



Autologous Serum In Eye Disorders

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Introduction

Tear film is very important in the maintenance of an intact ocular surface and thus pathologies of the conjunctival and corneal epithelium are intimately related to tear film dysfunction. When tears are depleted various changes like squamous metaplasia, epithelial defects and infections of corneal epithelium can occur.

It is with this objective in mind that ophthalmologists in the world over started searching for substances which could act as replacement for tears. Artificial tears like hydroxypropyl methyl cellulose and carboxy methyl cellulose are effective in providing lubrication to the ocular surface, but are unable to substitute the nutrients and factors present in natural tears. Serum drops are non allergic and their biomechanical and biochemical properties are similar to normal tears. Their use was first described by Fox *et al* (1) in 1984 in their search for a tear substitute free of potentially harmful preservatives. Later, Tsubota *et al* (2) reported that growth factors and vitamins present in serum eye drops have true epitheliotropic potential for the ocular surface. Here we review the currently available literature on the use of this new approach.

Why Autologous Serum ?

Serum contains various factors that are also present in tears including Vitamin A, Epidermal growth factor, transforming growth factor beta, basic fibroblast growth factor, Insulin like growth factor, Substance P as well as proteins such as lactoferrin and lysozyme (3). All these factors are essential for healthy functioning of ocular surface. Biochemical properties of normal human tears and serum are approximately similar, with vitamin A, fibronectin, transforming growth factor beta being more in serum and surface immunoglobulin A more in tears (4). Also there is no difference between growth factor

levels of autologous serum of dry eye syndrome and normal person (5). Vitamin A plays a vital role in reducing the progress to squamous metaplasia as may be seen in Kertoconjunctivitis sicca. Epidermal growth factor facilitates acceleration of corneal epithelial proliferation because of its anti-apoptotic properties (3). Transforming growth factor beta is also believed to control epithelial proliferation and to maintain cells in an undifferentiated state. Neural factors such as substance P are important for corneal epithelial migration. Human serum stimulates corneal fibroblast migration, proliferation and matrix metalloproteinase activity (6). In neurotropic ulcers these substances are decreased and may be supplemented by topical serum. Serum also contains antiproteases such as beta-2 macroglobulins which are thought to inhibit corneal collagenase and therefore, beneficial in conditions like alkali burns. Presence of oil in serum may act as a replacement for the lipid tear component produced by the meibomian glands. Proteins are also present in a high concentration in tears. Pre-albumin acts as a stabilizer for tear film and hence is important in the maintenance of its stability. Albumins and globulins protect the degradation of important cytokines. Although the mechanism is unknown the prolonged preservation of these components in serum makes autologous application clinically possible. Impression cytology studies also suggest that autologous serum leads to upregulation of goblet cells and mucin expression in a dose dependent manner. Since Rose Bengal staining is believed to be due to the lack of mucin, the increased mucin expression can explain the dramatic effect on the improvement of vital staining in clinical investigations (7). Autologous serum eye drops improve conjunctival status also in dry eye syndrome patients (8).

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Indications

The basic indications for use of autologous serum are the following:- Dry eye as seen in Sjogren's syndrome (2) and Keratoconjunctivitis Sicca (1). Persistent epithelial defects (9) where conventional therapy thereby has failed as in epithelial stem cell deficiency, diabetes post herpetic ulcer due to neurotropic problems. Ocular surface reconstruction. Corneal limbal transplantation and use of patients autologous serum as a tear substitute in patients with Steven's Johnson syndrome and ocular cicatricial pemphigoid. Alkali burn not responding to conventional treatment. *Other Indications are:* Superior limbic keratoconjunctivitis (10). Dry eye due to graft versus host disease (3). Treatment of macular holes: It has been found that after flattening of macular holes using surgical techniques, instillation of a drop of freshly prepared autologous serum (0.1ml) over the macular hole followed by perfluorocarbon gas tamponade and head positioning for 2 weeks result in higher closure rate of the macular hole.

Preparation and Use

A total of 40ml of blood is procured by venepuncture and is collected in a sterile container. Blood is left standing for two hours at room temperature to allow clotting to take place. Blood is centrifuged at 4000 rpm for 10 minutes. Serum is separated (40 ml blood yields 20ml serum). In a laminar flow cabinet 3ml aliquots of serum are removed and then packed into sterile dropper bottle with ultraviolet protection since vitamin A is easily degraded by light. The bottle is preloaded with 3 ml unpreserved chloramphenicol (0.5% chloramphenicol with boric acid 1.5%, borax 0.3% and purified water) or 3 ml saline. If 100% serum is desired saline or chloramphenicol is not used. Three (6 ml) bottles of 100% serum or six (6 ml) bottles of 50% serum are thus dispensed to the patients. There are various published variations in preparation and use of autologous serum with respect to centrifugal force (1500 rpm to 5000 rpm), duration of centrifugation (5 to 20 minutes), dilution, diluent and storage (1-3, 9).

Pre-Treatment Evaluation of the Patient

Prior to starting of autologous serum on a patient the following evaluations must be performed.

a. Subjective Evaluation (2)

Patient may be asked to grade their symptoms on the below mentioned format. The grading can then be redone on each follow up and benefit from treatment assessed

Symptom Grade

Grade 0	- No symptoms
Grade 1	- Mild symptoms with no discomfort
Grade 3	- Moderate symptoms with discomfort but no interference with daily activities
Grade 4	- Severe symptoms with discomfort, but no interference with daily activities
Grade 5	- Very severe symptoms with discomfort and interference with daily activities

b. Objective Evaluation (2)

1. **Tear Evaluation**
 - Schermir's I test
 - Schermir's II test
 - Tear break Up time
2. **Ocular Surface Svaluation**
 - Conjunctival impression cytology
 - Fluorescein staining
 - Rosebengal staining
 - Measurement of epithelial defect in two dimensions, longest and perpendicular

Storage and Stability of Autologous Serum

Patients are asked to store the autologous serum in a dark area as this prevents degradation of Vitamin A. The bottle in use is kept in the home refrigerator at 5⁰ C. The remaining bottles are stored in a freezer at -4 C (upto 1 month) or -20 C (upto 3 months) (2). Each bottle is discontinued after 7 days of use. Tsubota et al reported that the concentration of growth factors, vitamin A, and fibronectin in 100% and 20% serum diluted with NaCl stored at 4°C remained unchanged for 1 month and at -20°C for at least 3 months. Transforming growth factor beta, nerve growth factor, epidermal growth factor and insulin like growth factor-1 are more temperature and time resistant, but substance P degrades significantly at 4⁰ C in 24 hours (11). In a study by Prabhasawat *et al* (12) no contamination was reported upto 12 weeks for 20% autologous serum.

Clinical Results - Evaluation Criteria

Success is defined either as number of all eyes/patients improving or as reduction of mean baseline of fluorescein, rose bengal positive epitheliopathy (objective), or symptoms (subjective) score

Side Effects of Autologous serum

Side effects after the use of autologous serum have been reported but are rare. Immune complex deposition is known to occur especially in persistent epithelial defects due to rheumatoid arthritis or in cases with past history of herpes simplex. This has been attributed mainly due to



the auto antibodies in serum against the rheumatoid factor and the HSV antigen respectively. There may be swelling and redness. Itching, pain and discharge as well as allergy to chloramphenicol is also reported. Infective keratitis and conjunctivitis may also occur if serum is infected.

Legal Aspects

Medical Doctors can produce and apply autologous serum under their immediate responsibility. If the doctors do not actually produce such medications themselves, they must select appropriate personnel to do so.

In United States, producers of drugs and medical devices have to be registered with the Food and Drug Administration. In European Union and German legislation exemption is granted to medical doctors for production of autologous serum, but are responsible for the quality of production (13)

Contraindications

Use of autologous serum is contraindicated in the following patients:

- a. Rheumatoid arthritis
- b. Past history of herpes simplex keratitis
- c. Mooren's ulcer
- d. Microbial keratitis
- e. Infection of patients of Hepatitis B or C and HIV is also a contraindication for use of serum

Conclusion

While pharmaceutical lubricants offer little to no nutrition, eye drops made from autologous serum have a tear like biochemical character and supply nutritional components. In vitro studies have shown that serum supports viability, proliferation and migration of ocular surface epithelial cells better than unpreserved pharmaceutical tear substitutes. The production parameters should be optimized before a meaningful randomized, controlled clinical trial attempts to evaluate the real therapeutic potential of this approach in ocular surface disorder. Meanwhile, the use of serum eye drops remains an experimental approach. It is a cost effective way of treating dry eye syndrome with minimal side effects.

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