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Effect of the p38 MAPK Inhibitor on the Expression of Metalloproteinases and Their Inhibitors during the Formation of Abdominal Adhesions

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Abstract

Background: The aim of this study was to assess the effect of blockade of the p38 mitogen-activated protein kinase (MAPK) on the expression of genes encoding metalloproteinases (MMPs) during the formation of adhesions in the abdominal cavity.

Methods and Results: The experiments were carried out on male Wistar rats (*n*=70). The studies were carried out in two groups: Group 1 (control, n=35) – modelling the adhesive process; Group 2 (experimental, n=35) – modelling the adhesive process with intraperitoneal administration of Seroguard®—a prolonged form of the p38 MAPK inhibitor. The expression of the *MMP1a*, *MMP2*, *MMP7*, *MMP9*, and *TIMP* genes was assessed using real-time PCR.

In the control group, overexpression of the *MMP1a* and *MMP7* genes began from 6 hours after modeling the adhesive process, *MMP9* – from Day 1, *MMP2* – from Day 7 and persisted until the end of observation. With local blockade of p38 MAPK, the level of overexpression of genes encoding MMPs in the early stages was higher than in the control group (*MMP1a* – by Day 1; *MMP7* – by 6 hours and Day 1, *MMP9* – by 12 hours). From Day 3 to Day 14, the *MMP1a* and *MMP7* expression in the experimental group was significantly lower than in the control group.

Conclusion: The performed study demonstrated the involvement of different types of MMPs—collagenases (MMP1a), gelatinases (MMP2 and 9), matrilysins (MMP7)—in the rearrangement of the extracellular matrix during the process of adhesion formation in the abdominal cavity.(**International Journal of Biomedicine. 2021;11(4):446-450.**)

Key Words: adhesive process • p38 MAPK • MMP • TIMP

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Introduction

The formation and remodelling of the extracellular matrix are involved in the development of a range of diseases. Matrix metalloproteinases (MMPs) and their inhibitors play an important role in this process. MMPs are classified based on various criteria such as preferred substrate, enzymatic reaction mechanism, soluble or transmembrane domains, and structural homology.[1] Collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and others are isolated.[2]

For humans, 23 MMPs are known.[3] Collagenases (MMPs 1, 8, 13, 18) are able to break down collagens of types I, II and III. The main substrates of gelatinases (MMPs 2 and 9) are type IV collagen and gelatin. Stromelysins (MMPs 3, 10 and 11) have a broad ability to break down extracellular matrix proteins but are unable to split triple-helical fibrillar collagens. Matrilysins (MMPs 7 and 26) break down some components of the extracellular matrix. Membrane-type MMPs (MMPs 14, 15, 16, 17, 24, and 25) are expressed on the cell surface and activate proMMP. Other MMPs not classified in the previous categories include MMPs 12, 19, 20, 21, 23, 27, and 28.[2] However, it is becoming more and more obvious that this division is somewhat artificial, since there is a whole range of MMPs that do not fit into any of the traditional groups.

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The catalytic activity of MMPs is regulated by tissue inhibitors of metalloproteinases (TIMPs).[4,5] Traditionally, TIMPs have been thought to control the extracellular matrix by directly inhibiting MMP-dependent proteolysis. This classical role of TIMP suggests that elevated TIMP levels lead to fibrosis, while loss of TIMP leads to increased proteolysis of the matrix. Currently, it is believed that the interaction of MMP and TIMP is much more complex and depends on the specific tissue.[6]

The balance between MMP and TIMP levels controls the degree of local degradation of the extracellular matrix at the periphery of cells and thereby influences cellular processes such as migration, proliferation, and survival.[7]

MMPs are widely studied in the context of their involvement in extracellular matrix remodelling in acute and chronic diseases of inflammatory genesis.[8] However, at present, their biological functions are being reassessed, which has revealed many unexpected targets for MMPs, including the processing of chemokines, cytokines, and cell surface receptors.[9,10]

Considering the role of MMPs in extracellular matrix remodelling, MMPs are the object of study in diseases characterized by excessive growth of connective tissue. MMPs play a complex dual role in the development of fibrosis. MMPs can reduce fibrosis by proteolytic cleavage of extracellular matrix components, but under special circumstances, upregulation of certain MMPs also has an adverse effect that leads to the progression of fibrosis in the liver, lungs, and kidneys. Thus, it has been shown that dysregulation and overexpression of MMPs lead to excessive growth of connective tissue and the formation of rough scars.[11]

MMP production is activated in idiopathic pulmonary fibrosis along with inflammatory agents such as TGF-β and INF-γ. The increased levels of expression of MMP9, TIMP1, MMP1 were noted.[12,13] It is believed that increased regulation of MMP1, 2, 10, 11, and 14 is responsible for the progression of fibrosis.[14, 15] At the same time, an increase in MMP19 reduces the severity of fibrosis.[16]

MMP2, 3, 8, 10, 12, 13, and 14 are significantly increased with liver damage, which, according to some authors, accelerates the process of fibrosis formation.[17,18] Interestingly, an increase in MMP9 expression is associated with both the ability to stimulate development and eliminate fibrosis in the liver.[19] An increase in MMP9 expression in myocardial infarction has been proven.[20, 21]

The role of MMPs has also been studied in adhesions in the abdominal cavity. It has been shown that the levels of MMP2 and 9 in the blood serum in an experimental model can serve as prognostic markers for the detection of postoperative adhesions.[22] In clinical observation, it was shown that the concentration of MMP9 in the peritoneal fluid was significantly lower in women with adhesions in the pelvis than in healthy women, and the MMP9/TIMP1 ratio was significantly higher in women with significant adhesions during repeated laparoscopy compared with women with minimal adhesions or without adhesions.[23]

The aim of this study was to assess the effect of blockade of the p38 mitogen-activated protein kinase (MAPK) on the expression of genes encoding MMPs during the formation of adhesions in the abdominal cavity.

Materials and Methods

In this study, we used 70 nine-month-old male Wistar rats weighing 220–250 g. The rats were sedated using Ketamine 50 mg/kg, Droperidol 2.5 mg/kg and Atropine 0.4 mg/kg. An aseptic inflammatory process in the abdominal cavity was simulated by opening the serous-muscular layer of the cecum with a 1 cm incision, followed by closing the wound using screw sutures and the scarification of the right lateral canal.[24, 25]

The animals were kept in accordance with good laboratory practice. The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the Irkutsk Scientific Center of Surgery and Traumatology (Protocol No. 6 of 04.18.2017).

The studies were carried out in two groups: Group 1 (control, n=35) – modelling the adhesive process; Group 2 (experimental, n=35) – modelling the adhesive process with administration of Seroguard® (conjugate the 4-[4-(4-fluorophenyl)-2-(4-methylsulfylphenyl)-1H-imidazole-5-pyridine with polyvinyl-imidazole, JSC "Pharmasyntez") in a volume of 3 ml during the completion of the operation.[26]

The animals were sacrificed, and tissues were collected for examination at 7 time points, ranging from 6 hours to 30 days.

The study of gene expression in the serous-muscular layer of the cecum of intact animals (n=5) was used to determine the basic expression of genes.

The samples of the caecum lesion zone were collected from the experimental animals, placed in RNAlater solution (Ambion, Canada, Cat. N 7020), and then crushed in liquid nitrogen. RNA extraction was performed using the RNeasy Mini Kit (Qiagen GmbH, Germany, Cat. N 74104). RNA purification was performed with the Rnase-Free DNase Set (Qiagen GmbH, Germany, Cat. N 79254). cDNA synthesis was performed using the cDNA – RT2 First Strand Kit (Qiagen GmbH, Germany, Cat. N 330401).

The gene expression analysis was performed using real-time polymerase chain reaction (PCR) on a CFX96 Bio Rad device (USA). Gene expression was determined using the RT2-Profiler ™ Array Rat Wound Healing Kit (Qiagen GmbH, Germany). The genes are listed in Table 1. We used the RT2 SYBR Green qPCR Mastermix oligonucleotide kit (Qiagen GmbH, Germany, Cat. N 330503). The relative fold difference in gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Table 1.
Genes tested using real-time PCR

Notation	Gene name	GenBank	Unigene
MMP1a	Matrix metallopeptidase 1a (interstitial collagenase)	NM_001134530	Rn.79007
MMP2	Matrix metallopeptidase 2	NM_031054	Rn.6422
MMP7	Matrix metallopeptidase 7	NM_012864	Rn.10282
MMP9	Matrix metallopeptidase 9	NM_031055	Rn.10209
TIMP1	TIMP metallopeptidase inhibitor 1	NM_053819	Rn.25754

Results

We have evaluated the effect of local application of the p38 MAPK inhibitor on the expression of genes encoding metalloproteinases MMP1a, MMP2, MMP7, MMP9, and the TIMP1 inhibitor in the adhesion formation zone. For this purpose, Seroguard® was used. It is known that Seroguard® is a prolonged form of the p38 MAPK inhibitor intended for intraperitoneal administration.[26, 27]

It was found that in the control group, overexpression of the *MMP1*a and *MMP7* genes began as early as 6 hours after modeling the adhesive process, *MMP9* – from Day 1, *MMP2* – from Day 7 and persisted until the end of observation, indicating the ongoing restructuring of tissues in the damaged zone. The differences were significant with the indices of intact animals for the *MMP1a* and *MMP7* genes at all observation periods, for the *MMP9* gene – from Day 1 to Day 30, for the *MMP2* gene – from Day 7 to Day 30.

The overexpression of the *MMP1a*, *MMP7* and *MMP9* genes has two peaks, on Days 3 and 14, and the overexpression of *MMP2* has one peak – on Day 14 (Figure 1).

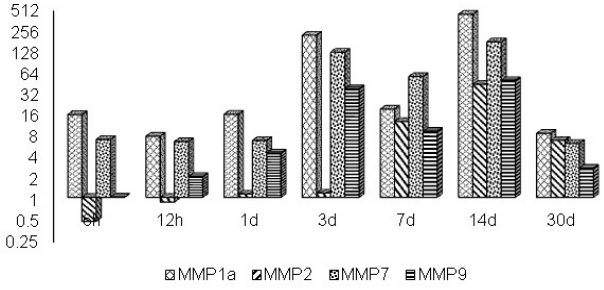

Fig. 1. *Expression of genes encoding MMPs in animals of the control group.*

In the experimental group, all genes encoding MMPs, except for *MMP2* (increased from Day 3), were overexpressed as early as 6 hours after modeling the adhesive process (Figure 2), and gene activity was increased until the end of observation. The differences were significant with the indices of intact animals for all genes encoding MMPs, except for *MMP2*, at all periods of observation. The first peak of activity for *MMP1a* falls on Day 1, *MMP9* – on Day 3, and *MMP7* – on Day 7. The second peak of activity for all genes encoding MMPs is observed on Day 14.

In the experimental group, from 6 hours to a day after injury, the level of overexpression of genes encoding MMPs in the injury zone was higher than in the control group. The differences were significant for *MMP1a* in a period of Day 1, for *MMP7* – in a period of 6 hours and Day 1, for *MMP9* – in a period of 12 hours. From Day 3 to Day 14, the activity of genes encoding MMPs in the experimental group was significantly lower than in the control group. Differences were significant for *MMP1a* at Day 3 and Day 14, and for *MMP7* – at Day 3. However, by the end of the observation (30 days), the expression in the experimental group again exceeds that of the control group. The differences were significant for the *MMP7* and *MMP9* genes.

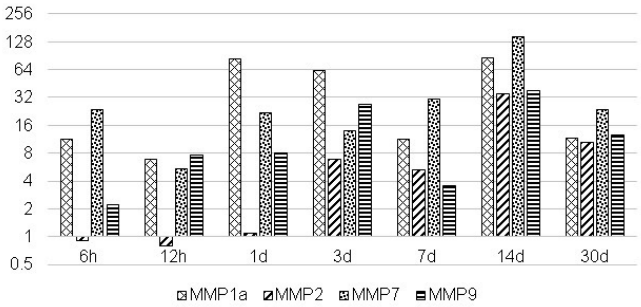

Fig. 2. *Expression of genes encoding MMPs in animals of the experimental group.*

At the same time, in the experimental group, the expression level of *TIMP1* (known as an inhibitor of MMP1) in the early stages (Day 3) was significantly higher than in the control group. In the rest of the periods, no significant differences were found between the groups (Figure 3).

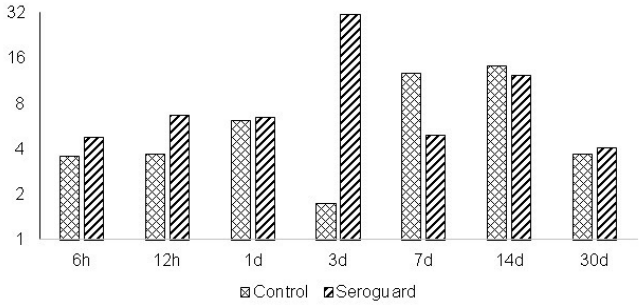

Fig. 3. *The TIMP1gene expression in animals of the control and experimental groups.*

Interestingly, in animals of the control group, the severity of overexpression of the *MMP1a* gene was significantly higher than the *TIMP1* gene, with the maximum severity of differences on Days 3 and 14 (Figure 4).

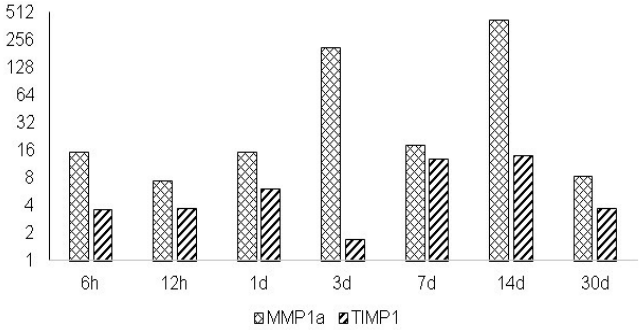

Fig. 4. *Expression of the TIMP1 and MMP1a genes in animals of the control group.*

In animals of the experimental group, the difference was less pronounced (Figure 5).

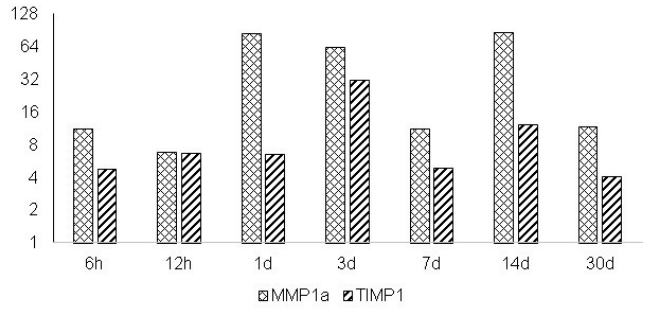

Fig. 5. *Expression of TIMP1 and MMP1a in animals of the experimental group.*

Conclusion

The performed study demonstrated the involvement of different types of MMPs—collagenases (MMP1a), gelatinases (MMP2 and MMP9), matrilysins (MMP7)—in the rearrangement of the extracellular matrix during the process of adhesion formation in the abdominal cavity. Moreover, the peaks of metalloproteinase activity coincide with the periods of active restructuring and formation of the extracellular matrix and fall on Days 3 and 14 (control group). The overexpression of the *MMP*2 and *MMP7* genes are most pronounced.

Local blockade of p38 MAPK affects the overexpression of MMPs in the peritoneal injury zone, leading to early pronounced overexpression (up to Day 1) of the *MMP1a*, *MMP7*, and *MM9* genes. However, in the control group, the expression of the *MMP1*a and *MMP7* genes on Days 3 and 14 is higher than in the experimental group with local blockade of p38 MAPK.

Competing Interests

The authors declare that they have no competing interests.

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