Probl Cryobiol Cryomed 2023; 33(4):293
https://doi.org/10.15407/cryo33.04.293

Фіпроніл погіршує проліферацію в нюховому нейрогенному відділі та змінює чутливість до низьких температур у дорослих мишей

К. Фабіанова[1], Й. Піско[2], Д. Фабіан[2],
М. Мартончикова[1], А. Рачек[1], Е. Рачекова[1]
[1]Інститут нейробіології, Центр біомедичних досліджень, Словацька академія наук, м. Кошице, Словацька Республіка
[2]Інститут фізіології тварин, Центр біологічних наук, Словацька академія наук, м. Кошице, Словацька Республіка

Fipronil Impairs Proliferation in the Olfactory Neurogenic Region and Alters Low Temperature Sensitivity in Adult Mice

K. Fabianová[1], J. Pisko[2], D. Fabian[2],
M. Martončíková[1], A. Raček[1], E. Račeková[1]
[1]Institute of Neurobiology, Biomedical Research Center, Slovak Academy of Sciences, Košice, Slovak Republic
[2]Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice, Slovak Republic

Pesticides are widely used in agricultural and household environments to kill vectors of disease, such as pests in agriculture. They are potentially toxic to other organisms, including humans and need to be used safely and disposed of properly. Fipronil (FPN) [5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-fluoromethylsulfinyl pyrazole] is a broad-spectrum insecticide that belongs to the phenylpyrazole chemical family. It is effective in controlling the insects that are resistant to other insecticides by blocking gamma aminobutyric acid chloride channels in insect's central nervous system. The neurotoxic effect of FPN was tested in the present study with emphasis on proliferation of cells in the olfactory neurogenic region involving the subventricular zone (SVZ) and the rostral migratory stream (RMS). Besides that, changes in low temperature sensitivity were evaluated. FPN was administered to experimental mice (three oral applications) using a syringe. Then, the mice brains were removed from the skulls and stored in 30% sucrose for cryoprotection. Afterwards, thin section from deeply frozen brains (-30°C) were cut on cryomicrotome. Subsequent Ki-67 immunohistochemical labelling was performed on slices stored by 4°C, in order to preserve the immunoreactivity of the sample. To evaluate the changes in low temperature sensitivity, the performances in the cold plate test were monitored. In this test, the limbs of animals were exposed to low temperatures (0°C) and the withdrawal latency was scored. Microscopic evaluation showed differences in the density of Ki-67+ cells within the SVZ/RMS neurogenic region in experimental mice in comparison with controls. The density of proliferating cells within the RMS was markedly reduced in FPN administered mice when compared to control mice. The reduction in proliferating cells density in experimental mice was even more striking within the SVZ. FPN administered mice were tested for low temperature sensitivity using the cold plate assay. Although mice from experimental group showed a little longer cold plate withdrawal latency than control group, no significant differences in latencies to lick a paw or jump off were observed. Our findings demonstrate a neurotoxic effect of FPN on cell proliferation in the SVZ/RMS neurogenic region and behavioural tasks related to nociception.

Probl Cryobiol Cryomed 2023; 33(4):293
https://doi.org/10.15407/cryo33.04.293a

Вплив середовища, кондиціонованого кріоконсервованими клітинами плаценти, на органотипову культуру яєчників

Т. Михальчук, В. Прокопюк, М. Шевченко, О. Прокопюк
Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

Effect of Medium Conditioned by Cryopreserved Placental Cells on Organotypic Ovarian Culture

T. Mykhalchuk, V. Prokopiuk,
M. Shevchenko, O. Prokopiuk
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

The need to find new methods of therapy for diseases of the female reproductive system and to increase fertility is explained by difficult demographic situation, significant spread of pathology and insufficient effectiveness of its treatment. Recent studies demonstrate the feasibility of using cryopreserved cell products of placental origin. Under *in vitro* conditions, a convenient model for studying the effect of placental products, reproducing the pathological processes of the female reproductive system is the organotypic cultivation of tissue fragments, that ensures the preservation of the histological and three-dimensional structure with inter- and extracellular interactions, and the cellular matrix components.

The aim of the work was to study the effect of the medium conditioned with cryopreserved placental cells on the mouse organotypic ovarian culture (OOC).

For the OOC obtaining the ovaries of BALB/c mice in an estrous phase were isolated, cut into 3 mm fragments and cultured in 24-well plates at the rate of 10 mg tissue/ml of DMEM enriched with 10% FBS (control) or the medium conditioned with cryopreserved placental cells (MCCPC), in a CO_2 incubator at 37°C in 5% CO_2 atmosphere. The OOC was morphologically analysed by histological examination of ovarian tissue fragments (OTF) stained by hematoxylin and eosin, OTF metabolic activity was assessed by the resazurin reduction test.

Primary OTF had a typical structure: the hilum of the ovaries consisted of connective tissue with vessels, generative elements were present at various stages of development (primordial, primary and secondary follicles, corpora lutea), oocytes, theca and granulosa cells were clearly visualized in the follicles. During OTF cultivation in DMEM, the corpus luteum and primordial follicles retained their structure, however, hyperhydration, ruptures in the stroma and hilum of the ovary, destruction of oocytes, and separation of granulosa cells from the theca were observed. When cultivating in MCCPC, the general structure of OTF did not change, there was a slight, without tissue ruptures, hyperhydration; metabolic activity was significantly lower compared to control. The effect of MCCPC on OTF is similar to processes that occur during pregnancy (ovarian function is suppressed, folliculogenesis is inhibited, hormonal function is carried out by the placenta) and can be explained by the effect of humoral factors released into the conditioned medium by placental cells.

The data obtained showed that OTF cultivation in MCCPC decreased the level of metabolic activity while preserving the general structure. The revealed effect of the medium conditioned with cryopreserved placental cells on the organotypic ovarian culture in the *in vitro* system is typical of the processes occurring in the ovaries *in vivo* during pregnancy.