Changes in gene expression of metalloproteinases-2 and -9 and their inhibitors TIMP2 and TIMP3 in human glioma cells exposed to low levels of fluoride

Magdalena Nowak *, Marta Skórka-Majewicz *, Wojciech Żwierełło *
Pomeranian Medical University in Szczecin, Department of Medical Chemistry, Powst. Wlkp. 72, Szczecin 70-111, Poland

INTRODUCTION

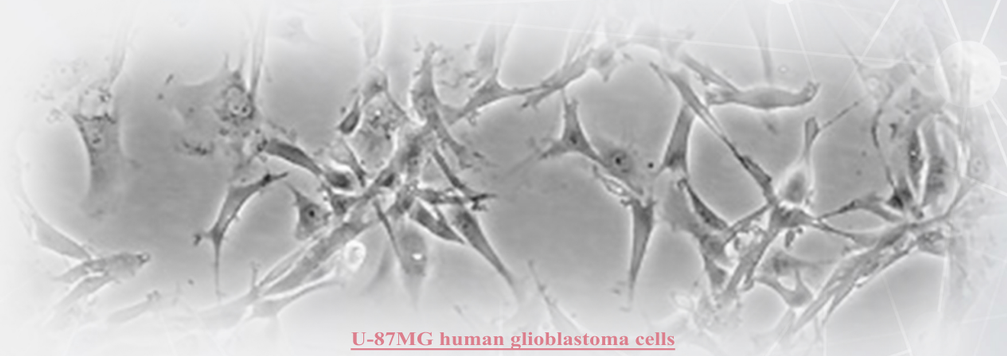
Fluorine compounds are common environmental pollutants and may excessively penetrate the human body, especially the brain (fluoride penetrates the blood-brain barrier). Some of the latest studies have shown that fluoride may interfere with some of the metabolic pathways involved in the development of invasive potential in many types of cancer (eg Wnt/catenin or NF-κB). One of the stages of tumor invasion is the degradation of the extracellular matrix by metalloproteinases (MMP-2 and MMP-9), which allows the migration and metastasis of cancer cells. Taking into account the above facts, we decided to check whether low concentrations of fluoride affect the expression level of genes encoding MMP-2, MMP-9, and their TIMP-2 and TIMP-3 inhibitors in human glioblastoma cells.

METHODS

U-87MG human glioblastoma cells were cultured with EMEM medium (10% FBS, 2 mM glutamine, 1% NEAA), 1 mM sodium pyruvate, 100 IU / ml penicillin, 10 μg / ml streptomycin) under optimal conditions (at 37 ° C, in an atmosphere of 5% CO2, with 95% humidity). Cells were treated with sodium fluoride (NaF; 1-5 μM) for 24, 48 and 72 hours.

The verification of the level of expression of genes that encoding MMP-2, MMP-9, and their TIMP-2 and TIMP-3 inhibitors were doing to be conducted using the PCR technique in real-time (RT-PCR). The total RNA was isolated from both control and study cells and were used in the reaction of reverse transcription to synthesize cDNA. The resulting cDNA matrix strand was used in the RT-PCR reaction to determine the level of expression of the studied genes with the use of specific starters. To monitor the number of products that are created during subsequent cycles, the SYBR Green (Roche) dye was used. To determine the relative level of expression of the studied genes, a comparative method was applied (2^-ΔCt) with the analysis of the mRNA level of the reference gene of glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

The analysis of the expression level of the MMP-2, MMP-9, Timp-2, and Timp-3 genes was carried out by RT-PCR.



U-87MG human glioblastoma cells
(https://www.lgcstandards-atcc.org/products/all/HTB-14.aspx?geo_country=pl)

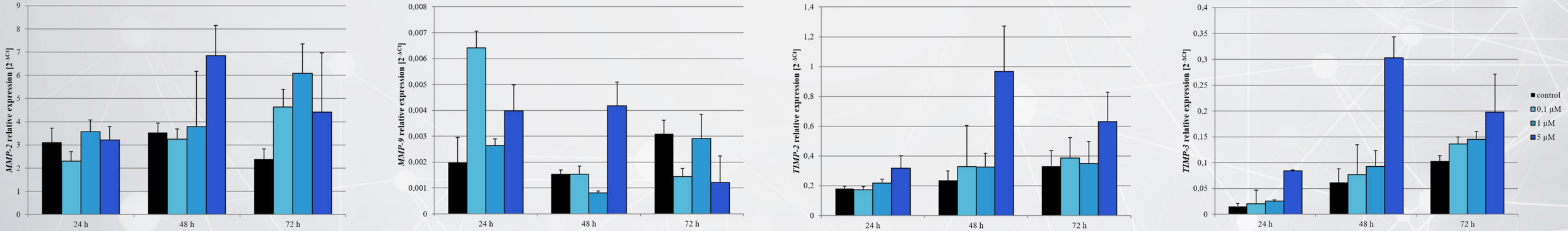


Fig 1. Relative expression

RESULTS

The results indicate that NaF (0.1-5 μM) can disrupt the expression of MMP-2, MMP-9, Timp-2, and Timp-3. In the case of MMP-2, there was an approx. 2-fold increase in expression in 48h (5 μM NaF) and about 2.5-fold increase in expression in 72h (0.1-5 μM NaF). For MMP-9, an approximately 3-fold increase in expression was observed in 24h (0.1 μM NaF) and 48h (5 μM NaF). Both Timp-2 and Timp-3 showed a significant increase in expressio observed at all time points especially at the highest concentration of NaF (5 μM).

CONCLUSIONS

The obtained results may suggest that even low concentrations of fluorine compounds may have an undesirable influence promoting the invasive potential of human glioblastoma cells.

DISCUSSION

Even though the studies conducted over the last 30 years have not shown a clear contribution of fluoride to the formation of neoplasms, a lot of controversies have recently been raised about its influence on the regulation of numerous metabolic pathways which are significant from anticancer therapies. In light of recent studies, the influence of fluoride on the invasiveness of cancer cells seems highly probable but is practically unexplored. Considering the numerous processes taking place in the brain under the influence of fluoride, it seems extremely important to investigate the influence of this environmental toxin on the progression and development of brain tumours. The results of this project may increase the knowledge about the course of brain tumour invasiveness and the signal pathways that control it in people exposed to fluoride. The results can also play a role in raising public awareness of the toxic effects of fluoride on human health, especially in the context of CNS and prevention among people exposed to fluoride.

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