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EXPERIMENTAL STUDY

Low concentrations of methamphetamine can suppress inflammation in trophoblast JEG-3 cell line induced by nicotine

Zhaleh H[1], Amer Ridha A[2], Amini K[3]

Substance Abuse Prevention Research Center, Institute of Health, Kermanshah University of Medical Sciences, Kermanshah, Iran. **hossain_jale@yahoo.com**

ABSTRACT

AIM: Nicotine at high concentrations induces apoptosis in trophoblastic cells through induction of cell cytotoxicity and Reactive Oxygen Species (ROS). Methamphetamine in low dose has pharmaceutical properties. It seems that this components in low dose can protect the trophoblastic cells from nicotine-induced cell death.

METHOD: Trophoblastic (JEG-3) cells grown in DMEM culture medium. MTT assay test detected the cell viability and Lactate Dehydrogenase test measured the cells cytotoxicity. Griess reaction was used for NO production analysis. Cell migration traced by wounding technique. Human Cytokine Array Focused 13-plex was also used for analysis of IL-1α, IL-1β, IL-6, INFγ, and TNFα pre-inflammatory cytokines.

RESULTS: Methamphetamine, in very low dose (pM), increased the cell viability and NO production, and decreased cell cytotoxicity, IL-1α, IL-1β, IL-6, INFγ, and TNFα pre-inflammatory cytokines of JEG-3 cell which were exposed to high dose of nicotine, respectively. Cell migration was enhanced by low dose of methamphetamine in JEG-3 cells.

CONCLUSION: Methamphetamine in very low dose suppressed the JEG-3 cell death induced by high dose of nicotine *(Fig. 5, Ref. 48)*. Text in PDF *www.elis.sk.*

KEY WORDS: methamphetamine, nicotine, cell death, NO.

Introduction

Nicotine is an alkaloid found in the tobacco plant as a most important addictive compound and it has devastating effects on global health because of rising smoking rates (1, 2). Nicotine concentration in the blood increases in people who have long-term smoked, and often acts as an agonist for nicotinic acetylcholine receptors (3–5). Although in various organs, such as heart, kidneys, intestines and lungs it has adverse effects and causes diseases, it's most important effects are in the central nervous system, causing various disorders and anomalies (6–8). On the other hand, nicotine has the potential to induce mutations in the genetic material of the cells, in particular the epithelial cells of the intestine, cervix, urinary bladder, stomach, lungs, colon, breast, and mouth and can trigger the first step in cancer development by stimulating cellular transfusion (9–13). High dose nicotine can cause ROS production and has an apoptotic effect on cell cultures, but low dose has protective effect on neuronal cells. Gao, T et, al in a study, stated that nicotine has no effect on cell growth in low concentrations (0.01 μM), but at high concentrations (0.1 μM, 1 μM and 10 μM) significantly inhibits cell growth of A549 cells (14). Nicotine activates the AChRs receptor as a neuronal nicotinic acetylcholine receptor, which induces p53 protein as a tumor suppressor and proteins p21 as a cell cycle inhibitor (15). Nicotinic acetylcholine receptors of Purkinje cell's which are activated by nicotine can trigger apoptotic process that ultimately reduces the Purkinje cell population (16).

Methamphetamine is a stimulant used in the several past decades as a drug for treating attention-deficit hyperactivity, narcolepsy and obesity, and increased consciousness (17–19). Methamphetamine leads to dopamine, norepinephrine, and serotonin release (20, 21), also it cause an increase in satisfaction, attention and strength. Myocardial infarction, concussion, annexation, rhabdomyolysis and psychosis are the most important methamphetamine side effects which may eventually cause death (22–24).

JEG-3 choriocarcinoma cell line as a monoclonal and highly proliferative trophoblastic model was selected, which is derived from a human choriocarcinoma and shows the biological and biochemical properties of syncytiotrophoblasts (25, 26). Although

[1]Substance Abuse Prevention Research Center, Institute of Health, Kermanshah University of Medical Sciences, Kermanshah, Iran, [2]Department of Dentistry, AL-Zahrawi University College, Holy Karbala, Iraq, and [3]Department of Biotechnology, Faculty of Advanced Science and Technology, University of Isfahan, Isfahan, Iran

Address for correspondence: H. Zhaleh, Dr, PhD, Substance Abuse Prevention Research Center, Institute of Health, Kermanshah University of Medical Sciences, Kermanshah, Iran.

morphine and methamphetamine in high concentrations can cause cell death due to cell cytotoxicity and apoptotic events, in very low concentration both of them seem to protect cultured cells from induced death. Therefore, we tried to study the possible protective effects of morphine and methamphetamine in different concentrations on nicotine-induced cell death of JEG-3 cell line. In this study cell viability, cytotoxicity, NO production, cell migration and neurotic factors such as IL1α, IL1β, IL6, INFγ and TNFα, in different concentrations of methamphetamine and constant concentration of nicotine, which leads to cell death, were obtained and protective potency of both drugs was analyzed.

Materials and methods

Cell culture

In this study, DMEM culture medium (Gibco) was used in T-25 cm^2 tissue culture flasks and JEG-3 cell were cultured in this media which contained supplementary compounds as listed below: 10 % fetal bovine serum (FBS, Gibco), 1 % non-essential amino acid (NEAA, Sigma), 2 mM L-glutamine (Sigma), 100 IU/ml penicillin (Sigma), and 100 µg/ml streptomycin (Sigma). Incubation of the cell culture was performed at 37 ºC in 5 % CO_2 medium, once in every two days. Trypsin-EDTA 0.25 % (Sigma) was used for trypsination of cell cultures. 1×10^4 cells/well was final density of the cells in 24-well culture plates.

Treatment

The cells were washed by PBS, pH 7.4 after a day of cell plating. Two groups of treatments were performed: Control: culture medium, Treatment 1: 1 mM nicotine, Treatment 2: 1 mM nicotine and 1 pM Methamphetamine, Treatment 3: 1 mM nicotine and 10 pM Methamphetamine, Treatment 4: 1 mM nicotine and 100 pM Methamphetamine, Treatment 5: 1 mM nicotine and 1 nM Methamphetamine, Treatment 6: 1 mM nicotine and 10 nM Methamphetamine, and Treatment 7: 1 mM nicotine and 100 nM Methamphetamine.

Cell viability (%) (MTT assay)

15×10^3 cells were loaded into 96-well plates. 200 µL of DMEM media containing 0.2 % BSA was added to each plate. An enzyme-linked immunosorbent assay (ELISA) reader measured the optical density of the wells at 570 and 630 nm.

Cytotoxicity

The cells were plated in 24 well culture plates with density of 10^4 cells/mL. Damaged cells release Lactate dehydrogenase (LDH) into the environment, so concentration of LDH indicates the cell cytotoxicity. An ELISA Reader (EL800; USA) measured the activity of LDH at 490 or 492 nm and reference wavelength of more than 600 nm. All experiments were replicated at least three times.

Nitric oxide test

An ELISA Reader measured the level of nitric oxide in different treatments after incubation of the cells in 37 °C for 30min. In this study, Griess reaction was performed for measurement of the amount of NO production in different treatments (27).

Migration assay

In this study, for assessment of cell migration, cells were plated in 6 well culture plates at confluent density. A sterile razor blade was used to perform the wounding in different treatments, after pressure induction. After washing the monolayer of wounded cells, the regions for more analyzing were selected based on the previous study (28). The cells were then incubated for 24 h with culture medium, fixed and cells that had migrated from demarcation line were counted in random six fields under phase contrast microscopy.

Cytokines

In this study, Human Cytokine Array Focused 13-plex was used for measurement of IL-1α, IL-1β, IL-6, INFγ, and TNFα pre-inflammatory cytokines in different treatments. The cells were exposed to different treatments for 48 h and activity of these cytokines were measured based on the company's protocol.

Results

Cell viability

MTT results showed that viability of treatment1 reached zero percent. It means that nicotine disrupted the neurotic cells at a high dose. Results of treatments 2–7 revealed that methamphetamine has improved the viability of the cells in very low concentration. An Intragroup significant difference was observed in cell viability of treatments 2–7 ($p < 0.05$) (Fig. 1).

Cell cytotoxicity

LDH test revealed that nicotine increased the cell cytotoxicity of JEG-3 cells in high dose. The cell cytotoxicity of treatment 1 (nicotine) was near to 100 %. Methamphetamine in low concentration decreased cell cytotoxicity of the cells which were exposed to cytotoxic concentration of nicotine. The cell cytotoxicity of treatments 2–5 was significantly different with treatment 1 (nicotine group). A significant intragroup difference was observed between treatments 2–7 ($p < 0.05$) (Fig. 2).

Nitric oxide

Production of NO in treatment1 (nicotine group) was so high. High concentration of NO leads to cell death. Methamphetamine

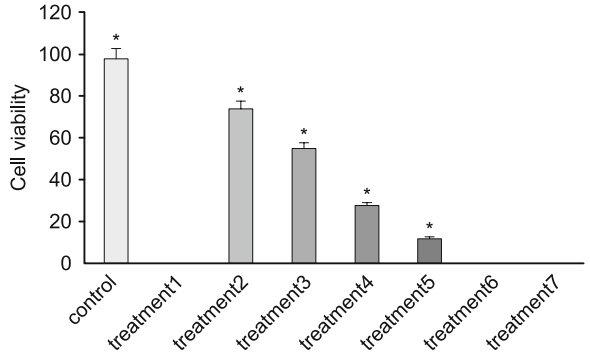

Fig. 1. The cell viability of different treatment media on JEG-3 cells after 48 h.

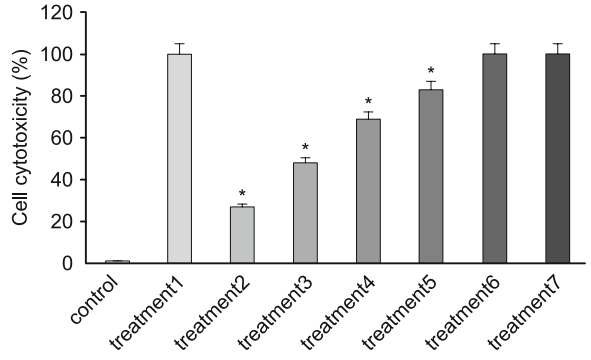

Fig. 2. The cell cytotoxicity of different treatment media on JEG-3 cells after 48 h.

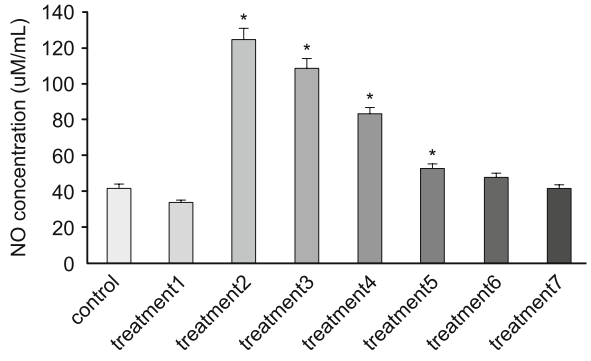

Fig. 3. The amount of nitric oxide production of different treatment media on JEG-3 cells after 48 h.

in very low concentration suppressed the production of NO. Treatment 2 suppressed NO production most effectively. NO production in treatments 2–5 were lower than treatment1 (nicotine group) significantly. A significant intragroup difference was observed between treatments 2–7 ($p < 0.05$) (Fig. 3).

Migration assay

High dose of nicotine in treatment1 suppressed cell migration significantly. Treatments 2–7 effectively suppressed the anti-migration efforts of high dose nicotine. The results of treatment 2 cell migration showed the most effective enhancement in cell migration in methamphetamine treatments. A significant intragroup difference was observed between treatments 2–7 ($p < 0.05$) (Fig. 4).

Cytokines

The concentration of IL-1α, IL-1β, IL-6, INFγ, and TNFα pre-inflammatory cytokines in treatment 1 (nicotine group) increased significantly. High doses of pre-inflammatory cytokines lead to inflammation and disruption of neurotic cells. Treatments 2–5 decreased the production of these pre-inflammatory cytokines significantly. Treatment 2 decreased the production of these cytokines more effectively than other treatments. A significant intragroup difference was observed between treatments 2–7 ($p < 0.05$) (Fig. 5).

Discussion

During pregnancy, smoking has devastating effects on the fetus. Nicotine deregulates the neurodevelopment when fetus is exposed to it. It may lead to psychiatric problems, such as substance abuse (29). Nicotine disrupts the trophoblastic cells and induces apoptosis in these cells. There is a hypothesis that explains nicotine initiates the production of free radicals. Free radicals result in reactive oxygen species which promote the inflammation through the increase of inflammatory cytokines production (30–32). Increase of knowledge about that can help us to control neurotic diseases induced by nicotine. A study revealed that nicotine promotes free radicals in JEG-3 cells, a transformed cell line of trophoblastic origin, and vitamin C and E can scavenge these radicals and protect the placental cells from nicotine induced cell death (33). Methamphetamine was initially used at very low-dose as a drug for sleep deprivation (34). It also increases awareness, weight and self-confidence (35–37). High concentrations of methamphetamine can induce cell death in neuronal cell lines, but in low concentrations, it may exhibit different behavioral patterns and protect

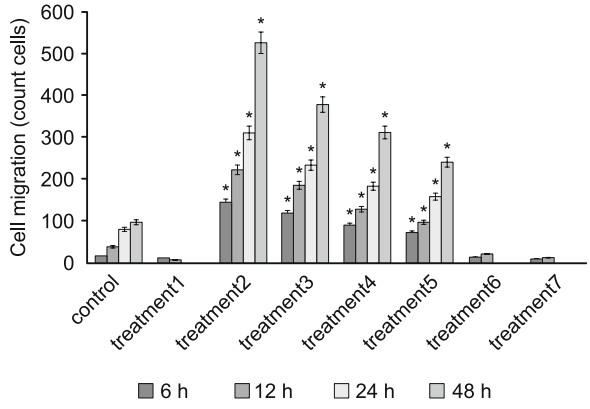

Fig. 4. Results of migration assay of different treatment media on JEG-3 cells after 6 h, 12 h, 24 h, and 48 h.

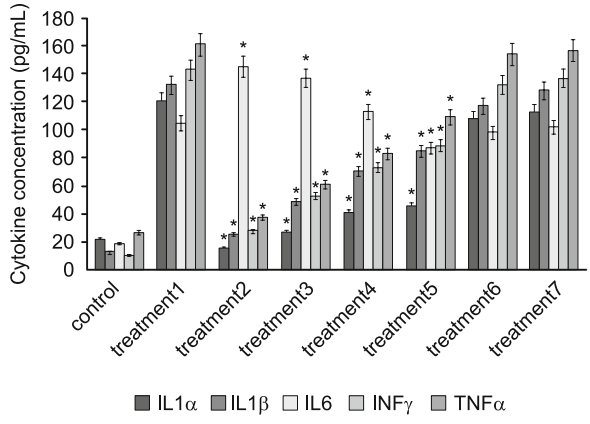

Fig. 5. Results of pre-inflammatory cytokines assay of different treatment media on JEG-3 cells after 6 h, 12 h, 24 h, and 48 h.

nerve cells from cell death (38). Dopamine and norepinephrine are involved in low-dose methamphetamine neuroprotective effects (39, 40). Nitric oxide signaling is suggested as main pathway which enhances the phosphorylation of PI3K. Also, it can increase the expression of pAKT (41). Our experiments showed that low dose methamphetamine increases the production of NO in a dose-dependent manner. Impact of NO observed in increase of reactive oxygen species and free radicals which start the inflammation process that leads to apoptosis of placenta and neuronal cells (42, 43). In this study, we reported that methamphetamine, in dose-dependent manner, induced the production of NO in JEG-3, whereas high concentration nicotine suppressed its production. NO affects the production of pre-inflammatory cytokines which lead to neuroinflammation as a most important cause of cell death in nerve system (44–46). NO, and ROS are involved in immune system regulation and they present complex modulating effects on the inflammatory process in the brain (47). A previous study indicated that infiltration of neutrophils and activation of microglia reduces when reuptake of serotonin is blocked. Furthermore, this blocking reduces the activity of NF-kappaB pro-inflammatory factor (48). In this study, we demonstrated that inflammation was suppressed by NO production induced by methamphetamine. Methamphetamine reduced the concentration of the IL1α, IL1β, IL6, INFγ, and TNFα proinflammatory cytokines in high-dose nicotine treated JEG-3 cells. Reduction of these cytokines by methamphetamine leads to suppression of inflammation and protection of nerve cells from nicotine induced cell death in a dose-dependent manner. A previous study showed that opioids decreased expression of X subunit, the immunoproteasome catalytic subunit LMP7, and activity of 20S proteasome which were increased by rotenone. These events lead to suppression of inflammation via NO. Increase of free ubiquitin by opioids indicates that it reduces the oxidized proteins through degradation and protects the neuronal cells from inflammation and death (45). Finally, the results of cell migration revealed that methamphetamine protects JEG-3 cells from nicotine-induced cell death in a dose-dependent manner.

Conclusion

In this study, we concluded that methamphetamine, in a dose-dependent manner, can protect JEG-3 cells from effects of high dose nicotine. Very low dose methamphetamine suppressed the JEG-3 cell death which was caused by high dose of nicotine through the production of NO and inhibition of pro-inflammatory cytokines.

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