Применение лектиновой гистохимии для изучения формирования и реактивности переходной зоны капсулы сустава

А. В. Федотченко, Н. А. Волошин

Цель работы – с помощью лектиновой гистохимии изучить формирование и реактивность переходной зоны капсулы сустава.

Материалы и методы. Объект исследования стал тазобедренный сустав лабораторных крыс. Для изучения реактивности переходной зоны капсулы сустава использовали методику внутриматочного введения антигена крысам на 18 сутки антенатального периода. Фрагменты тазобедренных суставов фиксировали в жидкости Буэна, декальцинировали, дегидратировали и заливали в смесь парафин:воск:каучук.

Результаты. В переходной зоне капсулы сустава выделяли regio superficialis и regio profunda. Установлено, что интенсивность экспрессии рецепторов к лектинам в regio superficialis была всегда выше, чем в париетальной части капсулы сустава. Переходная зона капсулы отличалась от прилегающих тканей сустава уже на 1 сутки, a regio superficialis и regio profunda дифференцируются между собой только на 7 сутки. В работе установлено, что формирование переходной зоны сопряжено с уменьшением экспрессии рецепторов к исследуемым лектинам в regio superficialis и увеличением содержания галактозо-, маннозо- и глюкозоконъюгатов в regio profunda, что имеет место с 14 суток. Реактивные изменения в переходной зоне капсулы сустава после воздействия антигенов более выражены.
The present time the problem of joint morphology research has received considerable attention due to its great importance in general morbidity. It is well-known that the initial signs of joints damage during its inflammation take place at the contact area of the joint capsule and articular cartilage. This area has a key role in maintaining morphological and functional integrity of the joint. A lot of scientists studied this zone using different terminology, namely “переходная зона синовиальной мембраны”, “marginal transitional zone”, “marginal synovi- um”, “synovium-cartilage junction”, etc [1,5,7,10,11].

We proposed to distinguish the visceral part (pars visceralis capsulae articularis), parietal part (pars parietalis capsulae articularis) and pars intermedia capsulae articularis (intermediate part, generally known term – “marginal transitional zone”). In pars intermedia two different zones are discerned: fibrous layer which entwines in the articular cartilage – regio profunda and the area of the fibrous layer, which contacts directly with the last one – regio superficialis [2,4,9]. Complex immunobiological relationship between the joint capsule and articular cartilage are provided by a number of mechanisms of non-specific immune resistance, in particular carbohydrate-protein factor. Lectin histochemistry are widely used to study the dynamics of carbohydrate residues distribution in organs and tissues. These methods provide an opportunity to assess the formation of non-specific immune protective barrier of the marginal transitional zone of the joint capsule which is aimed to maintain normal physiological interaction between the joint capsule and the articular cartilage and prevent the excessive invasion of the joint capsule into the articular cartilage.

**Aim**

To study the formation and reactivity of the marginal transitional zone (intermediate part, further in text) of the joint capsule by means of lectin histochemistry.

**Materials and methods**

We used hip joints of white laboratory rats. Three groups of animals were studied. The first group – intact animals. We injected 0.05 ml of immunoglobulin human normal the second experimental group – antigen-affected rats in order to study reactivity of the marginal transitional zone of the joint capsule. The third group were rats of control group (injected with 0.05 ml of physiological saline). Injections of antigens and physiological saline were given for foetus on the 18th day of gestation. On the 22nd day after conception animals were put to death under ether anaesthesia by means of decapitation on the 1st, 7th, 14th, 30th, 60th and 90th days of postnatal life in the afternoon from 13.00 to 14.00 (8 rats in each group, 144 rats were used in total). We guided by “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” Strasbourg, 18.III.1986. Fragments of the hip joints were fixed in picroformol, decalcinated in 20 % formic acid solution and dehydrated in alcohols and chloroforms. Pieces were immersed in a paraffin-wax-rubber mix (20:1 ratio). Histological specimens 3–5 microns thick were made by microtome. Peanut (PNA–HPR), vicia sativa (VSA–HPR), soybean (SBA–HPR), wheat germ (WGA–HPR), perca fluviatilis (PFA–HPR) agglutinins were used. Control histological sections were incubated in 1 % periodic acid solution for 30 minutes. Lectins binding sites visualization was carried out by diaminobenzidin-hydrogen-peroxide system. Obtained results were processed using semiquantitative analysis (from 0 to ++++) by means of χ10, χ40 and χ100 lens magnification. Distribution of lectin receptors was studied in regio profunda and regio superficialis of the intermediate part of the joint capsule and compared to obtained data of fibrous layer of the parietal part of joint capsule.

**Results of the study and discussion**

In newborn intact animals in the parietal part of the joint capsule there were low expression for the peanut (PNA), soybean (SBA) agglutinins: light levels for vicia sativa (VSA) and perca fluviatilis (PFA) lectins. Intensity of benzidine label in regio superficialis for studied lectins was less in comparison with parietal part of joint capsule and it is remarkable, that this phenomenon can be observed up to the 90th day (see Table 1). GlcNAc residues were not found in regio superficialis till the 14th day, but they could be seen in minimal volume in the parietal part. So, regio superficialis is distinguishable from adjoining area of joint capsule because of unequal lectins distribution. Minimal expression of glycoconjugates in regio profunda is also determined; it makes this area different from the adjacent articular cartilage. The clear border between regio superficialis and regio profunda was not defined due to absence of significant difference in lectins distribution in these parts.

On the 7th day in intact rats in regio superficialis we revealed appearance of PNA receptors expression with maximum on the 14th day, which allows us to distinguish this zone from regio profunda. Expression of VSA lectin receptors started to decrease and remained in the least quantity up to the 90th day.
One must note that fucose residues were not defined in regio superficialis from the 7th day till the end of observation. Meanwhile, they could be found in minimal volume in the parietal part (see Table 1).

On the 14th day in intact groups there was focus of intense expression of receptors for peanut and soybean lectins in regio profunda. From the 30th day strong expression of vicia sativa and wheat germ lectin receptors could be clearly seen in the same zone. These above-mentioned phenomena enable us to discern distinctly regio profunda from regio superficialis and neighboring articular cartilage as well.

From the 30th and up to the 90th day of observation, expression of receptors for studied lectins in parietal part of the joint capsule was light and in regio superficialis was minimal. Along with that, there was pronounced intensity of benzidine labeling for all investigated lectins except perca fluviatilis (PFA) lectin.

Antenatal antigen influence in newborns leads to increased β-D-galactose residues level and reducing of α-D-mannose residues number in regio superficialis and regio profunda as well. The distinct border between regio superficialis and regio profunda by analogy with intact newborns was not defined. Subsequently, antigenic influence did not affect the dynamics of the lectins receptors distribution in regio superficialis. In antigen-injected rats strong expression of receptors for peanut (PNA) and soybean (SBA) agglutinin in regio profunda can be observed as early as on the 7th day in comparison with intact rats. In addition, we observed a significant decrease in quantity both residues β-D-galactose (from the 14th to the 30th day) (Fig. 1) and α-D-mannose (from the 30th up to the 45th day) in regio profunda and found the appearance of significant quantity of fucose conjugates (from 30th to 45th day) (Fig. 2).

Thus, in the contact area of the joint capsule with articular cartilage there is zone, which is different from the adjacent part of the articular cartilage and the parietal part of the joint capsule as well. This difference derived from the distinction in lectin receptors distribution in it. Such changes in carbohydrate residues saturation are aimed for organization of, so-called, non-specific anatomical and physiological wall to regulate complex immunobiological interactions of joint capsule and articular cartilage, being as bar for immune cells. Regio superficialis formation passes against the background of decreasing lectin receptors expression in it. Meanwhile, regio profunda formation is accompanied by pronounced expression for all studied lectins but perca fluviatilis (PFA) lectin (see Table 1). It should be noted, that during the formation of regio profunda, there first occurred galactosylation of its matrix. From the 14th day one can observe the formation

### Table 1

| Parameters                  | Parietal part of the joint capsule (fibrous layer) | Intermediate part of the joint capsule (marginal transitional zone) |
|-----------------------------|----------------------------------------------------|-------------------------------------------------------------------|
|                             | norm                  | antigen influence               | norm                  | antigen influence               | norm                  | antigen influence               |
| day                         | lectins               | from 0/+ to +                   | from + to ++          | from 0/+ to +                   | from 0/+ to 0          | 0                                   |
| 1st                         | PNA                   | from 0/+ to +                   | from + to ++          | 0                               | +                     | 0                                   |
|                             | SBA                   | from 0/+ to +                   | from + to ++          | 0/+                             | 0/+                   | from 0/+ to 0                      |
|                             | VSA                   | from + to ++                   | 0/+                   | +                               | 0/+                   | 0/+                                 |
|                             | WGA                   | 0/+                              | 0/+                   | 0                               | 0                     | 0                                   |
|                             | PFA                   | +                                | +                     | +                               | 0/+                   | 0/+                                 |
| 7th                         | PNA                   | from + to +/+                   | ++                   | +                               | +                     | 0                                   |
|                             | SBA                   | +                                | ++                   | 0/+                             | 0/+                   | from 0/+ to 0                      |
|                             | VSA                   | +                                | +                    | 0/+                             | 0/+                   | 0                                   |
|                             | WGA                   | 0/+                              | 0/+                   | 0                               | 0                     | 0                                   |
|                             | PFA                   | 0/+                              | 0/+                   | 0                               | 0                     | 0                                   |
| 14th                        | PNA                   | from ++ to +                    | from ++ to +         | ++                              | ++                    | 0                                   |
|                             | SBA                   | from ++ to +                    | from ++ to +         | 0/+                             | 0/+                   | +++                                 |
|                             | VSA                   | +                                | +                    | 0/+                             | 0/+                   | 0                                   |
|                             | WGA                   | 0/+                              | 0/+                   | 0                               | 0                     | 0                                   |
|                             | PFA                   | 0/+                              | 0/+                   | 0                               | 0                     | 0                                   |
| 30th                        | PNA                   | from + to 0/+                   | from + to 0/+        | 0/+                             | 0/+                   | +++                                 |
|                             | SBA                   | +                                | +                    | 0/+                             | 0/+                   | +++                                 |
|                             | VSA                   | +                                | +                    | 0/+                             | 0/+                   | 0                                   |
|                             | WGA                   | +                                | +                    | 0/+                             | 0/+                   | +++                                 |
|                             | PFA                   | 0/+                              | 0/+                   | 0                               | 0                     | 0                                   |
| 60th and 90th               | PNA                   | from + to 0/+                   | from + to 0/+        | 0/+                             | 0/+                   | +++                                 |
|                             | SBA                   | +                                | +                    | 0/+                             | 0/+                   | +++                                 |
|                             | VSA                   | +                                | +                    | 0/+                             | 0/+                   | +++                                 |
|                             | WGA                   | +                                | +                    | 0/+                             | 0/+                   | +++                                 |
|                             | PFA                   | 0/+                              | 0/+                   | 0                               | 0                     | 0                                   |

Notes: 0 – absence of binding; 0/+ minimal lectin binding; + light lectin binding; ++ moderate lectin binding; +++ intensive lectin binding. Abbreviations: PNA – peanut agglutinin, VSA – vicia sativa agglutinin, SBA – soybean agglutinin, WGA – wheat germ agglutinin, PFA – perca fluviatilis agglutinin.
of double lectin-mediated barrier, consisting in reducing the lectin receptors expression in the one zone and appearance of strong lectin receptors expression in the other zone. It can be common biological feature of organs which are not recognized by immune cells in norm. Such phenomenon has been described in placenta [6]. Considering the obtained data, it is advisable to distinguish two zones in pars intermedia capsulae articularis, namely regio superficialis and regio profunda.

Antigen influence leads to a significant increase in the number of immune cells in the organs, in particular in joint capsule, which affects the rate and terms of organs formation [3]. Increased number of lymphoid cells in the joint capsule is the immunological risk due to possible breaching the barrier between the cartilage and the capsule and further penetration of joint capsule into the articular cartilage with subsequent development of immune inflammation in it.

Fig. 1. Fragment of intermediate part of hip joint capsule (showed by arrows) in acetabulum: a) intact rat; b) immunoglobulin-injected rat. 14th day. Histochemical reaction with peanut agglutinin (PNA–HRP), х40 lens magnification. Absence of peanut agglutinin expression in the intermediate part of the joint capsule after antigen exposure.

Fig. 2. Fragment of intermediate part of hip joint capsule (showed by arrows): a) intact rat; b) immunoglobulin-injected rat. 30th day. Histochemical reaction with percafluviatilis agglutinin (PFA–HRP), х40 lens magnification. Appearance of residues 6-L-fucose excessive amounts in the intermediate part after antigen exposure.
This leads to the development of compensatory and adaptive reactive processes concerning quantitative and qualitative changes in glycosylation of components of marginal transitional zone. These phenomena are strongly pronounced in regio profunda, in contrast to regio superficialis, that probably indicates regio profunda as most vulnerable structure. So, the premature appearance in regio profunda increased amounts of β-D-galactose residues can be considered as early formation of joint capsule intermediate part.

On the other hand, there are a number of matrix components in the joint capsule which serve as non-specific resistance factors because of having good adsorbing properties to stop activated lymphocytes, macrophages, etc., for example, fibronectin. Glycosylation apparently plays crucial role to provide biological function of matrix. Fibronectin has in its structure β-D-galactose residues can be considered as early formation of joint capsule intermediate part.

Aspects of further investigations. In the future some regularities of lectin receptors distribution in other components of joint will be also studied.

Conflict of Interest. All authors disclose no financial nor personal relationships with other people or organizations that could potentially and inappropriately influence (bias) their work and conclusions.

Conclusions
1. Thus, pars intermedia capsuleae articularis (joint capsule marginal transitional zone) is a structure that is morphologically different from the surrounding tissue and includes “regio superficialis” and “regio profunda”. Glycosylation of structures of the marginal transitional zone provides the formation of lectin-mediated barrier between the joint capsule and the articular cartilage that ensures the integrity of the joint as a whole. Formation of pars intermedia is accompanied by the decrease in expression of receptors to all investigated lectins in regio superficialis and the appearance of significant galactose-, mannose- and glucoseconjugates quantity in regio profunda.
2. Antenatal antigens influence leads to tension in immunobiological relationship between joint capsule and articular cartilage resulting in premature galactosylation of components of regio profunda, subsequently being accompanied by a decrease in the number of residues of galactose and mannose and appearance of excessive amounts of residues α-L-fucose.

References
1. Vagapova, V. Sh., & Rybalko, D. Yu. (2015) Funktsional'naya morfologiya e'lementov kolennogo sustava [Functional morphology of knee joint components]. Ufa: Gilem. [in Russian].
2. Voloshyn, M. A., & Grygor'eva, O. A. (2011) Suchasniy pohliad na budovu ta klasifikatsiiu struktur suhloba [Modern viewpoint on structure and terminology of joint]. Visnyk problebi biologii i medytsyny, 2(1), 56–59. [in Ukrainian].
3. Voloshyn, M. A., Hryhor'eva, E. A., Kushch, O. H., Vovchenko, M. B., Chuhin, S. V., Sviatlyts'kyi, A. O., et al. (2015) Limfotsyt – faktor morfogenezhu orhaniv [Lymphocyte is the factor of organs morphogenesis]. Morfolohichni doslidzhennia – vyklyky suchasnosti, (P. 5–6). Sumy. [in Ukrainian].
4. Voloshyn, M. A., & Fedotchenko, A. V. (2015) Dynamika stanovlenia struktur kul'chuvoto suhlobu protiatom poznat'noh periodu [Dynamics of hip joint forming during postnatal period]. Aktualni pytannia medychnyi nauk i praktyky. Proceedings of 6th Congress of anatomists, histologists, embryologists and topgraphoanatomists of Ukraine. (P. 17–24). Zaporizhchia. [in Ukrainian].
5. Dīlmukhametova, L. M., & Vagapova, V. Sh. (2014) Stroenie perekhodnoy zony siniavoi'joi membrany u plodov, detey i podrostkov [Structure of synovial membrane transitional zone in fetuses, children and teenagers]. Morfologiya – medicinski nauke i praktike. Proceedings of scientific conference for 85th anniversary of prof P.I. Lobko]. (P. 88–91). Minsk. [in Belarus].
6. Kushch, O. H. (2008) Zakonomirnosti budovy platsenty ta asotsiuvannoi' z neiu limfoidnoi' tkany' protiatom tretoho trymestru vahitnosti (anatomo-eksperymentalne doslidzhennia) (Avtoref. dis… dokt. biol. nauk) [Regularities of structure of placenta and lymphoid tissue associated with it during third trimester of pregnancy (anatomic and experimental research) Dr. biol. sci. diss.]. Ternopil. [in Ukrainian].
7. Allard, S. A., Bayliss, M. T., & Maini, R. N. (1990) The synovi- um-cartilage junction of the normal human knee. Implications for joint destruction and repair. Arthritis & Rheumatism, 33(8), 1170–1179. doi: 10.1002/art.1780330818.
8. Carsons, S., Lavietes, B. B., Slomiany, A., Diamond, H. S., & Berkowitz, E. (1987) Carbohydrate heterogeneity of fibronectins. Synovial fluid fibronectin resembles the form secreted by cultured synoviocytes, but differs from the plasma form. Journal of Clinical Investigation, 80, 1342–1349.
9. Fedotchenko, A. V. (2014) Features of joint capsule formation after antenatal action of antigen. Pain. Joints. Spine, 4(16), 79–86.
10. Gardner, D. L. (1994) Problems and paradigms in joint pathology. J. Anat, 184(3), 465–476.
11. Thompson, A. M., & Stockwell, R. A. (1983) An ultrastructural study of the marginal transitional zone in the rabbit knee joint. Journal of Anatomy, 156(4), 701–713.

Information about authors:
Fedotchenko A. V., MD, PhD, Assistant, Department of Human Anatomy, Operative Surgery and Topographic Anatomy, Zaporizhzhia State Medical University, Ukraine, E-mail: afedotchenko@ukr.net.
Voloshyn M. A., MD, PhD, DSci, Professor, Head of the Department of Human Anatomy, Operative Surgery and Topographic Anatomy, Zaporizhzhia State Medical University, Ukraine.

Відомості про авторів: Fedotchenko A. V., канд. мед. наук, асистент каф. анатомії людини, оперативної хірургії та топографічної анатомії, Запорізький державний медичний університет, Україна, E-mail: afedotchenko@ukr.net.
Voloshyn M. A., д-р мед. наук, професор, зав. каф. анатомії людини, оперативної хірургії та топографічної анатомії, Запорізький державний медичний університет, Україна.
Сведения об авторах:
Федотченко А. В., канд. мед. наук, ассистент каф. анатомии человека, оперативной хирургии и топографической анатомии, Запорожский государственный медицинский университет, Украина, E-mail: afedotchenko@ukr.net.
Волошин Н. А., д-р мед. наук, профессор, зав. каф. анатомии человека, оперативной хирургии и топографической анатомии, Запорожский государственный медицинский университет, Украина.

Поступила в редакцию 03.10.2016 г.