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A METHOD FOR IDENTIFYING THE ENDOCRINE CELLS OF THE GASTROINTESTINAL TRACT ON EPOXY THIN SECTIONS

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СПОСІБ ІДЕНТИФІКАЦІЇ ЕНДОКРИНОЦИТІВ ШЛУНКОВО-КИШКОВОГО ТРАКТУ НА ЕПОКСИДНИХ ШЛІФАХ

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РЕЗЮМЕ

Запропонований нами спосіб використовується для виявлення та оцінки кількості, розмірів, локалізації та ступеня враження ендокриноцитів в слизовій оболонкамиці шлунково-кишкового тракту при запальних процесах. Спосіб ідентифікації ендокриноцитів на тотальних препаратах включає в себе методику ущільнення тотального препарату в ЕПОН-812. Такий метод ущільнення, а потім імпрегнації солями срібла дає змогу ідентифікувати, визначити гістотопографію, кількісний та якісний склад ендокриноцитів на тотальному препараті в різних відділах шлунково-кишкового тракту та визначити ділянки для прицільного подальшого гістохімічного та електрономікроскопічного дослідження.

СПОСОБ ИДЕНТИФИКАЦИИ ЕНДОКРИНОЦИТОВ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА НА ЭПОКСИДНЫХ ШЛИФАХ

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РЕЗЮМЕ

Предложенный нами метод может применяться для выявления и оценки количества, размеров, локализации и степени поражения ендокриноцитив в слизистой оболочке желудочно-кишечного тракта при воспалительных процессах. Данный способ идентификации ендокриноцитив на тотальных препаратах включает в себя методику помещения тотального препарата в ЭПОН-812. Такой метод уплотнения, а затем импрегнации солями серебра позволяет идентифицировать, определить гистотопографию, количественный и качественный состав ендокриноцитив на тотальном препарате в различных отделах желудочно-кишечного тракта и определить участки для прицельного дальнейшего гистохимического и електрономикроскопичного исследования.

Keywords: endocrine cells, epoxy thin sections, gastrointestinal tract, impregnation.

The diagnosis and treatment of digestive deseases remain an urgent problem given their prevalence in Ukraine and abroad. Statistical studies show that more than half of the working population in more developed countries suffers from gastrointestinal pathology [1]. Leading morphologists emphasize that the most likely accurate diagnosis in the gastrointestinal pathology can be established only after the morphological study, which allows to determine morphometric accurately and according to the objective position the presence and the nature of structural changes, the direction of regenerative process; understanding of the normal structure is a prerequisite for successful analysis of the pathogenesis of digestive deseases [2].

Recently, there is an increase of interest in the research and study of endocrine cells in the mucosa of gastrointestinal (GI) tract. Numerous studies in this field have shown that in case of GI pathology, one of the leading roles played by APUD cells. Under these conditions, the quantity and quality of endocrine cells have great importance for the digestion process; their identification and morphological study are (done) performed by silvering that is due to the ability of APUD cells to accumulate the silver salts. Among the endocrine cells of (GI) tract distinguished: alpha cells, which secrete enteroglucagon; D cells that produce somatostatin; P/D1 cells that stimulate vasoactive intestinal polypeptide; enterochromaffin (EC) cells that produce serotonin and motilin; ECL cells that produce histamine; G cells that secrete gastrin; I cells that produce cholecystokinin; K cells that produce the gastric inhibitory polypeptide; L cells that produce enteroglucagon; M cells that produce motilin; N cells that produce neurotensin; P-cells that produce bombesin; PP cells that produce pancreatic polypeptide; S cells that release secretin [3]. All of the above mentioned endocrine cells have great importance in the digestion, absorption, and neuroregulation in the GI tract. Therefore, the actual problem of modern medicine is to identify and to determine the qualitative structure of the endocrine cells of GI tract.

Objective. The aim of work is to modify the method for determining the endocrine cells in different parts of GI tract on the epoxy thin sections with a large survey surface of these parts.

MATERIALS AND METHODS

To date, there is a way to determine the endocrine cells on paraffin and semi-thin sections [4]. However,

a significant drawback is that it does not provide a complete picture of cytoarchitectonics, quantity, qualitative composition of different types of endocrine cells in mucous membranes of GI tract in general. Plastination [5, 6] of the biological material for microscopic study provides an opportunity to investigate the biological structures with a large survey surface on the total specimen. This method is that the biopsy sample is placed in an epoxy compound (EPON 812) and thin sections are made from them using the known techniques. But the significant drawback of the above methods is that they were carried out on the complexes of cartilage, bone tissues and were stained with methylene blue, which makes it impossible to identify the APUD cells of GI tract.

RESULTS AND DISCUSSION

A thorough study of silvering methods made it possible to conclude that G cells are detected by Grimelius silver stain, EC cells are detected by Sevier-Munger silver technique. Comparing the results of the immunofluorescence and the histochemical method for determining the endocrine cells of gastric mucosa, we concluded that identification and analysis of G cells are possible with the use of a Grimelius and Sevier-Munger complex method. Developing the principle of integrated use of histochemical methods in the study of endocrine cells of the mucosa of GI tract on epoxy thin sections, we proposed and approved the scheme of histochemical identification of G cells, EC cells and ECL cells that is based on a comparative evaluation of the data. The latest data obtained by the Sevier-Munger method, which detects the argentaffin cells, and by Masson-Hamperl method that detects only argentophile cells and opens the possibility to identify on the thin sections of ECL cells. Also, evaluating the enterochromaffin cells of GI tract, we concluded that the inclusion in the histochemical analysis diazo reactions is needed, namely diazonium pink. The need to use the latter is determined by qualitative differences of EC cells of GI tract. Our studies have shown that the Masson-Hamperl method effectively detects the EC cells, while diazol pink stains well the granules of enterochromaffin cells of GI tract on the epoxy thin sections. The use of the proposed scheme for identification the endocrine cells on the epoxy thin sections allowed us to identify a number of new factors revealing, in varying degrees, the role of G cells, EC cells and ECL cells in normal and pathological GI tract.

The quantitative evaluation of endocrine cells can be performed in different ways fundamentally irrelevant. The morphometry of endocrine cells was conducted by counting their number to the square of section from the epoxy microsection, using a microscope with an Olympus C 3040-ADU digital micro nozzle with the program adopted for this research (Olympus DP – Soft, license № VJ285302, VT310403, 1AV4U13B26802) and BIOREX 3 (serial number 5604) with further recalculation to 1mm2 of mucosa area. Statistical analysis, in our choice, is the most suitable using the nonparametric criteria.

Biopsies were compacted in the epoxy according to generally accepted method for electron-microscopic examination without soaking the tissues by osmium salts. To study the APUD cells on the total specimens of different parts of GI tract, the compaction is performed for the whole organocomplex in EPON 812 in order to obtain a complete picture of the place and role of different types of APUD cells in the mucosa of the GI tract.

Our short method for detecting the endocrine cells on epoxy thin sections gradually is performed as follows:

The specimens of tissue from the different parts of GI tract were fixed in glutaraldehyde solution of 2,5% and compacted in EPON 812 according to the standard technique for electron-microscopic examinations including the contrasting phase in osmium salts;

The thin sections with thickness from 0,5 to 0,8 mm were made of snab blocks;

Within 5 minutes, the thin sections were immersed in the phosphate buffer a solution pH 5,6;

Subsequently, the thin sections were kept for 10 minutes in a freshly prepared Buen solution;

Then thin sections were kept for 1 hour (under visual control) in a 0.3% silver nitrate solution on the phosphate buffer (pH 5, 6) at a temperature of 370°C;

Later thin sections were developed for 3 minutes in 1% hydroquinone solution on the 5% sodium sulfite solution in the thermostat at 450°C;

Preparations were washed with distilled water for 10 minutes, dried in the thermostat a temperature of 370°C and investigated in the light microscope at different magnifications.

The granules of endocrine cells have been staining dark brown, other structures stained yellow that makes it possible to identify and to define the cytotopography, the qualitative and quantitative composition of the cells of diffuse endocrine system of GI tract. This compaction method and further impregnation with silver salts according to different methods makes it possible to identify and to define the cytotopography, the qualitative and quantitative composition of the endocrine cells on the total specimen in the different parts of GI tract and to define the areas for the further impact histochemical and electron-microscopic examination.

CONCLUSIONS

Thus, the above methods of histochemical identification of endocrine cells of the mucosa of GI tract on the epoxy thin sections are relatively simple, do not require the use of expensive reagents and instruments and can be widely used in practice of morphological laboratories to assess the endocrine system of GI mucous membranes.

POSSIBILITIES OF FURTHER STUDIES

This method is promising and visionary. Despite its simplicity in implementation, the researcher is able to use this method not only to identify the cells of the endocrine system of GI tract but also to detect the APUD cells of other organ specificity. In further studies, this method is planned to test at the diffuse endocrine system of respiratory system.

REFERENCES

1. Передерий В.Г. Современные представления о причинах возникновения и лечении язвенной болезни / В.Г. Передерий, С.М. Ткач // Журнал "Мистецтво лікування". Украина.– 2007.– 2 (38). С-4–6.

2. Білаш.С.М. Вивчення морфо-функціональних змін шлунку щурів при запаленні викликані λ-карагенин та корекції його трансплантацією кріоконсервованої плаценти / С.М. Білаш, В.І. Шепітько // Світ медицини та біології.– 2011.—№ 2.– С.169–173.

3. Струков А.І. Патологічна анатомія: Підручник. Пер. з рос. 4-е вид., стереотипне / Струков А.І., Сєров В.В..– Х.: Факт, 2004.– 760 с.

4. Старченко І.І. К методике гистохимической идентификации эндокринных клеток слизистой оболочки желудка и двенадцатиперстной кишки / І.І. Старченко, В.М. Успенский, В.Ю. Голефеевский //Архив патологи.– № 1.– 1996.– С.78–80.

5. Старченко І.І. Застосування методу пластинації в стереоморфологічних дослідженнях / І.І. Старченко, О.К. Прилуцький // Вісник проблем біології і медицини.– 2006, Вип. 2.– С. 420–422.

6. С.М. Білаш Значення та місце ендокриноцитів асоційованих зі слизовою оболонкою шлунку в патогенезі гострого гастриту / С.М. Білаш, В.І. Шепітько, Г.А. Єрошенко [та ін.] // Актуальні проблеми сучасної медицини: Вісник Української медичної стоматологічної академії.– Т. 12, Вип. 4 (40).– С 206–210.