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THE ROLE OF MATRIX METALLOPROTEINASE MMP-9, ITS INHIBITOR TIMP-1 AND INTERLEUKINE-1β IN PATHOGENESIS OF TRAUMATIC BRAIN INJURY

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Resume

Traumatic brain injury (TBI) is accompanied by high rates of morbidity and mortality in both developed and undeveloped countries that makes it one of the most actual medical and social problems. In recent years matrix metalloproteinases are in increasing interest while studying TBI pathogenesis because of their ability to increase permeability of the blood-brain barrier and to cause nervous tissue matrix reorganization. **The goal** of given study was to investigate the role of matrix metalloproteinase MMP-9, its inhibitor TIMP-1 and interleukin IL-1β in pathogenesis of TBI. **Methods**: The study was performed on 98 mature white rats. Moderate severity TBI was modeled with one blow on the cranial vault by means of free-falling plummet. Control group included 30 rats. Cytokines (IL-1b, IL-6, IL-8, TNF-a), MMP-9 and TIMP-1 levels were investigated in animals blood by means of ELISA on 1st, 3rd, 7th, 14th and 21st days after trauma. **Results and discussion:** MMP-9 levels increased by only 38,2% on the 1st day, but on the 3rd day there was its marked increase to 538%. It is known that metalloproteinases are released from the cells under the influence of various factors, including cytokines. On the 1st day after trauma it was IL-1β which increased by 705% showing the highest rise among other cytokines and exceeding increase in MMP-9 levels. This might indicate regulatory role of IL-1β. A marked increase in MMP-9 levels in turn lead to TIMP-1

activation. Significant increase in TIMP-1 levels was determined on the 3rd day after trauma. On the 7th day there was a critical period with the highest levels of IL-1β (2147,2%), MMP-9 (720,3%) and TIMR-1 (339,3%). Then all research indicators were decreasing with the most pronounced decrease in IL-1β and MMP-9. Conclusion: MMP-9 levels began to increase on the 1st day after trauma due to influence mainly IL-1β. An abrupt increase in MMP-9 in its turn caused an increase in TIMR-1 levels. **Conclusion**: Identified changes in IL-1β, MMP-9 та TIMP-1levels at TBI indicate complex relationships between cytokines and intercellular matrix reorganization regulators in formation of intercellular cooperation and inflammation development.

Key words: traumatic brain injury, MMP-9, TIMP-1, IL-1β

Introduction. According to epidemiological studies traumatic brain injury (TBI) is one of the actual medical and social problems worldwide [7, 9, 16, 19]. TBI is widespread in people of all ages and its prevalence does not depend on countries' economic development levels [15]. TBI is of particular importance among young people of working age because it is accompanied by high rates of morbidity and mortality [8, 10, 17]. According to Khalin et al. (2016) TBI incidence has increased from 235 to 326 per 100 000 Europe's population in recent years [13]. In Ukraine it is also noticed high rate of traumatic brain injuries [1, 3]. Long-term complications of TBI include motor and cognitive disorders that can significantly reduce quality of life [6, 13, 18]. TBI is accompanied by neuroinflammation and main link of its pathogenesis is an impairment of the blood-brain barrier. Increased permeability of the blood-brain barrier and a release of proinflammatory cytokines cause additional tissue alteration [11]. Matrix metalloproteinases and their tissue inhibitor TIMP play essential roles in neuroinflammation pathogenesis at TBI causing an increase in the blood-brain barrier permeability, as well as in nervous tissue matrix reorganization. Thus **the goal** of given study was to investigate the role of matrix metalloproteinase MMP-9, its inhibitor TIMP-1 and IL-1β in pathogenesis of TBI.

Materials and Methods

Experimental TBI was performed according to Jelsky-Zablitsev model (2005) [2]. The study involved 98 mature white rats. Moderate severity TBI was modeled with one blow on the cranial vault by means of free-falling plummet. Control group included 30 false-injured rats. Animal experiment was carried out according to European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1985). Cytokines (IL-1b, IL-6, IL-8, TNF-a), MMP-9 and TIMP-1 levels were investigated in

animals blood by means of ELISA kit from Biosource (Belgium) and Bender Medsystems (Austria).The study results were processed using the program Statistics 10 (StatSoft, Inc., USA).

Results and Discussion

Analysis of the received data showed that within the 1st day after trauma MMP-9 levels in rats' blood increased by 38,2% ($p < 0,05$) compared with the control group (Fig. 1).

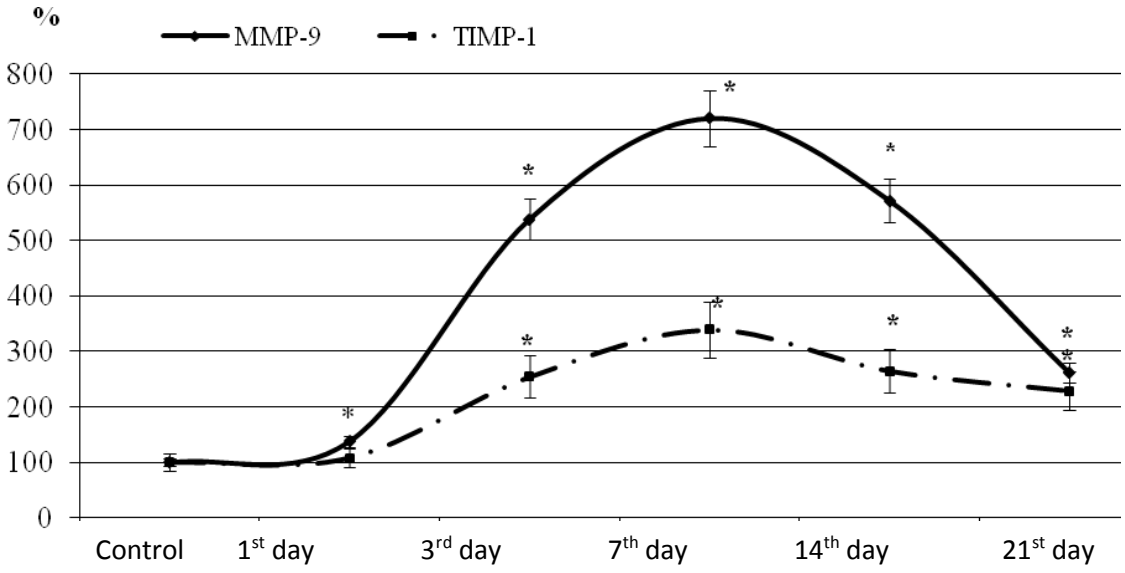

Figure 1. Dynamics (% of control values) of MMP-9 and TIMR-1 levels after TBI;
* - $P < 0,05$ compared with the average values of the control group

MMP-9 is a zinc-containing proteinase with gelatinase activity. Several types of cells including neutrophils, fibroblasts, lymphocytes, macrophages and others secrete small amounts of MMP-9 [4], but the secretion can be increased greatly due to effects of cytokines released at traumatic tissue injury. In our study on the 3rd day MMP-9 levels increased more than 5 times compared with the control group ($p < 0,01$). This is consistent with literature date about essential role of MMP-9 in development of inflammation in the brain. MMP-9 is involved in microvessels basement membrane reorganization, as well as it causes an increase in blood-brain barrier permeability [14]. In addition, MMP-9 is found to cause destruction of myelin components [20].

At TBI marked increase in MMP-9 activity is associated with nervous tissue matrix reorganization and cell-cell cooperation. Increased MMP-9 activity at the beginning of TBI is protective because it contributes leukocyte emigration to the site of injury. But further

increase in MMP-9 levels leads to tissue destruction. Peak rise of MMP-9 levels was observed on the 7th day after trauma (to 720%; p <0,01).

Anti-inflammatory cytokines levels also started to increase on the 1st day after trauma (Fig. 2).

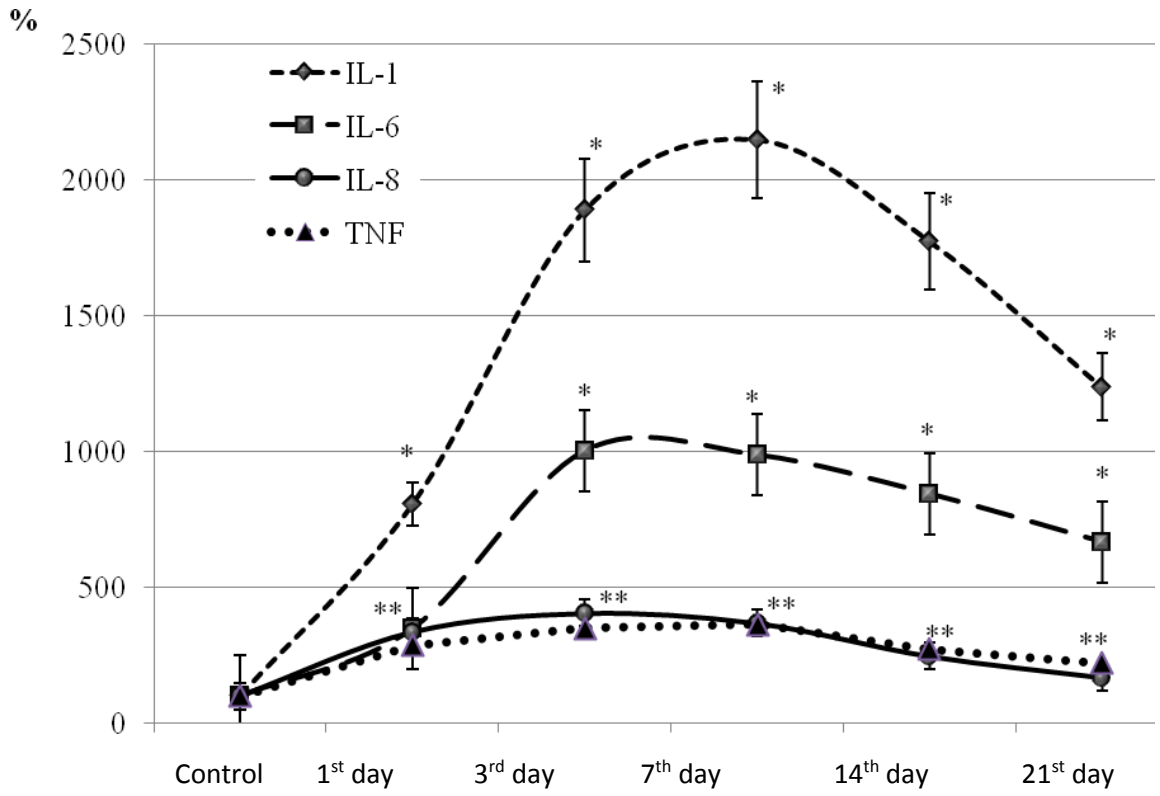

Figure 2. Dynamics (% of control values) of cytokines IL-1 β, IL-6, IL-8 and TNF-α levels; * - P <0,05 compared with the average values of the control group

It was IL-1β showing the highest rise among other cytokines during all TBI course (21,5 times; p <0,001). In our study, we investigate cytokines to determine their correlation with MMP-9. It was found that during the entire observation period- from 1st to 21st days after injury- changes in IL-1β levels were correlated with changes in MMP-9 levels. Thus it can be argued that IL-1β presumably was the main regulator of endopepidases synthesis. IL-6 and IL-8 demonstrated the maximum increase on the 3rd day after trauma that might be probably connected with their role as active chemoattractants for different types of leukocytes.

On the 14th day MMP-9 levels decreased by 149% (p <0,05) compared with those on the 7th day, but it was still 472% (p <0,01) higher its control levels. In our study changes in TIMP-1 levels were correlated with changes in MMP-9 levels. On the1st day TIMP-1 levels remained within the control values because of slight increase in MMP-9. An abrupt increase

in MMP-9 levels on the 3rd day after trauma caused an increase in TIMP-1 levels by 154 % ($p < 0,05$). In its turn TIMP-1 inhibits the activity of MMP-9 via binding to its catalytic site and thus forming a tide complex with metalloproteinase [20].

On the 21st day there was a further decrease in MMP-9 from 572% to 261% ($p < 0,01$). Apart from TIMP-1, endopeptidase levels can be reduced by influence of γ-interferon, corticosteroids, α2-macroglobulin [4]. According to literature date MMP-9 is directly correlated to neutrophils count and is inversely correlated to fibroblasts count [4].

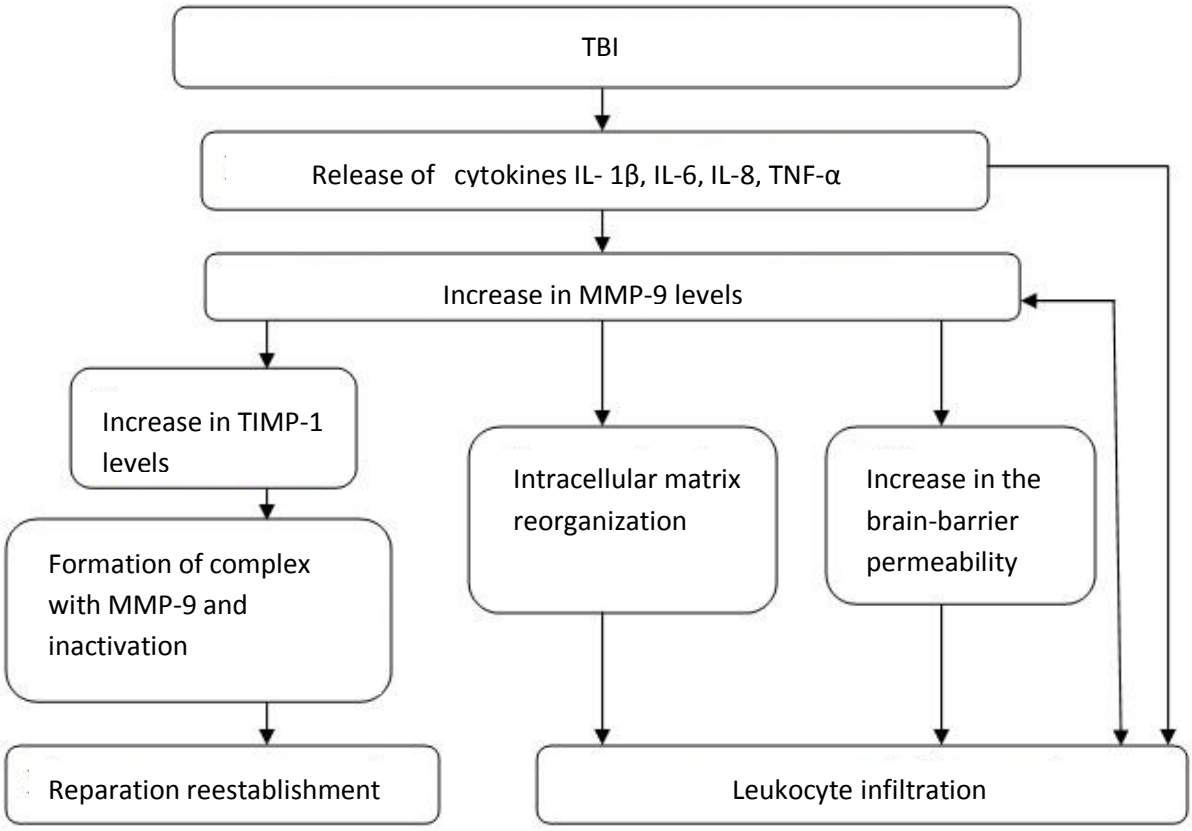

Figure 3. Scheme of relations between matrix metalloproteinases MMP-9 and its inhibitor TIMP-1 in the pathogenesis of TBI; BBB - blood-brain barrier

Thus our data confirm the key role of IL-1β to initiate the increase in MMP-9 levels at experimental TBI. In their turn the increase in MMP-9 and TIMP-1caused by trauma is essential link of pathogenesis of inflammation at TBI because they are involved in both nervous tissue matrix reorganization and intracellular cooperation and repair processes of damaged tissue (Fig. 3).

Zhang H. et al. (2010) suggest that the likely source of MMP-9 at TBI are neutrophils which first come from the systemic circulation and infiltrate the affected brain tissue [20]. In our opinion a decrease in MMP-9 levels on 21stday might indicate alleviation of inflammation

and rebuilding of repair mechanisms. On the other hand metalloproteinase is also a kind of signaling molecules that have the ability to activate various cells. It is established that neutrophils' membrane has receptors for proenzyme pro-MMP-9 and blockade of these receptors impairs migration neutrophils migration [20]. In experimental study J.Y. Hsu at al. (2008) showed that metalloproteinases, paticular MMP-9, are involved in astrocytes activation and migration and glial scar formation at traumatic injury of the spinal cord [12].

Conclusion

MMP-9 levels began to increase on the 1st day after trauma due to influence mainly IL-1β. An abrupt increase in MMP-9 in its turn caused an increase in TIMR-1 levels. Identified changes in IL-1β, MMP-9 та TIMP-1levels at TBI indicate complex relationships between cytokines and intercellular matrix reorganization regulators in formation of intercellular cooperation and inflammation development.

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