Determination of the effect of selected low fluoride concentrations on migratory abilities of human glioma U-87MG cell line

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INTRODUCTION

Fluorine (F) is an element that belongs to the group of halogens. Small amounts of fluorides are necessary for the proper development of bones and teeth. However, increased intake of fluorine and continuous exposure has negative effects on the human organism. Its effects on many organs, such as the liver, pancreas, lungs, heart, skeletal muscles, and kidneys, are well documented. It is well documented that fluorine ions cross the blood–brain barrier. Fluoride also causes neurodegenerative changes in the cerebellum, hippocampus, and cerebral cortex. By inhibiting the synthesis of neurotransmitters and reducing the number of their receptors, it reduces the ability to learn and remember. Some hypotheses that prolonged exposure to fluoride may hurt intelligence, especially in children. Primary brain tumors are the leading solid tumors in children and the second most common cause of death after leukemia in this age group. Since it is suggested that fluoride may have a strong influence on the development of CNS already in the prenatal and postnatal periods, it may be one of the adverse factors influencing the course of CNS tumors in young people.

Some recent work has shown that fluoride affects many metabolic pathways that can theoretically be involved in the development of invasive potential in many types of neoplasms, including brain neoplasms. These include the Wnt/β-catenin, PI3K/Akt, and NF-kB pathways.

It is known that fluoride stimulates bone formation and although these mechanisms are still unknown, research on bone formation in rats exposed to high doses of fluoride has shown that it promotes osteoblast differentiation through the Akt and GSK-3β-dependent activation of the Wnt/β-catenin signaling pathway. Moreover, the results of another study indicate that long-term exposure to high levels of fluoride may cause a decrease in Dkk-1 and SOST, physiological inhibitors of the Wnt/β-catenin pathway activation. The increased activity of this pathway leads to GSK-3β inhibition and β-catenin degradation. This relationship is also confirmed by studies in which it was demonstrated that with increased levels of exposure to fluoride and arsenic, the activation of the Wnt/β-catenin pathway gradually increased, while the content of Dkk-1 decreased drastically. Other studies on murine tooth fluorosis have confirmed that both Wnt and Rho pathways have increased activity when ameloblasts (ALC) were treated with sodium fluoride. Finally, this year’s study for the first time revealed abnormal changes in GSK-3β/β-catenin signaling in rat brain exposed to fluoride, emphasizing the important role of GSK-3β/β-catenin signaling in fluoride-induced neurotoxicity.

The transcription factor NF-kB controls the expression of many genes whose protein products are involved in a wide range of processes associated with neoplastic invasiveness such as proliferation, apoptosis, angiogenesis, and metastasis. It has been proven that fluoride can play a role in the regulation of the NF-kB pathway. Studies on skeletal muscle cells have shown that fluoride increased the expression of PI3K, BAD, Bel-2, Bax, and caspase-9, while the expression of PDK1 and P-Akt1 was significantly reduced. The results of studies on mice exposed to different doses of fluoride showed that NaF exceeding 12 mg/kg induced inflammatory reactions in the kidneys by activating NF-kB and reducing the expression of anti-inflammatory cytokines (IL-4 and IL-10) and increasing the levels of PGE2, iNOS, COX-2, IL-6, IL-8 in comparison to control. A similar study in mice exposed to sodium fluoride showed that the liver had a strong inflammatory response with the formation of numerous brain disorders such as IL-1β, IL-6, IL-8, COX-2, and MCP-1 by activating the MAPK and NF-kB pathways.

METHODS

"Wound healing" assay

After 72 hours or three months of passaging in appropriate NaF concentrations (0.1-10 µM), U-87MG cells were grown in 6-well plates (controls + NaF concentrations indicated). After reaching confluence (~ 80%), the cell layers were scratched with 200 µl pipette tips and washed with PBS to remove cell debris. Fresh medium without serum was added to each well and the wound closure was visualized at 0, 3, 6, 12, and 24 hours using a microscope.

Cell migration test

After 72 hours or three months of passage in appropriate NaF concentrations (0.1-10 µM), a total of 1 × 10⁵ U-87MG cells (controls + 0.1-10 µM concentration of NaF; in serum-free EMEM containing 1% serum albumin bovine species) were inoculated in the upper chamber of a 24-well Transwell system with a pore size of 8.0 μm. EMEM containing 10% FBS was added to the lower chamber. After incubation, non-migrating and non-invasive cells on the upper surface were removed with a cotton swab and cells on the lower surface were fixed with 4% paraformaldehyde and stained with Giemsa. Photographs were taken and cells were counted under the microscope.

RESULTS

Our observations showed that both in the case of short-term and long-term culture in the presence of sodium fluoride, the mobility of glioblastoma cells significantly increased. Importantly, the effect was visible at the lowest concentration (0.1 µM) and increased at higher concentrations (1-10 µM) of NaF.

CONCLUSIONS

The results of these studies can shed new light on the therapeutic approach in people with brain tumors and draw attention to environmental factors such as fluoride, which may already hamper the treatment of patients at low doses. 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 tumors.