The impact of hydroxybutyl chitosan and 5-Fluorouracil on the procedure of endoscopic endonasal dacryocystorhinostomy.

1. Background

Endoscopic endonasal dacryocystorhinostomy (Endo-DCR) has the advantages of less surgical trauma, shorter time, less postoperative bleeding, faster recovery, and no skin scarring. However, the success rate of Endo-DCR surgery still varies widely between 58% and 94%, and the reason for surgical failure is mainly due to the membranous closure of the lacrimal sac-nasal anastomosis. The application of new filling materials to the lacrimal sac-nasal anastomosis provides a new way to improve the success rate of Endo-DCR.

Chitosan (CS) is a natural cationic polysaccharide, chemically named poly-2-amino-2-deoxy B-D-glucose, which has good biocompatibility and biodegradability, and is a new type of natural medical biomaterials. CS also has the advantages of abundant sources, no need for refrigeration, can be sterilized by autoclaving, and low price. Several studies have demonstrated the safety and efficacy of CS products for Endo-DCR.CS as hemostatic dressing for Endo-DCR is superior to collagen hemostatic agents. The success rate of CS-assisted Endo-DCR is higher than that of unassisted control group. However, chitosan is not easily soluble in water, making its application somewhat limited.

Hydroxybutyl chitosan (HBCS), a CS ether synthesized by substituting hydroxybutyl for the hydroxyl and amino groups of CS, maintains the nontoxic, biocompatible, biodegradable, antimicrobial, and moisturizing properties of CS, and the hydroxybutylation confers water-solubility and temperature-sensitivity to CS. Therefore, it is also widely used in biomedical applications such as postoperative adhesion prevention, tissue engineering, drug delivery and wound healing, etc. Materials made of HBCS, complexes of HBCS, and derivatives of HBCS have been shown to be safe and effective wound dressings with antimicrobial and blood coagulation promoting effects. Used for this trial proposed to be used is a heterophasic system of synthesized hydroxybutyl chitosan, this HBCS undergoes a sol-gel transition at a critical temperature (37 degrees Celsius), which facilitates clinical manipulation while accurately localizing around the anastomosis, preventing postoperative adhesions, and inhibiting the growth of fibroblasts.The role of HBCS in wound healing has been shown to have definitive therapeutic efficacy in clinical applications, and in ophthalmology also has some applications.

5-Fluorouracil (5-FU) is an antimetabolic drug that inhibits protein synthesis by suppressing the biochemical activities of rRNA and mRNA in the nucleus; it also induces the growth of apoptotic genes. In recent years, 5-FU has been found to be effective in the treatment of keloid

scars. It has now been widely used both at home and abroad to inhibit fibroblast proliferation and scar formation in the filtration vesicles after glaucoma filtration surgery, with good safety and efficacy. In antimetabolites as DCR adjuvant therapy significantly increased the size of the opening and improved the success rate of surgery.

2. Aims:

To investigate the effects of HBCS with and without 5-FU during Endo-DCR.

3. Induction and Exclusion Criteria

3.1 Induction Criteria:

1) Age: 18 years or above;

2) Gender: unlimited;

3) Disease duration: unlimited;

4) Indications: patients diagnosed with chronic dacryocystitis;

5) Subjects have a strong need for treatment, fully understand the condition and sign an informed consent form.

3.2 Exclusion Criteria:

1) Those who do not meet the inclusion criteria;

2) Those who are allergic to hydroxybutyl chitosan and 5-fluorouracil;

3) Those who have suffered from blepharitis, blepharitis, entropion, ectropion, keratitis, lacrimal tumors and other diseases of the external eye, ocular surface and other lacrimal apparatus within three months, or

those who have undergone ocular surgery;

4) Those who suffer from chronic dacryocystitis and have a history of lacrimal duct placement;

5) Those who suffered from lacrimal surgery or lacrimal fistula;

6) Those who have used glucocorticoid sprays such as Cochlearia within three months.

7) Those with a history of facial trauma (eyelid trauma, nasal trauma, etc.) or facial surgery within three months;

8) Those with nasal abnormalities or nasal diseases (nasal polyps, nasal tumors, sinusitis, turbinate hypertrophy, nasal bone fracture, etc.) within three months or those who have undergone nasal surgery;

9) Facial paralysis or severe eyelid laxity or other causes of tearing;

10) Those who also suffer from autoimmune diseases, bleeding disorders, renal insufficiency dialysis treatment and other serious systemic diseases, or those who have a debilitating general condition;

11) Those with blisters or shingles;

12) Those who also have more serious skin diseases such as acne, rosacea or previous history of physical scarring;

13) Medical, personal and allergic history that, in the judgment of the investigator, may interfere with the results of the test or increase the risk to the patient;

14) Participation in a clinical trial of another drug within the last 3

months;

15) Those who are unable to follow up on the scheduled visits or who are unable to cooperate with the investigator.

4. Exit Criteria

1）Patients who could not or did not undergo the procedure for various reasons, such as the occurrence of certain comorbidities, complications, or specific physiological changes in the subject that make continuation of the procedure inadvisable;

2）Patients whose surgical procedure was changed for various reasons.

3）Withdrawal of informed consent before the end of the study, including:

a. Withdrawal from the study due to unsatisfactory efficacy or without any reason;

b. The occurrence of an adverse event or serious adverse event that, in the opinion of the physician, warrants termination of further clinical trials;

c. Poor compliance and inability to complete the review, resulting in incomplete information and affecting the determination of efficacy.

5．Test Groups

The trial was divided into 3 groups, 40 cases were to be collected in each group, totaling 120 cases.

1）Control group: gelatin sponge wrapped thrombin and tobramycin dexamethasone ophthalmic ointment group.

2）Test group A: temperature-sensitive hydroxybutyl chitosan (HBCS) group

3）Test group B: HBHS combined with 5-fluorouracil (5-FU) group

6. Treatment

With random table, the patients were randomly divided into three groups. In group A, HBCS was applied to the surface of the anastomosis of the nasal mucosal flap and the lacrimal sac mucosal flap. During the aforesaid procedure, 0.3 ml of 5-FU solution (25 mg/ml) was injected under the nasal mucosa around the anastomosis in group B. (Sup.1) Anastomoses in Group C patients were covered with gelatin sponges encapsulated with thrombin, tobramycin dexamethasone, and oculentum.

7. Study Endpoint

The end of 6 months after surgery was used as the study endpoint.

8. Flow-up

		1 day	1 week (±1 day)	2 weeks (±1 day)	4 weeks (±3 days)	8 weeks (±3 days)	12 weeks (±7 days)	>24 weeks
Subjective perception	**Comfort**	√						
	Bleeding	√						
	Epiphora, mucopurulent discharge	√	√	√	√	√	√	√
Specimen collection	**conjunctival sac**		√	√	√	√		
	nasal cavity				√			
	Endoscope					√	√	√

9. Expected Risks and Measures to Address Them

1）En-DCR is an invasive procedure and there is a risk of blood leakage from the lacrimal sac-nasal anastomosis in the early postoperative period.

Most of the distant cases fail due to mucosal epithelial tissue hyperplasia, scarring and obstruction of the anastomosis. HBCS can stop diffuse bleeding, inhibit bacteria, and promote wound healing, etc. 5-FU can effectively inhibit the proliferation of fibroblasts. However, the effect of the two as dressings at the anastomosis of the lacrimal sac and nasal mucosa applied to En-DCR is uncertain. We will take appropriate measures if the following situations occur.

a. Localized infection: intensive anti-infection treatment.

b. Severe postoperative bleeding: Use other drugs and dressings to stop bleeding.

c. Granulation tissue, scar proliferation and anastomotic adhesion: excise or separate them, and apply antiproliferative drugs to the wound.

d. Delayed healing: use other dressings and medications.

2）Adequate and repeated education, obtaining informed consent, establishing timely and effective communication with patients, and standardized follow-up.

10. Statistical methods

To compare the differences between the adjuvant therapeutic effects of different En-DCR in the three groups: data were analyzed using SPSS 19.0 software, one-way ANOVA was used for age comparison, and Kruskal- Wallis H-test was used for comparison of postoperative comfort and blood leakage. Comparison of gender, complications, and cure rate

was performed by chi-square test, and the difference was considered statistically significant at $P<0.05$. Effective rate was compared by Fisher's exact probability method, and $P<0.05$ was regarded as statistically significant difference, and $P<0.0167$ was regarded as statistically significant difference for two-by-two comparison. The collected conjunctival sac and nasal flora were analyzed: alpha diversity analysis, PCA analysis, taxonomic composition analysis, and Lefse analysis were used, and differences were considered statistically significant at $P<0.05$. Comparisons between groups were made using chi-square test or fisher's exact probability method for adverse events and serious adverse events when necessary.

Version number 1.0
Version Date 2021.08.10

Informed Consent Form ● Informed Informational Page

Dear Patient:

Your doctor has diagnosed you or a family member with chronic dacryocystitis. We would like to invite you to participate in a drug clinical observation, namely "A randomized, contemporaneous controlled clinical trial of the safety and efficacy of temperature-sensitive hydroxybutyl chitosan and 5-fluorouracil assisted endoscopic lachrymal nasolacrimal anastomosis in the treatment of chronic dacryocystitis". This clinical observation method has been reviewed and approved by the Ethics Committee of Tianjin Medical University Eye Hospital.

Before you decide whether to participate, please read the following as carefully as possible. It will help you understand what the method is about as well as its significance, the procedure and duration of treatment, the possible benefits, risks and discomforts to you. If you wish, you can also discuss it with your relatives and friends or ask your doctor to give explanations to help you make your decision.

Background and significance

Current status of disease treatment

Chronic dacryocystitis is a common ocular disease, and surgical reconstruction of tear drainage channels is the key to treating this disease. Endoscopic dacryocystorhinostomy (En-DCR) has the advantages of less surgical trauma, shorter time, less postoperative bleeding, faster recovery, and no skin scarring. However, the success rate of En-DCR surgery still varies widely between 58% and 94%, and the reason for surgical failure is mainly due to the membranous closure of the lacrimal sac nasolacrimal anastomosis.

In recent years, with the development of material science and tissue engineering, new synthetic and biological materials have been appearing, and intraoperative anastomotic filler materials have been improved continuously, from non-absorbable materials (iodoform gauze, petroleum jelly gauze, non-absorbable expanding sponges, etc.) to absorbable materials (gelatine sponges, nanoabsorbent sponges, melaleuca gel, self-crosslinked sodium hyaluronate gel, chitosan and its derivatives, etc.), in search of an economical, Safe and stable anastomotic filler material that can promote epithelialization of the anastomosis and the surrounding mucosa, as well as effectively reduce postoperative complications, provides a new way to improve the success rate of surgery.

Hydroxybutyl chitosan (HBCS), a water-soluble CS ether synthesized by replacing the hydroxyl and amino groups of CS with hydroxybutyl groups, has biological properties such as promoting blood coagulation, antibacterial, moisturizing, promoting vascular endothelial growth, and inhibiting the growth of fibroblasts, etc. It has good biocompatibility and no cytotoxicity, and has been widely used in biomedicine for the prevention of adhesion and wound healing after surgery. 5-FU, an anastomotic filler material, can promote the epithelialization of the anastomosis and surrounding mucosa and effectively reduce postoperative complications, providing a new way to improve the success of surgery. antimetabolites such as 5-FU have also been shown to be safe and effective in other ophthalmic surgical settings (e.g., glaucoma and corneal surgery) to reduce fibrosis and improve clinical outcomes.

We tried to apply HBCS dressing or HBCS dressing combined with local mucosal injection

of 5-FU solution to En-DCR in order to further reduce the complication rate and improve the success rate of the procedure.

Purpose

To evaluate the efficacy of local application of HBCS dressing and local mucosal injection of 5-FU solution by using prospective, randomized, controlled clinical observation method, and to compare the effectiveness and safety of gelatin sponge dressing, so as to bring a new solution for the adjuvant treatment of En-DCR.

Participating units and number of included patient cases

The center of this clinical observation project is Tianjin Medical University Eye Hospital, and it is expected that 120 subjects will participate in this project.

What do I need to do if I join the clinical observation?

1. Before your enrollment, the doctor will ask and record your medical history, and examine your physical condition, including blood test, chest X-ray, lacrimal imaging, heart rate and blood pressure examination, lesion assessment, and medical history assessment. If you pass the examination, you may participate voluntarily and sign the informed consent; if you do not wish to participate, we will administer the treatment according to your wishes.
2. If you are willing to participate, you can receive the gelatin sponge dressing wrapped with thrombin and tobramycin ophthalmic ointment, HBCS dressing-assisted En-DCR, or HBCS dressing and 5-FU solution combined with local mucosal injection, and the efficacy of the treatment will be evaluated at 6 months after the surgery.
3. Other matters requiring your cooperation

It is important that you come to the hospital at the follow-up appointments agreed upon by your doctor and you. Your follow-up visits are very important because your doctor will be able to determine if the treatment you are receiving is really working and will be able to guide you in a timely manner.

You must not use any other treatment for your wound during the clinical observation period. If you need any other treatment, please contact your doctor beforehand.

Possible benefits of participating in the program

1. By participating in the program, you will get good treatment and observation.
2. you may be treated with temperature-sensitive HBCS dressings free of charge, and the quality of the dressings and medicines are guaranteed.
3. You can consult the project team members about your disease and the project at any time free of charge.

Possible adverse reactions, risks, discomfort and inconvenience of participating in the program

No adjuvant treatment can guarantee that the new tear ducts created by surgery will be open. In this project, the temperature-sensitive HBCS dressing is a product developed by Huizhong International Medical Devices (Beijing) Co., Ltd. and has passed the Beijing Innovative Medical Device Review. The dressing is non-toxic, biocompatible, biodegradable, antimicrobial and moisturizing with temperature-sensitive properties. It is composed of HBCS, glycerol and purified water, which is safe and reliable. 5-FU is an antimetabolite drug that is

effective in the treatment of keloids. Now it has been widely used both at home and abroad to inhibit fibroblast proliferation and scar formation in the filtration vesicles after glaucoma filtration surgery, with good safety and efficacy.

The biggest ethical risk of this project is that HBCS dressing or local mucosal injection of HBCS dressing and 5-FU solution cannot effectively promote the healing of postoperative wound and prevent the proliferation of scar tissue and granulation tissue, which will result in poor tear drainage. In order to protect your rights and interests to the greatest extent possible, when the development of the disease is likely to be healthy, we will terminate the project and give the treatment with appropriate measures.

You need to follow up in the hospital on time during the clinical observation period and do some tests, these take up some of your time and may cause you trouble or inconvenience.

Is personal information confidential?

Your medical records (program charts/patient files, lab tests, etc.) will be kept intact at the hospital where you are seen. Laboratory test results will be recorded by your doctor in your medical record. The project implementer, ethics committee and drug regulatory authorities will be allowed to access your medical records. Any public reports of the results of the program will not disclose your personal identity. We will make every effort to protect the privacy of your personal medical information to the extent permitted by law.

How can I get more information?

You may ask any questions about this program at any time and have them answered accordingly.

Your doctor will notify you in a timely manner if there is any important new information during the course of the project that may affect your willingness to continue participating in the project.

If you have any questions about your rights and interests during your participation in this study, you may contact the Ethics Committee of our organization. Tel: 022-86428817.

You can voluntarily choose to participate in the program and withdraw from the program.

Participation in the program is entirely at your discretion. You may refuse to participate in the Program or withdraw from the Program at any time during the Program without affecting your relationship with the Physician and without prejudice to any loss of medical or other benefits to you.

Your continued participation in the Program may be discontinued at any time during the course of the Program by the Physician or the Program Provider in the best interests of you. If you are withdrawn from the Program for any reason, you may be questioned about gelatin sponge dressings wrapped with thrombin and tobramycin ophthalmic ointment with oligocemethasone, HBCS dressings, or topical mucosal injections of HBCS dressings and 5-FU solution to assist with En-DCR therapy. You may want to undergo related tests if your doctor thinks they are needed.

Informed Consent Form ● Consent Signature Page

Statement of Consent

I have read the above description of this program and have had the opportunity to discuss and ask questions about this program with my doctor. All questions I have asked have been answered to my satisfaction.

I am aware of the possible risks and benefits of participating in this program. I understand that participation in the program is voluntary and I confirm that I have had sufficient time to consider this and understand that:

● I can ask my doctor for more information at any time.

● I can withdraw from the program at any time without discrimination or retaliation, and that my medical treatment and rights will not be affected.

I am equally aware that if I withdraw from the Program, especially if I am withdrawn from the Program due to medication, it would be very beneficial to the Program as a whole if I informed my doctor of any changes in my condition and completed the appropriate physical and physical-chemical examinations.

If I need to take any other medication due to a change in my condition, I will seek my doctor's advice beforehand or tell him/her truthfully afterwards.

I give my consent for the Ethics Committee of the Drug Administration or the sponsor's representative to have access to my project information.

I will be given a signed and dated copy of the informed consent form.

Finally, I have decided to agree to participate in this project and I promise to follow my doctor's instructions as much as possible.

Patient Signature: _____
Patient ID number: _____
Contact phone number:_____

I confirm that I have explained the details of the program to the patient, including his/her rights and possible benefits and risks, and given him/her a copy of the signed informed consent form.

Signature of program implementer: _____
Contact phone number: _____