МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
ФАКУЛЬТЕТ ЕКОЛОГІЧНОЇ БЕЗПЕКИ, ІНЖЕНЕРІЇ ТА ТЕХНОЛОГІЙ
КАФЕДРА БІОТЕХНОЛОГІЇ

ДОПУСТИТИ ДО ЗАХИСТУ
Завідувач випускової кафедри
_____ М.М. Барановський
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ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

ЗДОБУВАЧА ВИЩОЇ ОСВІТИ ОСВІТНЬОГО СТУПЕНЯ «БАКАЛАВР»
СПЕЦІАЛЬНІСТЬ 162 «БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ»
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Тема: «Технологія отримання хондроїтину сульфатів із хрящових тканин риб»

Виконавець: студентка 404 гр. ФЕБІТ Третьякова А.О.

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MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
NATIONAL AVIATION UNIVERSITY
FACULTY ENVIRONMENTAL SAFETY, ENGINEERING AND TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

ALLOWED TO DEFENCE
Head of the graduate department
_____M.M. Baranovsky
«___»_____2021

BACHELOR THESIS

(EXPLANATORY NOTE)
OF GRADUATING STUDENT OF EDUCATIONAL DEGREE «BACHELOR»
SPECIALTY 162 «BIOTECHNOLOGY AND BIOENGINEERING»
EDUCATIONAL PROFESSIONAL PROGRAM «PHARMACEUTICAL BIOTECHNOLOGY»

Theme: «Obtaining chondroitin sulfates from cartilaginous tissues of fish»

Executor: student of group 404, FESET Tretyakova A.O.

Supervisor of studies: Ph.D., Associate Professor Glushko J.M.

Standards inspector: Drazhnikova A.V.

KYIV 2021

NATIONAL AVIATION UNIVERSITY

Faculty Environmental Safety, Engineering and Technologies

Department of Biotechnology

Speciality: 162 «Biotechnology and bioengineering»

EPP: «Pharmaceutical Biotechnology»

APPROVED

Head of the Department

_____ Baranovsky M.M.

«___» _____2021

TASK

to perform a thesis

Tretyakova Anastasiia Alexandrovna

1. Theme of degree work: «Obtaining chondroitin sulfates from cartilaginous tissues of fish» approved by the Rector «11» of May 2021 № 715/ст

2. Term of work execution: from «10» of May 2021 and «20» of June 2021.

3. Content of explanatory note: Introduction. Literature review. Analysis of methods of obtaining chondroitin sulfate. Analysis of the main areas of application and prospects for the use of chondroitin sulfate. Conclusions. References.

4. List of necessary graphic (illustrative) material: 8 fig.

НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ

Факультет екологічно безпеки, інженерії та технологій

Кафедра біотехнології

Спеціальність: 162 «Біотехнології та біоінженерія»

Освітньо–професійна програма "Фармацевтична біотехнологія"

ЗАТВЕРДЖУЮ

Завідувач кафедри

_____Барановський М.М.

«___» _____2021 р.

ЗАВДАННЯ

на виконання дипломної роботи

Третьякова Анастасії Олександрівна

1. Тема дипломної роботи: «Технологія отримання хондроїтину сульфатів із хрящових тканин риб», затверджена ректором «11» від травня 2021 р. № 715 / ст.

2. Термін виконання роботи: з «10» травня 2021 р. Та «20» червня 2021 р.

3. Зміст пояснювальної роботи: Вступ. Літературний огляд. Аналіз методів отримання сульфату хондроітину. Аналіз основних сфер застосування та перспективи використання сульфату хондроитин. Висновки. Списки використаних джерел.

4. Список необхідного графічного (ілюстративного) матеріалу: 8 рис.

5. Schedule

№	Task	Execution term	Signature of the head
1	Coordination of the content of the thesis with the thesis supervisor	10.05.2021 − 12.05.2021	
2	Selection of information sources	12.05.2021 − 17.05.2021	
3	Development work concept	17.05.2021 − 20.05.2021	
4	Processing information sources	20.05.2021 − 25.05.2021	
5	Registration of the thesis	25.05.2021 − 30.05.2021	
6	Formulation of conclusions	30.05.2021 − 05.06.2021	
7	Thesis defense	14.06.2021	

6. Date of issue of the task: «10» of May 2021

Supervisor of degree work_____Glushko J.M.

Task for execution was taken over by _____Tretyakova A.O.

5. Календарний план–графік

№	Завдання	Термін виконання	Підпис керівника
1	Узгодження змісту дипломної роботи з дипломним керівником	10.05.2021 – 12.05.2021	
2	Підбір джерел інформації	12.05.2021 – 17.05.2021	
3	Розробка концепції роботи	17.05.2021 – 20.05.2021	
4	Обробка джерел інформації	20.05.2021 – 25.05.2021	
5	Оформлення дипломної роботи	25.05.2021 – 30.05.2021	
6	Формулювання висновків	30.05.2021 – 05.06.2021	
7	Захист дипломної роботи	14.06.2021	

6. Дата видачі завдання: «10» травня 2021 року

Керівник дипломної роботи _____ Глушко Ю.М.

Завдання прийняла до виконання_____ Третьякова А.О.

ABSTRACT

Explanatory note to the diploma thesis « Obtaining chondroitin sulfates from cartilaginous tissues of fish.», 45 p., 12 figures, 45 references.

Object of investigation – preparation and analysis of chondroitin sulfates isolated from fish cartilage and its use in medicine.

Subject of investigation – chondroitin sulfates isolated from cartilaginous tissues of fish.

Purpose of the work – there is an analysis of methods for obtaining and using chondroitin sulfates isolated from the cartilaginous tissues of fish.

Methods of investigation – analytical, biochemical, physiological.

The results of the thesis can be used during research and in the practice of specialists – biologists and specialists – biotechnologists.

METHODS OF ISOLATION OF CHONDROITIN SULFATE, CARTILAGE, ULTRAFILTRATION, CHONDROPROTECTORS, PHARMACOLOGY, PHARMACOKINETICS, HYDROLYSIS.

РЕФЕРАТ

Пояснювальна записка до дипломної роботи «Технологія отримання хондроїтину сульфатів із хрящових тканин риб.», 45 с., 8 рисунків, 45 посилання.

Об'єкт дослідження – отримання та аналіз хондроїтин сульфатів, виділених із хрящових тканин риб, та використання його в медицині.

Предмет дослідження – хондроїтин сульфат, виділений з хрящових тканин риб.

Мета роботи – є аналіз методів отримання та застосування хондроїтину сульфатів виділених з хрящових тканин риб.

Методи дослідження – аналітичні, біохімічні, фізіологічні.

Результати дипломної роботи можуть бути використані під час проведення наукових досліджень та в практичній діяльності фахівців — біологів та фахівців — біотехнологів.

МЕТОДИ ВИДІЛЕННЯ ХОНДРОЇТИН СУЛЬФАТУ, ХРЯЩОВА ТКАНИНА, УЛЬТРАФІЛЬТРАЦІЯ, ХОНДРОПРОТЕКТОРИ, ФАРМАКОЛОГІЯ, ФАРМОКІНЕТИКА, ГІДРОЛІЗ.

CONTENT

LIST OF SYMBOLS, ABBREVIATIONS, TERMS

CS − Chondroitin sulfate

OA – Osteoarthritis

ECM – Extracellular matrix

EMEA – European Medicine Agency

OARSI – Osteoarthritis Research Society International

EULAR – European League Against Rheumatism

GAG – Glycosaminoglycan

NO – Nitric oxide

COX – Cyclooxygenase

NF – Nuclear factor

mPGES – Microsomal prostaglandin synthase

TNF – Tumour necrosis factor

TLR − Toll− like receptor

PG − Prostaglandin

IL − Interleukin

MMP − Matrix metalloproteinase

TIMP − Tissue inhibitor of metalloproteases

GL − Glucosamine

CH − Chondroitin

INTRODUCTION

Actuality of theme. Development of modern technologies for processing aquatic organisms is an urgent task of the fishing industry. creation of technologies for the production of fortified foods, preventive and medicinal products derived from marine aquatic organisms, includes research of the composition of raw materials, identification and quantitative analysis of its components, study of physicochemical properties of biologically active components of raw materials. Natural polysaccharide chondroitin sulfate (cholesterol), contained in cartilage tissue, is a sulfated glycosaminoglycan, the macromolecules of which consist of alternating monomer units of sulfated N− acetyl− D− galactosamine and D− glucuronic acid. Linear cholesterol macromolecules help to make cartilage more resistant to the pressure exerted on it by body weight, participate in the formation of bone tissue, ligaments, as well as in maintaining the elasticity and resilience of blood vessel walls. Cholesterol is a widely used dietary supplement for the treatment of degenerative dystrophic diseases of the joints and spine, such as osteoarthritis and osteochondrosis. Currently, commercial drugs of cholesterol are obtained mainly from the cartilage of mammals. In recent years, aquatic tissue has also been used for its production. Problems of isolation of cholesterol from various natural objects and its application in medicine and biotechnology are dealt with by such foreign scientists as A. Kinoshita,. H. R. Morris, K. Sugahara, M. J. Miller, C. E Costello .; H. Takai,. T. Kono, C. Amornrut, A. B. Khare, S. A. Houliston, F. Abdel and Russian researchers TN Shkarin, IM Sorokoumov and others. Modern methods of obtaining cholesterol are multi− stage extraction processes, and the yield of the final product and its purity are not always high. It should be noted that the physicochemical properties of cholesterol from aquatic organisms, methods of their identification and quantitative analysis, quantitative patterns of chemical hydrolysis have not been studied. In this regard, the development of new and improvement of known technologies for the isolation of cholesterol from marine aquatic organisms, the study of their physicochemical properties, methods of identification and quantitative analysis are urgent tasks.

Object of investigation – obtaining and analysis of chondroitin sulfates isolated from cartilaginous tissues of fish..

Subject of investigation – chondroitin sulfates isolated from cartilaginous tissues of fish.

Purpose of the work – Analysis of the use of chondroitin sulfate isolated from chondria fish from the literature.

Tasks for execution of the bachelor thesis:

1. To find out information concerning the properties of chondroitin sulfate;.

2. Analyze methods of obtaining of chondroitin sulfates;

3. Analyze the main spheres of application and prospects for the use of chondroitin sulfate isolated from cartilaginous tissues of fish.

Methods of investigation – analytical, biochemical, physiological.

ВСТУП

Актуальність теми. Розробка сучасних технологій переробки гідробіонтів є актуальним завданням рибної галузі. створення технологій виробництва збагачених харчових продуктів, профілактичних і медичних препаратів, отриманих з морських гідробіонтів, включає в себе дослідження складу сировини, ідентифікацію та кількісний аналіз його компонентів, вивчення фізико− хімічних властивостей біологічно активних компонентів сировини. Природний полісахарид хондроитинсульфат (ХС), що міститься в хрящової тканини, являє собою сульфатованих глікозаміноглікан, макромолекули якого складаються з чергуються мономерних ланок Сульфатовані N− ацетил− D− галактозаміну і D− глюкуроновою кислоти . Лінійні макромолекули ХС допомагають зробити хрящ більш стійким до тиску, який чинить на нього вагу тіла, приймають участь у формуванні кісткової тканини, зв'язок, а також у підтримці пружності та еластичності стінок кровоносних судин. ХС є широко використовуваної харчовою добавкою для лікування дегенеративнодістрофіческіх захворювань суглобів і хребта, наприклад, артрозу і остеохондрозу. В даний час комерційні препарати ХС отримують головним чином з хрящової тканини ссавців. В останні роки для його виробництва стали також використовувати тканини гідробіонтів. Проблемами виділення ХС з різних природних об'єктів і його застосування в медицині і біотехнології займаються такі зарубіжні вчені як A. Kinoshita ,. H. R. Morris, K. Sugahara, M. J. Miller, C. E Costello .; H. Takai ,. T. Kono, C. Amornrut, A. B. Khare, S. A. Houliston, F. Abdel і російські дослідники Т. Н. Шкаріна, І. М. Сорокоумов і інші. Сучасні методи отримання ХС є багатостадійні процеси екстракції, а вихід кінцевого продукту і його чистота не завжди високі. Слід зазначити, що фізико− хімічні властивості ХС з гідробіонтів, методи їх ідентифікації та кількісного аналізу, кількісні закономірності хімічного гідролізу практично не вивчені. У зв'язку з цим, розробка нових і вдосконалення відомих технологій виділення ХС з морських гідробіонтів, вивчення їх фізико−

хімічних властивостей, методів ідентифікації та кількісного аналізу є актуальними завданнями.

Об'єкт дослідження – аналіз методів отримання хондроїтину сульфатів із хрящових тканин риб.

Предмет дослідження – хондроїтину сульфати, виділені з хрящових тканин риб.

Мета роботи – аналіз використання хондроїтину сульфату, виділеного з хондрієвих риб завдяки літературі.

Завдання на виконання бакалаврської роботи:

1. З’ясувати інформацію щодо властивостей хондроїтин сульфату.

2. Проаналізувати методи отримання хондроїтину сульфати.

3. Проаналізувати основні сфери застосування та перспективи використання хондроїтину сульфату, виділеного з хрящових тканин риб.

Методи дослідження – аналітичні, біохімічні, біотехнологічні.

CHAPTER 1
LITERATURE REVIEW

1.1. Chondroitin sulfates

Chondroitin sulfates are polymeric sulfated glycosaminoglycans (Fig.1.1). They are specific components of cartilage. Produced by the cartilage tissue of the joints, they are part of the synovial fluid and various chondroprotective drugs. .a necessary building component of chondroitin sulfate is glucosamine, with a lack of glucosamine in the synovial fluid, a lack of chondroitin sulfate is formed, which impairs the quality of the synovial fluid and can cause crunchingjoint.

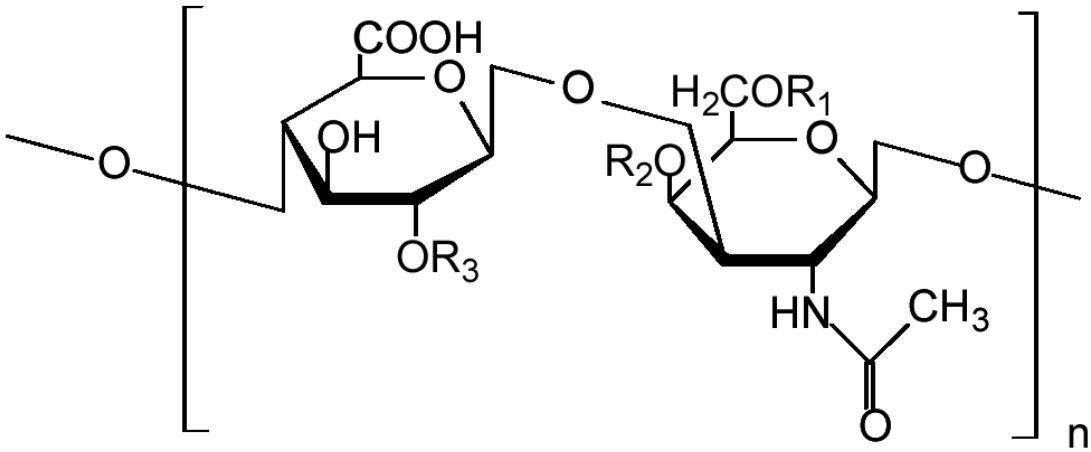

Fig. 1.1. Chemical structure of one unit in a chondroitin sulfate chain

Glycosaminoglycans, mucopolysaccharides are the carbohydrate part of proteoglycans, polysaccharides, which include amino sugar− hexosamines. In the body, glycosaminoglycans are covalently bound to the protein part of proteoglycans and do not occur in free form.

Drugs based on chondroitin sulfates, positioned by manufacturers as chondroprotectors, do not differ in their action from placebo and are not recommended for the treatment of osteoarthritis (osteoarthritis).

Osteoarthritis (OA) is characterized by progressive structural and metabolic changes in articular tissues, predominantly cartilage degradation, subchondral bone sclerosis, and synovial inflammation. Treatment for OA involves a multimodal therapeutic intervention as no cure has been found to date. Treating OA requires medications that can slow, stop, or even avoid joint degradation. Many of the recommended interventions have only a symptom− modifying effect and a few structure− altering effects.

Chondroitin sulfate is used in the complex therapy of osteoarthritis. It is necessary to take into account that the structure and properties of polysaccharides in the composition of chondroitin sulfate, as well as the source of its production, significantly affect the absorption, bioavailability and, as a consequence, the effectiveness and safety of drugsoral administration.

CS and other compounds, such as glucosamine, have been used for therapeutic purposes for more than 40 years. CS is sold as an over− the− counter dietary supplement in North America and is available by prescription according to European Medical Agency (EMEA) regulations in Europe. In recent decades, CS has aroused much interest as a potential therapeutic agent against OA. CS is part of the recommendations of the International Society for Osteoarthritis Research (OARSI) for the treatment of osteoarthritis of the knee. Cholesterol and glucosamine can be an excellent alternative for patients with OA.

1.2. Pharmacology

According to drug developers, chondroitin sulfate slows down bone resorption and reduces Ca^{2+} loss. Improves phosphorus− calcium metabolism in cartilaginous tissue, accelerates the processes of its restoration, inhibits the processes of degeneration of cartilaginous and connective tissue. Suppresses the activity of enzymes that cause damage to cartilage tissue, stimulates the synthesis of glycosaminoglycans, promotes the regeneration of the articular bag and cartilaginous surfaces of the joints, increases the production of intra− articular fluid. Reduces soreness and increases the mobility of the

affected joints. Structurally similar to heparin, it can potentially prevent the formation of fibrin thrombi in the synovial and subchondral microvasculature [1].

Chondroitin sulfate consists of several fractions with different molecular weights, while high molecular weight fractions are decomposed in the gastrointestinal tract. In this regard, the first preparations of chondroitin sulfate were suitable only for intravenous use. However, over time, technologies have been developed for obtaining low molecular weight fractions of chondroitin sulfate, which are almost completely absorbed in the gastrointestinal tract, retaining their structure, and are embedded in cartilage tissue. Modern biologically active food additives contain low molecular weight fractions of chondroitin sulfate, obtained mainly from the cartilage of salmonids.

When taken orally, chondroitin sulfate blocks the activity of pancreatic lipase and slows down the absorption of fats in the intestine. Long− term use of chondroitin sulfate may result in decreased levels of hyperlipidemia and hypercholesterolemia, and even weight loss.

1.3. Pharmacokinetics

Natural CS has a molecular weight of 50− 100 kDa. After the extraction process, the molecular weight is 10− 40 kDa, depending on the raw material. CS is mainly derived from bovine, porcine or marine (shark) cartilage.

CS is mainly administered orally in doses from 800 to 1200 mg / day. CS is rapidly absorbed in the gastrointestinal tract. Fully occupied CS reaches the blood compartment in the form of 10% CS and 90% depolymerized low molecular weight derivatives.

Different bioavailability and pharmacokinetic variables have been reported depending on the characteristics and origin of CS [2].

The bioavailability of CS ranges from 10% to 20% after oral administration [3]. CS uptake may depend on sulfation status. CS pharmacokinetic parameters measured in healthy male volunteers [4] after oral administration showed a significant increase in plasma CS levels (over 200%) compared to dose levels at 24 hours. The maximum concentration was observed 2:00 after dosing, and the increase is significant from 2 to

6:00 after dosing. First order kinetics were observed at doses up to 3000 mg. Multiple doses of 800 mg in patients with OA do not alter CS kinetics. Another characteristic of CS is its ability to accumulate in the articular tissue [5]. High levels of labeled CS have been found in articular tissues including synovial fluid and cartilage after ingestion in humans.

It is difficult to assess the correspondence of the maximum concentration achieved in the blood compartment (Cmax) for this type of drug, since in vivo the response may be the result of the influence of CS and disaccharides. Moreover, CS is a long− acting drug that results in a slow onset of action with a maximum effect achieved after a few months. In addition, CS is a biological drug, which means that its measurements in biological fluids do not distinguish the drug from endogenous molecules. The maximum effect (Emax) was predicted using an alternative method [6]. The Emax effect was calculated based on clinical efficacy. It has been estimated that 50% EMAC is achieved after 35 days in patients with mild OA. The approximate plasma half− life of CS and its derivatives in humans is 15 hours. Steady state is achieved in 3− 4 days, and it may take 3− 6 months of treatment to achieve maximum effect. CS is not metabolized by cytochrome P450. This is in the interest of the very low risk of drug interactions.

Finally, the carryover effect is described for CS. Indeed, after a delayed onset of action, the maximum effect persists after cessation of therapy.

1.4. Physical and chemical properties

Chondroitin sulfate chains are unbranched polysaccharides of variable length containing two alternating monosaccharides: D− glucuronic acid (GlcA) and N− acetyl− D− galactosamine (GalNAc). Some GlcA residues are epimerized to L− iduronic acid (IdoA); the resulting disaccharide is then called dermatan sulfate.

Chondroitin sulfate is obtained from natural products with high variability in terms of chain length and the nature of sulfation. Differences in the composition of chondroitin sulfate extend to its origin, which distinguishes chondroitin sulfate from terrestrial and marine sources. One way to look at this difference is the ratio of disaccharide units: terrestrial chondroitin sulfate consists almost exclusively of unsulfated (O) and

monosulfated A and C (carbon 4 of the N− acetylgalactosamine (GalNAc) sugar and carbon 6 of the GalNAc sugar) units, while marine species have a higher proportion of disulfated D, E (carbon 2 of the glucuronic acid and 6 of the GalNAc sugar and carbons 4 and 6 of the GalNAc sugar) units. In addition, marine chondroitin sulfate chains tend to be longer, with a molecular weight of up to 70 kDa in shark chondroitin sulfate, while in terrestrial animals the molecular weight is usually below 45 kDa [7]

Chondroitin sulfate chains are associated with hydroxyl groups of serine residues of some proteins. It is not known exactly how proteins are selected to attach glycosaminoglycans. Glycosylated serine are often accompanied by glycine and have adjacent acid residues, but this motif does not always involve glycosylation.

The addition of the GAG chain begins with four monosaccharides in a fixed structure: Xyl − Gal − Gal − GlcA. Each sugar is associated with a specific enzyme, which allows you to control the synthesis of GAG at several levels. Xylose begins to attach to proteins in the EPR, while other sugars attach to the Golgi apparatus [8].

The Golgi complex (also called the Golgi apparatus, the Golgi body, and others) is a single− membrane organelle found predominantly in eukaryotes.(Fig 1.2).

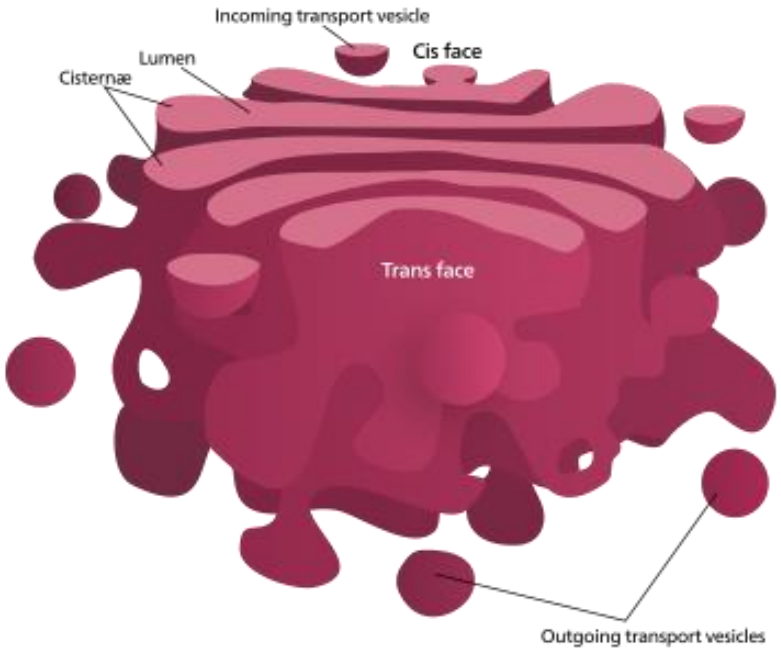

Fig 1.2. The Golgi apparatus

It was discovered in 1898 by the Italian physician Camille Golgi and was named after him. The main function of the Golgi complex is glycosylation and phosphorization of substances from the endoplasmic reticulum. It is a system of parallel and flattened tanks and tubes, to which are attached membrane bubbles that transport substances from the endoplasmic reticulum.

In the tanks of the Golgi apparatus mature proteins intended for secretion, transmembrane proteins of the plasma membrane, proteins of lysosomes, etc. The reaching proteins are sequentially moved through the tanks of the organelles, where their modifications take place − glycosylation and phosphorylation. In O− glycosylation, complex sugars join the proteins through an oxygen atom. During phosphorylation, an orthophosphoric acid residue is attached to the proteins. Different tanks of the Golgi apparatus contain different resident catalytic enzymes and, therefore, with the maturing proteins in them, different processes take place sequentially. It is clear that such a step− by− step process must be controlled in some way. Indeed, mature proteins are "labeled" with special polysaccharide residues (mostly mannose), which obviously play the role of a kind of "quality mark". It is not entirely clear how mature proteins move through the tanks of the Golgi apparatus, while resident proteins remain more or less associated with a single tank.

The effect of CS on part inflammation has been described in its use in patients with osteoarthritis. However, anti− inflammatory effects have also been reported in several organs or systems, including the skin, liver, and digestive system [8]. These effects are largely mediated by the interaction of CS with proinflammatory enzymes and transcription factors associated with weakening of the inflammatory response [9]. Jeong− Sook Noh et al. Have shown that in liver tissues CS− E produces marked protection against inflammatory and oxidative damage by lipopolysaccharides due to downregulation of the liver inflammatory factors TNF− α, IL− 1β, COX− 2, and iNOS [10].

H. Kawashima et al. Found that CS− E has a strong binding affinity with both L− and P− selectin, as well as with some chemokine [11]. The resulting CS interactions effectively suppress the activity of these proteins when leukocytes are recruited to sites of inflammation during infection and inflammation. While this is an important immune

response for the prevention of infections and some controlled inflammatory processes, it is also a key factor in the pathology and progression of many autoimmune diseases such as psoriasis and bowel disease [12]. The balanced activity of inflammatory phenomena is necessary for the restoration of systemic homeostasis, and this stage is very often not achieved after some autoimmune reactions or pathological inflammatory processes. In these uncontrolled events of the immune and inflammatory response, medical intervention is needed to regulate the processes down. CS of various types of sulfation was a key factor in these complications [9].

Determining the mechanisms by which active carbohydrate− protein complexes are regulated and stabilized, and therefore how they function at different stages of the disease, is critical for understanding the basic biochemistry and pharmacology of GalAG, as well as for a complete study of their medicinal properties, especially CS with different models of sulfation. In this regard, Xu Wang et al. characterized by improved methods of nuclear magnetic resonance (NMR) binding properties of two CS hexasaccharides: one is completely 4− sulfated in three disaccharides (CS444), and the other with 6− sulfation only on a non− reductive final disaccharide (CS644), complexed with the pro− inflammatory chemokine CCL5 / RANTES [13]. This slight change in the sulfation structure was enough to cause large changes in the interaction. It has been shown that CS444 is the best ligand for interaction and consistent structural mapping of the CCL5: CS hexasaccharide complex.

Chondroitin sulfate is well soluble in water.

1.5. Anticatabolic and anabolic effects

The first anabolic effect of CS was described over 30 years ago. CS increased the synthesis of hyaluronate in synovial cells [14]. Since then, it has also been demonstrated to increase the synthesis of collagen and type II proteoglycan in human chondrocytes [15] and GAG in bovine chondrocytes [16]. This effect may also be secondary to downregulation of matrix metalloproteinase (MMP) [17]. CS has also been shown to inhibit MMP− 1, − 3 and − 13 and ADAMTS− 4 and − 5 (aggrecanases) in human,

porcine and bovine chondrocytes.Inhibition of MMP− 13 may be due to inhibition of p38 and activation of Erk1 / 2 [18]. Various inhibitory potencies have been demonstrated for CS− 4 and CS− 6 against MMPs in murine chondrocytes [19]. Differences between CS− 6 and CS− 4 have also recently been shown in human cells. Indeed, CS− 6 (shark origin) can counteract the inhibition of IL− 1b inhibitor of tissue metalloproteases (TIMP) − 3 in human chondrocytes and TIMP− 1 in synovial fibroblasts, whereas CS− 4 (porcine trachea origin) did not have the same effect. CS can up− regulate hyaluronan synthase in fibroblast− like cells [20] and may be effective as a joint lubricant as shown in bovine cartilage explants [21]. It has also been reported that CS can influence the resorption process that occurs in the subchondral bone in OA. CS may well increase the osteoprotegerin (OPG): receptor activator ratio for the ratio of NF− κB ligands (RANKL) in OA osteoblasts in favor of subchondral bone homeostasis [22]. CS has been demonstrated to act on most of the articular tissues involved in the pathophysiology of OA. Finally, a recent in vitro study on bovine cartilage explants treated with CS− 4 and CS− 6 for 4 weeks showed a negative effect of CS, showing that it decreases GAG [23]. It is important to note that this study was conducted with the highest concentration of CS ever investigated (10− 100 mg / ml).

The anabolic and anti− catabolic effects of CS have also been found in vivo. CS may increase PG production in the rabbit cartilage degradation model [24], to prevent an increase in MMP− 9 in Freund's adjuvant arthritis in rats when administered as a dietary supplement [25] or inhibit MMP− 13 in collagen− induced arthritis in mice [26].

It has been shown that CS has antiapoptotic properties both in vitro and in vivo. It has been shown that CS (200 (μg / ml) reduces the sensitivity of rabbit chondrocytes to apoptosis [27]). This effect was associated with a decrease in NF− κB translocation and a decrease in MAP kinase signaling. pathways via p38 and Erk1 / 2. Another study indicated an antiapoptotic effect of CS− 4 and CS− 6 in the articular chondrocytes of mice [28]. In vivo, this has been shown in a mouse model of spontaneous OA, in which 12− day treatment with cholesterol (0.3 mg / day) reduces the number of apoptotic chondrocytes when evaluated after 30 days [29]. It has also been shown to be able to inhibit caspase− 3

and − 7. activation in collagen− induced arthritis in mice [27]. These results support the chondroprotective effect of CS.

Antioxidant effects were considered. CS has been shown to have antioxidant properties in vitro in human skin fibroblasts [30] and in vivo in collagen− induced arthritis in rats. CS provides protection against hydrogen peroxide and superoxide anions. Indeed, these studies have shown that it can limit cell death, reduce DNA fragmentation and protein oxidation, reduce the formation of free radicals and act as a scavenger of free radicals. It reduces lipid peroxidation and improves antioxidant protection by restoring endogenous antioxidants, reduced glutathione (GSH) and superoxide dismutase (SOD).

1.6. Conclusions to chapter

Despite the moderate effects of CS on pain and function, CS is an interesting product for the management of knee OA. Clinical evidence is in favour of a slow− acting effect on symptoms in moderate knee OA. CS is recommended by the most popular guidelines. Its safety profile is surely one of its main benefits for the treatment of aging patient with some comorbidity. There is then no limitation to its use in OA patients, if we ignore the economical impact. Nevertheless, caution should be exercise with regards to the type and the formulation of CS. Of course, some questions remain regarding its mechanism of action. The effect of CS on subchondral bone and synovium inflammation could be better documented.

The positive effects of chondroitin sulfate on the pathophysiology of osteoarthritis may be related to its contribution to the right balance between anabolism / catabolism in joint tissues.

CHAPTER 2
ANALYSIS OF METHODS OF OBTAINING CHONDROITIN SULFATE

2.1. Method for obtaining chondroitin sulphate from tissue of marine hydrobionts

The invention relates to biochemistry and biotechnology, in particular to methods for producing chondroitin sulfate from tissues of marine aquatic organisms, such as cartilage tissue of fish. The method provides for the preparation of raw materials for enzymatic hydrolysis. Alkaline hydrolysis is carried out with proteolytic enzyme preparations with neutralization of the resulting solution to pH 7. Salt is added to the resulting enzymatic hydrolyzate to a value of at least 0.1 mol / L. Conduct its sequential ultrafiltration first on a membrane with a retention limit of 50 kDa with the separation of high molecular weight impurities, then on a membrane with a retention limit of 5 kDa with a separation of low molecular weight substances. The solution of chondroitin sulfate retained on the membrane is washed on the same membrane with distilled water until the salts are completely removed. A final washing with distilled water is carried out on a membrane with a retention limit of 50 kDa.

The invention relates to the fishing industry, in particular to methods for producing chondroitin sulfate from tissues of marine aquatic organisms, such as fish cartilage tissue, muscular− muscular sac of molluscs, etc., and can be used in food, cosmetic industries, in medicine. The main properties of chondroitin sulfate, which are of decisive importance for its successful application in various fields, are high bioavailability, biological compatibility, low toxicity, the ability to selectively accumulate in cartilage tissue.

To obtain chondroitin sulfate, the most widely used method is the dissolution of chondroitin sulfate in an alkaline medium, enzymatic hydrolysis of proteins, separation of the high molecular weight carbohydrate fraction by precipitation from low molecular weight protein hydrolysis products remaining in solution, washing the resulting precipitate and drying the finished product.

This generally accepted technology is implemented by different authors in different ways: the sequence, the number of operations, temperature conditions, the nature and concentration of the reagents used change. The closest technical solution is a method for producing chondroitin sulfate using ultrafiltration.

This method of obtaining chondroitin sulfate includes the collection (preparation) of a feedstock, including connective tissue, hydrolysis of the feedstock with proteolytic enzyme preparations to obtain a solution of a hydrolyzate and an undissolved substance, treatment of a liquid hydrolyzate with a reagent containing divalent alkaline earth metal hydroxide with a pH greater than 10 to precipitate protein impurities from hydrolyzate, separation of at least part of the precipitate from the hydrolyzate solution and treatment of the liquid hydrolyzate using a membrane with the formation of a filtrate (permeate) of low molecular weight substances and a "delayed" concentrate, which contains a high molecular weight fraction − chondroitin sulfate. The patent proposes to use membranes with a molecular weight retention limit of 5 to 15 kDa (preferably 8 to 10 kDa).

The product obtained in this way is a concentrate, which, among other substances, contains chondroitin sulfate. Thus, the degree of purification of the target product is not high enough. The inventive method is also based on ultrafiltration separation of hydrolyzate fractions with different molecular weights and uses the property of high molecular weight molecules of chondroitin sulfate to be separated from − low molecular weight products of protein hydrolysis on ultrafiltration membranes. The technical result of the present method is to increase the degree of purification of the target product by using the ability of chondroitin sulfate molecules to strongly change the hydrodynamic radius when changing the ionic strength of the solution (electrolyte concentration, for example, NaCl), which makes it possible to achieve a higher purification of the target product than the prototype by means of sequential ultrafiltration the obtained hydrolyzate on membranes with different retention thresholds. As a raw material for obtaining chondroitin sulfate, raw materials containing cartilage tissue obtained as a result of processing various marine aquatic organisms can be used.

When using frozen raw materials, it is preliminarily carried out defrosting. The prepared raw material is crushed and loaded into a reaction vessel, in which alkaline and

then enzymatic hydrolysis is carried out. Cartilage hydrolyzate contains various products of protein breakdown, salts, high− molecular polysaccharides (chondroitin sulfate). The dissolution of alkali− soluble substances, including proteins and chondroitin sulfate, is carried out at a temperature of 25 to 50 ° C for 3 hours with constant stirring. After the end of alkaline hydrolysis, the mixture is neutralized to pH 7 and the insoluble precipitate is separated by filtration or centrifugation. Carrying out alkaline hydrolysis provides a preliminary separation of undissolved impurities and, as a consequence, helps to increase the yield of the target product, increase its purity. An enzyme preparation (FP) or a previously prepared solution of FP with proteolytic activity, for example, an enzyme preparation obtained from the king crab hepatopancreas, is added to the resulting solution.

Enzymatic hydrolysis of proteins is carried out at the temperature of the incubation mixture and the duration of treatment that is optimal for a given FP (when using FP from Kamchatka hepatopancreas, the temperature is from 45 to 55 ° C and the duration of hydrolysis is from 4 to 8 h), the solid precipitate is separated.

A salt, for example sodium chloride, is added to the resulting solution, bringing the salt concentration to 0.1 mol. Then the solution is ultrafiltered through a membrane with a molecular weight retention limit of less than 50 kDa to separate high molecular weight proteins and suspended particles remaining after hydrolysis. The concentrated solution containing chondroitin sulfate is washed with a solution of salt, for example sodium chloride or other salt with a concentration of 0.1 mol / L. For this, sodium chloride or another salt is added to the resulting solution to maintain its concentration in the solution to a value of at least 0.1 mol / L. If, after neutralizing the alkali, the concentration of NaCl is higher than the indicated one, then no additional amount of sodium chloride is added. When the concentration of NaCl in the solution is above 0.1 mol / L, the molecules of chondroitin sulfate are strongly globulated, which does not allow separating the components of the hydrolyzate. The salt concentration used provides a decrease in the hydrodynamic radius of chondroitin sulfate molecules and the possibility of their passage through − membrane with a molecular weight retention limit of less than 50 kDa, while on the membrane non− hydrolyzed proteins such as collagen are retained. The resulting solution of chondroitin sulfate, low molecular weight peptides, amino acids and salts is

subjected to ultrafiltration separation on a membrane with a molecular weight retention limit of 5 kDa, which ensures the retention of chondroitin sulfate molecules and the separation of salt molecules, amino acids and low molecular weight peptides. It is known that when the salt concentration in the solution decreases to less than 0.001 mol / L, for example NaCl, the chondroitin sulfate molecules but unfold, which leads to an increase in their hydrodynamic radius.

The maximum radius is observed in distilled water. The chondroitin sulfate retained on the membrane is washed with distilled water; as a result, the salt concentration decreases and the chondroitin sulfate molecules, the hydrodynamic radius of which has increased significantly, can be concentrated on membranes with a molecular weight retention limit of 50 kDa. On a membrane with a retention limit of 50 kDa, chondroitin sulfate is finally washed with distilled water from the remaining peptides of average molecular weight, and its solution is concentrated by ultrafiltration. The high molecular weight fraction of chondroitin sulfate is concentrated on the membrane, while low molecular weight peptides and amino acids pass through it. The obtained concentrated solution of chondroitin sulfate is then used to isolate a dry preparation or as a solution in the preparation of preparations with chondroitin sulfate. Isolation of dry khodroitin sulfate is carried out by precipitation with the addition of an excess of a precipitant (for example, ethyl alcohol) or by drying (sublimation, spray drying, etc.).

For example, the resulting solution is precipitated by adding alcohol in a ratio of 1: 2, kept until complete precipitation of chondroitin sulfate, the precipitate is separated by filtration or centrifugation, washed with alcohol, acetone, dried in a freeze dryer, vacuum or other dryer. A preparation of purified chondroitin sulfate is obtained with a mass fraction of the main substance of at least 90%. The target product − chondroitin sulfate is a white, odorless, amorphous powder, hygroscopic, the mass fraction of water is not more than 10%, the mass fraction of chondroitin sulfate is 90− 95% The mass fraction of the main substance was determined by acid hydrolysis in hydrochloric acid (26% HCl, 100 ° C, 1 h) and determination of the formed glucuronic acid by the Dische method. Identification was carried out by infrared spectroscopy. As a standard, we used a preparation of chondroitin 6− sulfate sodium salt from shark cartilage. The comparison

results showed that the samples of chondroitin sulfate obtained by the inventive method have practically similar indicators with those of the standard sample, which confirms the high degree of purification of the target product. The use of ultrafiltration is possible immediately when the preparation of chondroitin sulfate is isolated after enzymatic hydrolysis of raw materials or when the preparation precipitated with ethanol is purified after its dissolution in water.

2.2. Ultrafiltration mechanism features

Ultrafiltration is a process of membrane filtration, similar to reverse osmosis, using hydrostatic pressure to push water through a semipermeable membrane. The pore size of the ultrafiltration membrane is usually 103− 106 Daltons. Ultrafiltration is a pressure barrier for suspended solids, bacteria, viruses, endotoxins and other pathogens to obtain water of very high purity and low sludge density.

Ultrafiltration is a type of membrane filtration in which hydrostatic pressure causes a liquid to come into contact with a semipermeable membrane(Fig 2.1.). Suspended solids and high molecular weight solutes are retained, while water and low molecular weight solutes pass through the membrane. Ultrafiltration is not fundamentally different from reverse osmosis, microfiltration or nanofiltration, except for the size of the retained molecules.

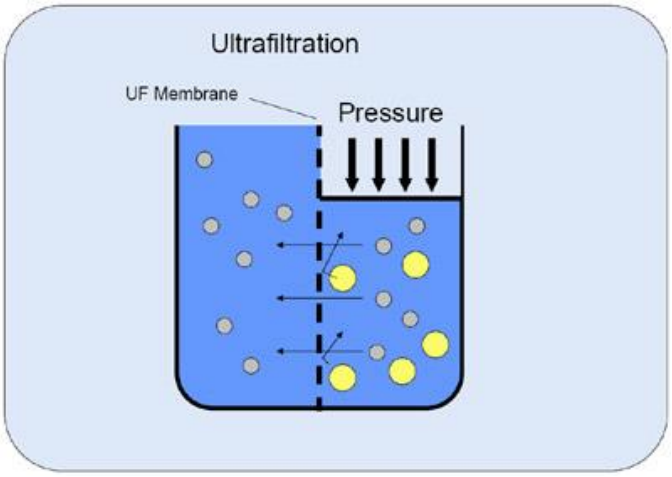

Fig. 2.1. Ultrafiltration schem

The membrane, or more precisely, the semipermeable membrane, is a thin layer of material capable of separating substances by applying a driving force to the membrane. Once considered a viable desalination− only technology, membrane processes are increasingly being used to remove bacteria and other microorganisms, solids and natural organic materials that can impart color, taste and odor to water and react with disinfectants to form disinfection by− products.

As progress is made in the production of membranes and the design of modules, capital and operating costs continue to decline. This newsletter covers pressurized membrane processes such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis .

2.3. Membrane arrangements vitrification

Depending on the shape and material of the membrane, different modules can be used for the ultrafiltration process [31]. Commercially available UF module designs vary depending on the required hydrodynamic and economic constraints as well as the mechanical stability of the system at specific operating pressures [32]. Key modules used in industry include:

- Tubular modules

The design of tubular modules uses polymer membranes molded on the inside of plastic or porous paper components, the diameter of which is usually 5− 25 mm with a length of 0.6 − 6.4 m [33]. Several tubes are placed in a PVC or steel sheath ... The supply of the module passes through the tubes, providing a radial transfer of the permeate to the side of the shell. This design allows easy cleaning, but the main disadvantages are low permeability, high membrane content and low packing density [33].

- Hollow fiber

This design is conceptually similar to a tubular module with a body− tube arrangement. One module can be composed of 50,000 hollow fibers and is therefore self− supporting as opposed to a tubular structure. The diameter of each fiber is 0.2 to 3 mm, with the feed flowing in the tube and the product penetration being radially collected from

the outside. Self− supporting membranes have the advantage of being easy to clean due to the backwash capability. However, replacement costs are high, since a single defective fiber would require replacement of an entire bundle. Given that the tubes are small in diameter, this design also makes the system prone to blockage.

– Spiral wound modules

They consist of a combination of flat membrane sheets separated by a thin mesh spacer material that serves as a porous plastic shield support. These letters are wrapped around a central perforated tube and installed in a tubular steel casing under pressure. The feed solution passes through the membrane surface and permeate spiral into a central collection tube (Fig.2.2).

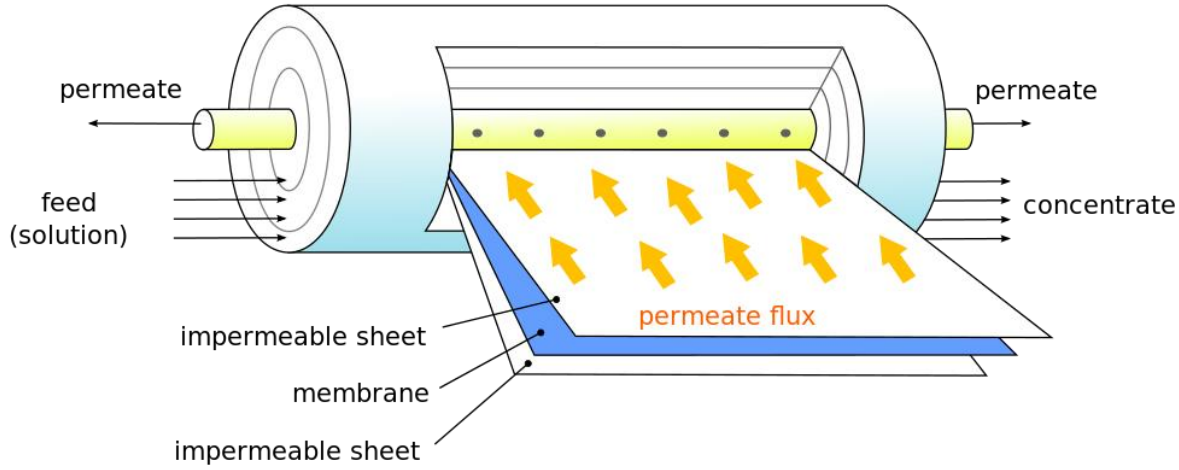

Fig. 2.2. Spiral− wound membrane module

Spirally wound modules provide a compact and low cost alternative to ultrafiltration design, offer high volumetric throughput and can also be easily cleaned [32]. However, it is limited to thin channels, where the supplied solutions with suspended solids can lead to partial blockage of the pores of the membrane.

– Plate and frame

For this, a membrane is used, located on a flat plate, separated by a mesh material. The feed is fed through a system from which the permeate is separated and collected from the edge of the plate. The length of the canal can be from 10 to 60 cm, and the height of the canal is from 0.5 to 1 mm [31]. This module provides a low retention volume,

relatively easy membrane change and the ability to deliver viscous solutions due to the low channel height, unique to this particular design.

2.4. Hydrolysis

Polymers break down into monomers in a process known as hydrolysis, which means "water cleavage", a reaction in which a water molecule is used during decomposition. During these reactions, the polymer breaks down into two components. If the components are non− ionized, one part receives a hydrogen atom (H−) and the other a hydroxyl group (OH−) from the cleaved water molecule. This is what happens when monosaccharides are released from complex carbohydrates as a result of hydrolysis.(Fig.2.3)

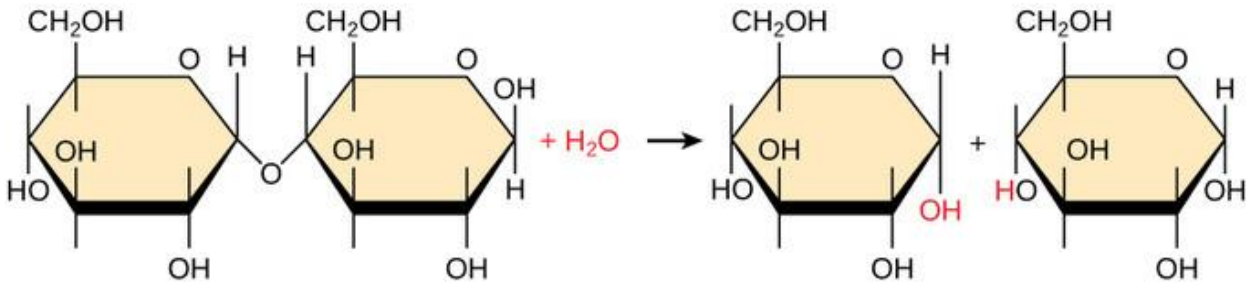

Fig. 2.3. Hydrolysis reaction, forms non− ionized products.: In the hydrolysis reaction shown here, the disaccharide maltose is cleaved to form two glucose monomers with the addition of a water molecule. One glucose gets a hydroxyl group in place of the former covalent bond, the other glucose gets a hydrogen atom. It is the reverse reaction of the synthesis of dehydration that combines these two monomers

If the components are ionized after the split, one part gains two hydrogen atoms and a positive charge, the other part gains an oxygen atom and a negative charge. This is what happens when amino acids are released from protein chains via hydrolysis.

- Enzymatic hydrolysis is a process in which cellulases are added to hydrolyze pre− treated lignocellulosic biomass to fermented sugars. The process

includes several key steps: transfer of enzymes from the main aqueous phase to the surface of the cellulose,

- adsorption of enzymes and formation of enzyme− substrate complexes,
- hydrolysis of cellulose,
- transfer of hydrolysis products from the surface of cellulose particles aqueous phase and
- hydrolysis of cellodextrins and cellobiose to glucose in the aqueous phase . The overall speed of the process is influenced by the structural features of lignocellulosic biomass, as well as the composition and source of cellulases.

Characteristics of cellulose, which most affect the rate of hydrolysis − is its crystallinity and available surface area . Cellulose fiber consists of both crystalline and amorphous regions. The crystallinity of cellulose affects the adsorption of enzymes, the synergy between the components of cellulases and the processivity of cellulases . When cellulase binds to crystalline cellulose, the rate of hydrolysis and yields is more than 100 times lower than when binding to amorphous cellulose . Available cellulose fiber surfaces include both outer and inner surfaces . The surface area is affected by the shape and size of the cellulose particles, while the inner surface area depends on the capillary structure of the cellulose fibers, the anatomical structure of the plant cell wall and the method of pretreatment (Fig.2.4.).

The rate of hydrolysis is also influenced by the sources of enzymes and the proportions of various enzyme components. A synergistic effect was observed among different components of cellulases, as well as among different glycosylhydrolases (eg, cellulases, hemicellulases and ligninases). Cellulase enzymes from different sources have different resistance to product inhibition, which, in turn, affects the rate of enzymatic hydrolysis.

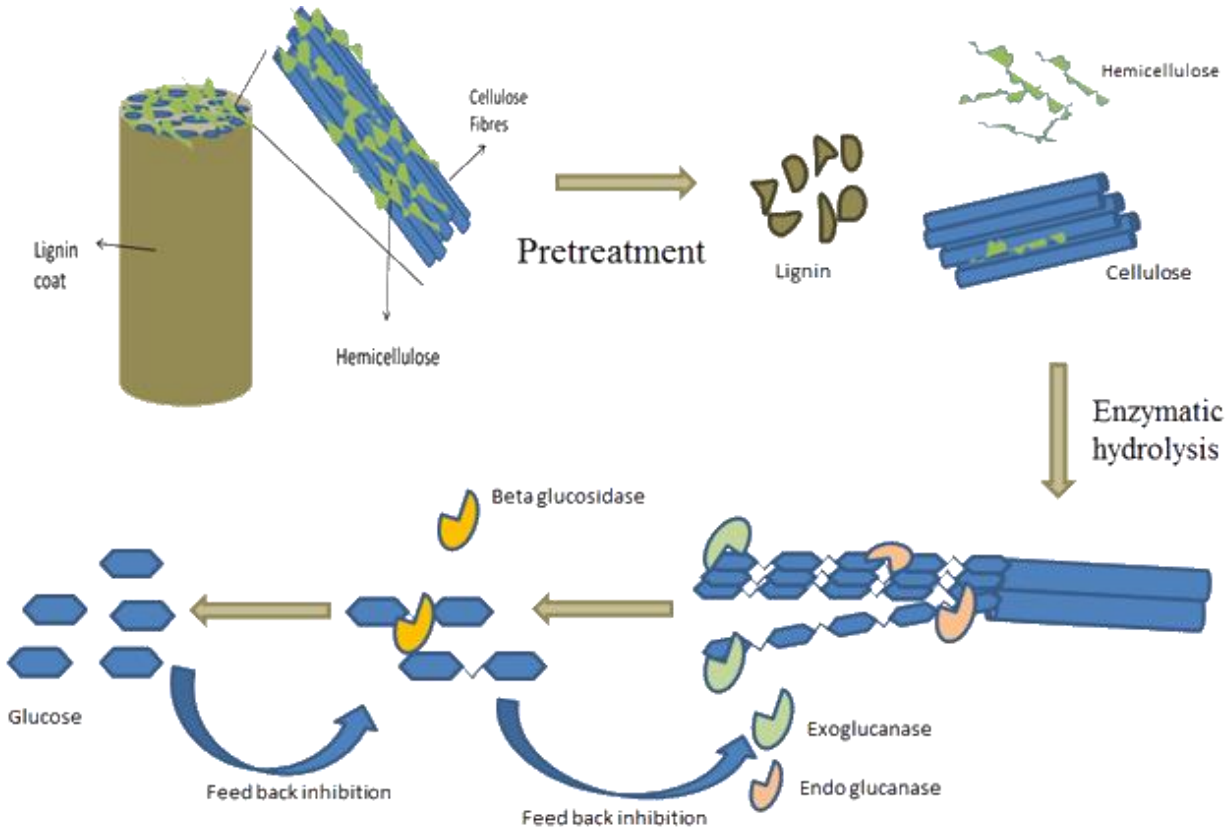

Fig. 2.4. Enzymatic Hydrolysis

2.5. Conclusions to chapter

The technology of obtaining CS from aquatic organisms using ultrafiltration membranes with different molecular weight retention limits has been analyzed. The parameters of the stage of precipitation of CS from the solution, close to the optimal ones, were determined. It was shown that CS obtained by the technology using ultrafiltration membranes is characterized by a high degree of purification and a narrower molecular weight distribution in comparison with CS obtained by a known technology.

CHAPTER 3
ANALYSIS OF THE MAIN AREAS OF APPLICATION AND PROSPECTS FOR THE USE OF CHONDROITIN SULFATE

3.1. Chondroprotectors

Chondroprotectors are designed to protect the articular cartilage from thinning and destruction, and also serve as a building material for synovial fluid − a lubricant that is inside the joints and promotes the sliding of the articular surfaces of the bones relative to each other.Chondroprotectors include chondroitin and glucosamine. These are the structural components of cartilage tissue that are essential for the continuous process of reproducing healthy cartilage.

The purpose of taking them is to reduce pain and stiffness in the joints, restore the structure of the cartilage.

There are 3 generations in the group of chondroprotectors:

Extracts from animal products (including cartilage of fish, crustaceans): alflutop, rumalon, mucartrin.

Monocomponent drugs:

- based on chondroitin sulfate: struktum, mucosat, chondroxide;
- based on glucosamine: don, elbon, stoparthrosis.

Combined drugs, which include glucosamine, chondroitin sulfate and additional components, such as vitamins, non− steroidal anti− inflammatory drugs: teraflex, arthroguard, geladrink.

Also, drugs differ in the method of administration:

- for oral administration in the form of powders, capsules or tablets, Teraflex capsules for example (Fig.3.1.)

Fig.3.1. Teraflex capsules

- for injection of intramuscular or intra− articular solutions;
- for external use – ointments, artiflex cream 40g as an example.(Fig 3.2.)

Fig.3.2. Artiflex cream

3.1. Future of Chondroprotectors

The aging process of the population is steadily accelerating not only in Russia, but also in most European countries. Already in many regions, people over 60 years old make up more than 30% of the population. The share of older people in the general population is growing faster than in any other age group.

One of the most common connective tissue diseases in the elderly is arthrosis (OA). Osteoarthritis (OA) (synonyms: osteoarthritis, erosive (osteo) arthrosis) is a degenerative−dystrophic disease of the joints caused by damage to the cartilage tissue of the articular surfaces. The symptom that primarily characterizes the disease is pain, which can be

accompanied by difficulties in movement and deformities. The most affected joints are the hips, knees, and shoulders. OA is the most common joint disease characterized by progressive cartilage degeneration, changes in the subchondral bone, and chronic synovitis [34]. Modern treatment of OA is limited to the use of drugs that affect the symptoms of the disease: analgesics, anti− inflammatory drugs, and treatment devices [35].

It is believed that the development, growth and regeneration of bones occur in the phases of ossification of the endochondral system. First, the cartilage cells and the surrounding matrix form a scaffold for bone formation [36]. Osteoblasts then penetrate this matrix and deposit the mineralized parts of the bone. Despite the long− term popularity of this model, it does not describe all the circumstances of the regeneration of the mesenchymal skeleton. A number of studies have shown that primitive chondrocytes not only form a temporary matrix, but can also differentiate into cartilage, mature bones, and even into adjacent stromal reticular cells [37]. As the bone lengthens, hematopoietic stem cells move from the liver to the bone marrow, supporting mature mesenchymal stem cells of the created population of perisinusoidal niches [36]. The articular cartilage, sandwiched between the synovial fluid and advanced by the pidchondral bone, becomes more and more distant from the bone marrow itself with an increase in ossification. This creates an articular compartment in a unique setting for unique function. Articular cartilage deserves special attention in the search for new cellular treatments for OA.

OA can be defined as degenerative processes in connective tissue. Degenerative− dystrophic changes in connective tissue is a disease of the musculoskeletal system and connective tissue (OA, osteochondropathy, dorsopathy, degenerative− dystrophic changes in osteochondral structures, consequences of trauma, dysplastic diseases, diseases of soft tissues (ligaments, tendons, muscles)) , various deformations of the musculoskeletal system, etc.

The main symptoms are defined by joint pain and stiffness. Joint pathology is diverse and includes focal lesions, synovial edema and inflammation, osteophytes (bone spurs), weakening of the periarticular muscles, coherent weakness, abnormal remodeling and thinning of the articular bone, and loss of articular cartilage It is widely believed that OA is an age− related dynamic response of a joint to injury or inflammation. All articular

tissues are damaged by OA, but the most noticeable loss of articular cartilage and changes in adjacent bone. OA is the destruction of the joint as an organ; in its effect on the body, it can be compared with renal or heart failure.

The aim of this review is to classify OA according to the severity and to study the effectiveness of known drugs with chondroprotective properties on the course of OA.

3.2. Bioavailability

As described above, both GL and CH are components of the extracellular matrix of articular cartilage. Experimental studies have also suggested additional effects on inflammatory pathways that contribute to the development of OA. Subject to external input, they have been widely considered as a treatment option for OA.

GL and CH have been used medicinally for almost 40 years [38]. However, their bioavailability after oral administration in humans remains a matter of controversy. The key issue would be the absorption of these agents through their release from the GI tract.

In mammals, the liver is the main site of their metabolism and degradation, but the exact mechanism is not clear [39]. The published information is quite contradictory. Early pharmacodynamic studies determined absorption only indirectly. Laboratory studies have suggested that GL significantly deteriorates in the gastrointestinal tract [40]. Other studies show that, despite the large molecular size that enters the body, CH is partially absorbed in the intestine, and some of them can reach the joints [42]. Pharmacokinetic studies in dogs have shown that GL (hydrochloride) is absorbed from a bioavailability of about 10% − 12% of single or multiple doses [42]. In humans, the serum GL level after taking a dose of 1.5 g of GL sulfate does not exceed 12 mmol / L. Animal studies have also shown that after ingestion of GL hydrochloride, the concentration of synovial GL is higher in joints due to synovial inflammation compared to levels achieved in healthy joints [43].

According to CS, different bioavailability and pharmacokinetic variables have been reported, usually depending on the study methodology or characteristics of the CS . Previous studies have reported a bioavailability of 10% − 20% [44]. Human studies have shown significant increases in plasma levels (over 200% compared to the dose level)

within 24 hours [40]. The use of labeled CS showed a high level of CS, which was observed in human synovial fluid and articular cartilage after administration. A limitation of the above studies is that both GL and CS are biologically derived. Thus, their measurements in biological fluids do not distinguish the drug from endogenous molecules.

3.3. Economic impact of glucosamine application

OA is most common in people over the age of 50, and with the gradual aging of the population in a number of countries, assessing the cost− effectiveness of treatment and the impact on health budgets is becoming increasingly important. Economic evaluation compares different treatment strategies, taking into account costs (costs of intervention and costs associated with the disease) and consequences, for example, the number of quality− adjusted life years (QALYs) that a patient received as a result of treatment. A 6− month cost− benefit analysis showed that pCGS was more cost− effective than paracetamol and placebo for knee OA in terms of the incremental cost− effectiveness ratio (ICER) [45]. In addition, a systematic review and economic assessment has shown that the added cost per QALY of adding GS to existing care throughout the patient's life is about £ 21,335 (approximately US $ 33,346) [45]. Sensitivity analyzes suggest that the cost− effectiveness of GS therapy is partly dependent on improved quality of life, changes in the likelihood of knee TJR, and the discount rate.

Observation of patients with OA in routine clinical practice has shown that continuous treatment with prescription pCGS leads to a decrease in the consumption of other concomitant OA drugs, the number of medical consultations and examinations in the long term [45]. Patients with OA who had previously participated in RCTs continued to be followed in the clinic (on average 5 years after the clinical study), during which the total average cost of treatment for OA was calculated during the year preceding the follow− up visit. It was almost 2 times lower among patients receiving prescription pCGS compared with those receiving placebo (€ 292 vs. € 605; p = 0.024). The total cost of OA drugs taken by patients in the placebo group (including analgesics and NSAIDs) was almost 2 times higher than that taken by patients in the pCGS group (€ 204 in the placebo group

versus € 108 in the pCGS group)), while the number of consultations with general practitioners, general practitioners (GPs) and paramedics, as well as examinations (radiographs, gastroscopy and non− OA examinations) performed in the previous year, were consistently higher for the placebo group, according to compared with patients taking pCGS [45].

3.4. Conclusions to chapter

In this review, OA is classified by severity, studied the effectiveness of known drugs with chondroprotective properties in the course of osteoarthritis. The main types of OA classification are considered. Sources of collagen− containing industrial animal and plant raw materials have been identified. The mechanisms of action of chondroprotective drugs (chondroitin sulfate, glucosamine sulfate or hydrochloride, hyaluronic acid, glycosaminoglycan, extraction drugs from animal or vegetable raw materials), their composition and properties are described. It is established that the correctors of bone and cartilage metabolism on the basis of chondroitin sulfate have chondroprotective and chondrostimulating effect. Currently, the treatment of OA is shifted towards cell therapy. Cell therapy has shifted from autologous chondrocytes to the use of readily available, highly proliferative and multipotent mesenchymal stromal cells. Global studies show that chondrocytes contain vitamin D receptors, which play an important role in regulating the production of matrix metalloproteinases and prostaglandin E2. The severity of the problem of treatment and prevention of degenerative− dystrophic processes of connective tissue proves the relevance of research on this topic.

CONCLUSIONS

1. The main effects of chondroitin sulfate are anti− inflammatory, analgesic, protective. CS improves phosphorus− calcium metabolism in cartilage tissue, inhibits enzymes that disrupt the structure and function of articular cartilage, inhibits the processes of degeneration of cartilage tissue; stimulates the synthesis of glycosaminoglycans (GAG), normalizes the metabolism of hyaline tissue, promotes the regeneration of cartilaginous surfaces and the articular bag. The appointment of correctors for the metabolism of bone and cartilage tissue is indicated at any stage of the degenerative process in the joints. By slowing down the resorption of bone tissue, reducing the loss of Ca^{2+} and accelerating the processes of bone tissue repair, they inhibit the progression of OA

2. Chondroitin sulfate is obtained from various sources but the main ones are fish. The main method of production is enzymatic hydrolysis and the next step is superfiltration to separate the hydrolyzate solution and then neutralization to obtain a precipitate and subsequent filtration to obtain a pure starting product.

3. One of the promising areas of pharmacotherapy for degenerative− dystrophic lesions of the joints, such as osteoarthritis (OA), is the use of chondroprotectors (CP). CP belong to the group of symptomatic delayed− acting drugs. The most acceptable drug capable of affecting metabolic processes in cartilage, synovial and bone tissue, suppressing the synthesis of pro− inflammatory mediators, is chondroitin sulfate (CS).

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