model

Daily CUA therapy of rats with AD-model for 1 month led to increase of levels of proinflammatory cytokines: IL-1β (by 22%) and TNFα (by 30%) and reducing of ambivalent IL-6 (by 38%) in blood serum compared of Control (Table 1).

In the Comparison group 2 (nasal administration of bidistillate instead of CUA solution to rats with AD animal model) content of IL-1β remained unchanged, but dynamic concentrations of TNFα and IL-6 coincided with the Main group 2. However, CUA renewed quite specifically concentration of anti-inflammatory IL-10 in serum of rats in contrast to the effect of bidistilled water. In general, cytokine response in blood circulation of experimental rats is activated by curcumin treatment.

Curcumin performance in the cerebral cortex revealed specific depressing effect on cytokines (Fig. 1). Namely, the normalized level of IL-1β and IL-6; TNFα reduced by 49% compared to Controls; IL-10 level did not change, but at the same time it did not differ from control indexes. The further aggravation of neuroinflammation induced by intrahippocampal injection of Aβ42_Human was observed in Comparison group 2, the concentrations of IL-1β and IL-10 in this area of the brain increased by 50% and 73%, respectively, after 1 month of bidistillate treatment, what corresponds to 2-month term impact of Aβ42_Human compared with Main group 1.

In the hippocampus of animals, CUA effect on cytokine appeared similar to the above, but below the level (Fig. 2). The cytokines concentration did not normalize, while level of IL-6 and IL-10 increased by 49% and 83% respectively, compared with the indexes month ago (Main group 1). But when comparing hippocampus cytokines of Main groups 2 rats of Comparison groups 2 becomes clear specific CUA induced inhibition levels of IL-1β (by 33%), TNFα (by 24%), IL-6 (by 34%) and IL-10 (by 99%). The observed anti-inflammatory activity of CUA led to the recovery of memory parameters, in particular concerning positive reactions of animals (Fig. 3).

3.3. Fragment_63Rat Docking with Curcumin

During experiments on prediction of the spatial structure of Fragment_63Rat (Ile661-Leu723 of APP) we have selected 8 models: one built by a server profile simulations SWISS-MODEL (63_SM), 1 built de novo using the tool conformational search QUARK (63_Q), 5 models were built using I-TASSER server (63_IT1-5) and 1 model was reconstructed using original de novo mathematical modeling tool - Molecular Constructor (63_MC).
Analysis of the physicochemical properties of built models using ProtParam tool provided us with information necessary for further parameterization of input files for molecular dynamics in Gromacs. Short-term molecular dynamics was carried out using explicit solvent in the box. Its size was oversized (10 Å) because of high mobility and the lack of information on conformational mobility of Fragment_63Rat. Geometry optimization via free energy minimization was performed using computational modules «grompp», «mdrum», ffmgx force field, and Steepest Descent Algorithm (maximum number of steps = 1000 and gradient = 0.1). Molecular dynamics simulations (5 ns at T = 310°K) were performed using full atomic force field.

There were no messages about incorrect or invalid values of conformational parameters during the calculation of Fragment_63Rat molecular dynamics. What in part demonstrates the high quality and stability of constructed models [75-76]. Results of molecular dynamics were analyzed taking into account two criteria: standard deviation between C-α-atoms (conformational fluctuations, RMSD) and protein conformational energy (total energy of van der Waals and Coulomb interactions, CE), using previously proven protocol (Fig. 4) [75, 77].

![Graph Image]

Figure 4. Standard deviation between Cα-atoms (RMSD) and conformational energy (CE) of built models of Fragment_63Rat according to results of molecular dynamics simulations.
High conformational mobility of some models during MD simulations is potentially associated with a large amount of unstructured elements. Despite high fluctuations of 63_IT3 and highest stability of 63_Q, their energy fluctuations are well correlated with all models except for 63.SM and 63.IT5. Subsequent analysis was performed for average normal energy models (t = 4-5 ns) using internal tools of Gromacs. Based on results of molecular dynamics, for docking with a molecule of curcumin, two models of target protein were selected (Fig. 5): 63_Q - a model with best performance and energy of conformational fluctuations, and 63.IT3 - a model, characterized by the presence of unique elements of secondary structure, inherent in this group of proteins [78]. Preliminary we have selected three sites of enzymatic restriction of Fragment_63Rat: I - Met11-Asp12 (for β-secretase); II - Lys27-Leu28 (for α-secretase); III - Val51-Ile52 (for γ-secretase).

It was found that according to constructed models, Site III located on unstructured and moveable part of peptide (Fragment_63Rat) preventing binding of CUA at this area. At the same time, Site I and Site II definitely have more favorable structure for binding such structures and were selected for subsequent molecular docking. As ligands, we used two conformations of CUA: 1) extracted from PDB-structure of TTR (PDB: 4PMF) and 2) chosen according results of conformational search. After checking accuracy of atoms and bonds, ligands were saved in full atomic format *.mol2, and prepared for docking using Hermes program by CCDC Software Ltd. Analysis of 63.IT3 and 63.Q sites of CUA binding was performed using a flexible molecular docking using CCDC GOLD genetic algorithm. Default settings of CCDC GOLD were used, except choice of site central atom and radius of docking region. In this case, choice of sites was caused by location of two amino acid residues - experimentally predicted sites of enzymatic restriction. In the case of the first site (Site I), centering was made on Met11, and CA of model 63_IT3 and CB of model 63.Q1 were used as central atoms of target. In the case of the second site (Site II), centering was carried out on Lys27, and CA of model 63_IT3 and CB of model 63.Q1 were used as central atoms of target. For docking, a complete mobility of the ligands was set. Amino acid residues of target were fixed, and the radius of docking site was increased up to 15 Å, covering the major part of model surface. In the first case, genetic algorithm was run in 100 time repetition, and in the second case, it was run in 10 times based on 10-best frames selected in the first stage. Internal evaluation functions of CCDC GOLD were used as selection criteria. Thus, the value of key function - GoldScore indicate affinity of ligand to binding site (higher value - better), and ASP value indicates comfort of conformational state of the ligand itself (smaller
value - better). Coincidence of favorable values of GoldScore and ASP was a marker for selection of leading models of curcumin-Fragment_63Rat interaction. According to aforementioned algorithm, we found coincidence of two conformational states of curcumin in complexes with 63_Q and 63_IT3. These conformations were selected based on statistical indicators of CCDC GOLD, and advantageous conformational states of selected ligands. In the leading models of curcumin-Fragment_63Rat complexes (Fig. 6) methyl group of CUA are placed within a hydrophobic pocket of the target, and oxygen groups form stable hydrogen bonds. We consider that conformation of the model 63_Q is more probable for curcumin binding, due to presence of conformationally stable elements of secondary structure, results of statistic and molecular dynamics. SOPMA method of secondary structure prediction, with a probability of 73% makes it possible to establish the main elements of primary structure of polypeptide chain: α-helix, β-fold, β-turns and irregular structure areas. Comparative analysis of Site I and Site II based on the ChemScore evaluation algorithm of CCDC GOLD has shown that position of curcumin in Site II is not only conformationally, but also energetically more favorable for ligand binding.

Our results indicate, that suppose of the possible interaction of curcumin with aforementioned sites (Site I for β-secretase and Site II for α-secretase) of enzymatic restriction has a right to exist.

4. Discussion

Ours results demonstrated activation of cytokines system in rat’s brain with AD animal model (Fig. 1-2). These data confirms numerous researches about neuroinflammation induced by Aβ deposits, fibriles or oligomers [79-84]. Aβ deposition is responsible for microglia activation [79]. Aβ enhances the inflammatory response to stimulation of NF-κB, nuclear factor that is involved in the regulation of ERK (extracellular signal-regulated kinase) and MAPK (mitogen-activated protein kinase) pathways that lead to the production of cytokines and chemokines [80]. Toll-like receptors (TLR), along with IL-1R and TNFR, are important for regulation of microglial responses to Aβ. Modification of the inflammatory state of microglia/macrophages may play an axial role in AD-
related pathology [81]. According to our data, chronic neuroinflammation induced by the administration of Aβ42_Human to hippocampus manifested changes of TNFα and IL-10 serum levels (Table 1) and decline in cognitive and memory indexes in rats (Fig. 3). Therefore, hyperproduction of cytokines may play a role of realizing mechanism in the early stages of amyloidosis and Alzheimer’s dementia.

Our suggestion that curcumin is an effective anti-inflammatory factor was confirmed by experimental results obtained in vivo and explain the mechanism described its effect on NF-κB. It is known, that CUA can inhibit activation of the proinflammatory transcription factor NF-κB, inhibiting phosphorylation and degradation of IκBα, an inhibitor of NF-κB. CUA is also a potent inhibitor of COP9 signalosome and associated kinases, casein kinase 2 and protein kinase D, all linked to the ubiquitin-proteasomal system (UPS) [85-86]. Effect of curcumin is inhibition of IκB kinase (IKK) activation, required for NF-κB activation [87-89]. That is what we explain the detected anticytokine effect curcumin in experimental animals (Fig. 1-2).

![Figure 7. Hypothetical scheme of anticytokine activity of curcumin in Aβ-induced animals and its binding to a fragment of AβPP.](image)

Hypothetical scheme of Aβ42_Human and curcumin influences through intersection point of cytokines signaling to the NF-κB pathway is shown in Fig. 7.

It has been shown that CUA inhibits the formation of Aβ fibrils and has potential anti-amyloidogenic effect [90-93]. Similarly, nanoparticles functionalized with CUA derivatives [94] exhibit high affinity toward Aβ42 monomers and the corresponding fibrils [95, 96]. Recent studies of the complex Aβ42 curcumin analysis of MD trajectories showed that the C-terminal β-strands at the site Ala21-Val40 are much rarer spirals and curcumin helps to stabilize the latter. In addition, calculations Aβ42-CUA complex showed its direct interaction with the hydrophobic cor Aβ42, which may explain why curcumin acts as an inhibitor of aggregation of Aβ42 [97, 98]. They are hydrophobic residues Phe 10, Phe 19, Phe 20 and Leu34, which do not belong to the sites of the proteolytic cleavage of AβPP and are in their neighborhood, interacting with the hydrophobic region of the phenolic rings of curcumin. These data are consistent with those obtained in our work to sites I and II: in particular, all three phenylalanine surround processing site II of AβPP, while Phe10 and Leu34 are closer to sites I and III, respectively, (Figure 6). Therefore, anti-amyloidogenic effect of curcumin is not blocking the excision sites Aβ40 or Aβ42 but a binding of the hydrophobic region in beta-amyloid peptide and aggregation preventing. Thus, some levels of curcumin effect have been shown in experiments in vivo and in silico (Fig. 7), its anti-amyloidogenic influence is realized.

5. Conclusions

The role of neuroinflammation as a mechanism of cognitive impairments development has been shown by the experimental model of Alzheimer's disease in rats. An anti-inflammatory effect of nasal treatment with an aqueous solution of curcumin has been determined. A scheme of curcumin action has been proposed taking into account the data of molecular docking with the AβPP fragment, containing area of Aβ.

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