USE OF AUTOLOGOUS SERUM IN OPHTHALMIC PRACTICE

APLICACIONES DEL SUERO AUTÓLOGO EN OFTALMOLOGÍA

LÓPEZ-GARCÍA JS, GARCÍA-LOZANO I, RIVAS L, MARTÍNEZ-GARCHITORENA J

ABSTRACT

Objective: To establish a protocol for the use of autologous serum in the ophthalmic practice.

Methods: Personal experience and a literature review.

Results: The use of autologous serum, in eye drop form, has been reported as a new treatment for several ocular surfaces diseases. These products have biomechanical and biochemical properties similar to normal tears. They contain components such as fibronectin, vitamin A and growth factors that have an epitheliotropic effect on the ocular surface epithelial cells.

Conclusions: The clinical studies performed showed a variable efficacy, with the preparative process and use of the autologous serum eye drops varying considerably between different studies (Arch Soc Esp Oftalmol 2007; 82: 9-20).

Key words: Autologous serum, ocular surface, dry eye, corneal epithelium, impression cytology.

RESUMEN

Objetivo: Establecer un protocolo razonado para la preparación y manejo de la terapia con suero autólogo en la práctica oftalmológica.

Métodos: Revisión bibliográfica y experiencia personal.

Resultados: La utilización de suero autólogo en colirio ha sido referida por muchos autores como una nueva forma de terapia en el manejo de enfermedades de la superficie ocular. El suero autólogo presenta unas propiedades mecánicas y bioquímicas similares a las de la lágrima, y como ésta contiene componentes como la fibronectina, vitamina A y factores de crecimiento que tienen un efecto epitheliotrófico sobre las células epiteliales de la superficie ocular.

Conclusiones: Los distintos estudios publicados muestran una importante variabilidad tanto en la eficacia de esta terapia como en la metodología de preparación y aplicación.

Palabras clave: Suero autólogo, superficie ocular, ojo seco, epitelio corneal, citología de impresión.
INTRODUCTION

The positive effects of the application of autologous serum in the treatment of dry eye patients are known since 1984 due to the research of Fox et al (1). However, the lack of knowledge about its action mechanism at the eye surface level kept its utilization in clinical practice from growing until the end of the decade, thanks to the work of Tsubota et al (2,3).

Tears have a great importance in the stability and feasibility of the corneal and conjunctival epithelium due to the interdependence between the various structures comprising the eye surface (4). The cornea obtains its main nutrients (glucose, electrolytes, etc.) from the aqueous humor, but growth factors, vitamins and neuropeptides which account for the proliferation, migration and differentiation of corneal and conjunctival epithelium come from the lachrymal gland which pours them into each tear (5,6). In addition, tears have antimicrobial, nutritive, mechanical and optical properties (7). In dry eye cases, the toxicity over epithelial cells increases (8,9) giving rise to common epithelial disorders. In these cases, artificial tears cannot on their own promote adequate epithelium regeneration. Some authors have made recourse of a variety of surgical procedures to stimulate the production of tears (1). However, said surgical techniques are complex and frequently involve associated complications.

The utilization of autologous serum in ophthalmology arose out of the need of finding lachrymal substitutes which, in addition to humidifying, are able to provide other components of tears which are reduced in dry eye cases. Bovine fetal serum and umbilical serum have been utilized for the same purpose (11). Numerous growth factors are obtained from the bovine fetal serum which are utilized for in vitro cell cultures (12). In addition, it has been utilized for treating corneal ulcers in dogs (13).

In what concerns umbilical serum, it is believed it has a larger concentration of growth factors but, as with bovine serum, possible allergies must be considered as well as the risk of transmitting infectious diseases (11).

In this regard, we believe it is preferable to utilize the patient’s own fluids such as autologous serum because it provides the same advantages, there is no risk of disease transmission and it also lacks antigenicity.

AUTOLOGOUS SERUM PROPERTIES

Even though the final action mechanisms of autologous serum over ocular epithelium are not yet clear (14), detailed knowledge about them is increasing. For instance, it is known that autologous serum contains some components involved in the proliferation, migration and differentiation of the eye surface epithelial cells.

It is believed that many serum components have some trophic effect on the eye surface epithelial cells due to their action on the epithelial dynamics, modeling the proliferation of epithelial cells in the limbus and cornea (15), to the extent that in vitro studies with conjunctival epithelial cells have proved an effect (dependent on dosage) of autologous serum on the expression of mucines, mainly mucine 1, regulated by EGF receptors in the cup-shaped cells (3).

Of all serum components it is believed that the most important are the Epithelial Growth Factor (EGF), Transformer Growth Fibroblast b-factor (TGF-b), vitamin A, fibronectine, albumin, a2 macroglobulin, the Platelet-derived Growth Factor (PDGF-AB), neuropeptides such as substance P and the insulin 1 type growth factor. Accordingly, EGF accelerates the migration process of epithelial cells (16,17), and has antiapoptotic effects (18,19). This factor is present in the basal as well as reflex lachrymal secretion (20), with a slightly lower concentration to the one it has in autologous serum. TGF-b is involved in epithelial and stromal repair processes (21-24), with its concentration in serum being 3 times more than the concentration found in tears. Vitamin A seems to prevent squamous metaplasia processes of epithelium (3). Its concentration in serum is much higher to that found in tears. On the other hand, proteins such as albumin have proved to have antiapoptotic activity (25), while a2 macroglobulin exhibits anticolagenase activity (26). Fibronectine is one of the most important factors in cellular migration (27,28), with its concentration in serum being well above that found in tears. In addition, autologous serum contains neuronal factors such as substance P and insulin 1 type growth factor which seem to
play a part in the migration and adhesion of the corneal epithelium (29). In turn, PDGF-AB is one of the 5 known isoforms of platelet-derivated growth factors. This factor is activated intra-cell and is secreted by the alpha granules of platelets after its activation, enhancing mitosis and scarring.

In addition, autologous serum contains immunoglobulines such as IgG, lisozyme and supplemental factors which endow it with a certain bactericide and bacteriostatic effect.

Table 1 shows the comparative relationship between some concentrations of the main epitheliotrophic factors found in tears and in autologous serum (7).

### EFFECTS OF AUTOLOGOUS SERUM

The effects of autologous serum on the eye surface are determined by its numerous properties. Serum exhibits characteristics which are very similar to those of tears as regards pH and osmolarity. Like tears, it contains abundant growth factors and bactericides which allow a treatment with topical autologous serum not only to humidify the eye surface, but also to provide nutritional and growth factors necessary to maintain cellular feasibility in the epithelial repair processes, and bactericide components which reduce the risk of contamination and infection (30).

Even though some authors do not find statistically significant differences between treatments with autologous serum and other conventional therapies with artificial tears or BSS (31,32), the application of autologous serum broadly exceeds substitutive therapy with artificial tears. Similarly, we found in these patients an improvement of almost two degrees of squamous metaplasia in corneal impression cytology. These results match those found by Noble et al (33), who found that autologous serum treatment improves the clinical symptoms and the conjunctival metaplasia degrees, but failed to find statistically significant effects with Rose Bengal, Schirmer and fluorescein clearing.

Although in aniridia keratopathy patients we have found a slight positive effect of autologous serum in the improvement of corneal transparency and the regression of neovessels (unpublished results), the main effect of this therapy takes place with the epithelium formation of the ocular surface. Accordingly, its main clinical application is for managing corneal epithelium disorders.

Notwithstanding the above mentioned research, it is not known yet which are the most beneficial factors and the optimum concentration thereof. Reviewing published results, authors like Geerling et al found a large variance in the effects of autologous serum, both on the subjective symptoms of patients as on the modification of tests such as the Schirmer test, Rose Bengal or fluorescein clearing. Said diversity of results could be due to the low homogeneity of the population of the studies and to variations in the preparation of autologous serum by different authors (7).
PREPARATION OF THE AUTOLOGOUS SERUM

The distribution of pharmaceutical products is regulated by government laws in most countries. In the European Union, although the Euro Parliament has issued guidelines each member country is empowered to authorize the distribution of drugs. In the US, the Food and Drug Administration (FDA) controls the marketing of pharmaceutical products.

As autologous serum is a product for individual use it is not considered to be a pharmaceutical product but a pharmaceutical compounding, and in our country the preparation thereof is regulated by Royal Decree 175 dated Feb. 23, 2001 (Official Gazette 065-2001 dated March 16, 2001: 9746-9755).

In other countries like Germany, the standard preparation protocol follows the guidelines of the Bundesärztekammer (Medical Society) and the Paul Ehrlich Institute for the donation and use of hematic products (7). In the United Kingdom, the standard preparation protocol follows the principles recommended by the National Blood Service and the medication control agency in agreement with the guidelines for depositing self-donations of blood by the British Committee for Standards in Hematology (38). Said protocols apply the same criteria as for blood donation, i.e., patients are screened for infectious diseases of parenteral transmission (AIDS, hepatitis B and C, syphilis, etc.) so that if any result is positive, he/she is excluded for treatment with autologous serum, as is the case with those who exhibit anemia or cardiovascular diseases.

Reviewing the literature we found a large variability in the preparation methodology, storage and dispensation of the autologous serum eye drops. In addition, most authors agree on the need of unifying criteria by means or multicentre randomized studies for optimizing the results obtained with this treatment.

The preparation of the autologous serum eye drops must involve the Hematology (Blood Bank) and Pharmacy services. Blood is extracted from patients’ veins with vacuum extraction tubes with gel and without anticoagulant (fig. 1A). The amount of blood extracted varies according to each article. In our case we extracted about 40 cc of blood which was distributed in 4 tubes. Subsequently we left the tubes in vertical position at 22°C for about 2 hours to allow coagulation. This coagulation time also differs in published articles; some authors directly centrifuge the blood, while others wait up to two days (33). In experimental studies carried out in cell cultures it was seen that a longer coagulation time is related to a larger effect of the autologous serum over the migration and differentiation of epithelial cells, with authors recommending a coagulation time of at least two hours (39). They also found a larger concentration of all examined factors, with significant results for EGF and TGF-b when the blood was allowed to rest over two hours.

After coagulating the blood it was centrifuged to separate the autologous serum (figs. 1 B and C). In this stage of the process we found in the literature wider variations in the centrifugation power and time. Authors like Tsubota et al (2) centrifuge for 5 minutes at 1500 rpm while others like García et al Centrifuge for 10 minutes at 5000 rpm (40), Geerling et al (7), centrifuge at 3000 rpm for 15 minutes whereas others like Malavazzi et al (32) centrifuge at 1500 rpm for a full hour. In this, it is important to consider that the centrifugal force not only depends on the rpm but also on the rotor diameter, which varies according to the type of machine. Accordingly, some authors like Poom et al prefer to utilize «g» force values (26). The G-force concept includes the rpm as well as the rotor diameter and accordingly it seems a more homogeneous and applicable measurement regardless of the centrifuge make or model. Many such machines display the centrifugal force in rpm as well as G-force. The time and power of centrifugation is important. For example, Geerling et al (7) consider that 3000 rpm for 15 minutes produces an important amount of autologous serum without producing hemolysis and, on the contrary, they consider that with less centrifugal force or less time not only less serum is obtained but it can also retain remains of platelet membranes which, if the dosage is large enough, can cause cell apoptosis. The same authors compare their results centrifuging at 4,000 G for 10 minutes with those obtained by Tsubota et al (2) centrifuging at 1500 rpm for 5 minutes, and found that they obtained a larger EGF concentration but a lower amount of TGF-b. Other authors such as Liu et al (39) found that, by increasing the centrifugal force from 500 to 3,000 G the concentration of EGF and Vitamin A increases considerably in the serum, in turn having a less spectacular effect over TGF-b.
In our case we opted for an intermediate solution: we centrifuged 10 minutes at 2500 rpm, an average speed which does not produce lysis, and obtained about 5cc of serum from each 10cc blood flask.

After separating the serum we go on to prepare the eye drops with fresh serum or store it in pipettes shielded from light by aluminum paper and adequately identified, storing them in a freezer at –80°C for subsequent preparation (fig. 1 D). In this stage we also found discrepancies amongst authors. Most prepared all the autologous serum eye drops with fresh serum, giving the flasks to the patient for their utilization, recommending them to keep the one in use in the refrigerator at 4°C and storing the rest at –20°C or –30°C (30,33). Some studies proved that autologous serum components remain stable for one month at temperatures of +4°C and for three months at –20°C (2). However, authors such as Sitaramamma et al (41) recommend keeping the flasks in the refrigerator awaiting use because the concentration of proteins in some bodily fluids goes down dramatically at temperatures below 4°C.

The eye drops are prepared in aseptic conditions and in a laminar flow container. After de-freezing the serum sample or using fresh serum, 2 cc are separated and introduced through a millipore filter into a sterile flask adapted for ophthalmological use.

Fig. 1: A: Extraction of blood by venous puncture and centrifugation. B and C: Separation of the serum from the remainder of blood elements.
Subsequently 8cc of physiological serum are added to obtain a final concentration of 20% (figs. 1 E, F and G). The container is wrapped in aluminum paper and tagged with the patient data (fig. 1 H). As in the rest of the process there is a large variability in literature regarding the use of filters and the final product concentration. Most authors do not use filters, but other like Fox et al (1) do recommend their use for separating from the autologous serum any remains of fibrin which seem to reduce the serum effects. The utilization of filters entails important advantages such as the elimination of remains or particles which are present in the serum and also, due to the size of the pore (0,22 µm), said filters add a sterilizing effect by retaining microorganisms such as bacteria.

However, on the other hand, said filters could retain molecules, thus altering the serum composition. To avoid this we recommend the utilization of millipore filters such as Durapore® or Millipore ExpressT. Both have a pore size of 0,22 µm and a low protein affinity and are useful for the volumes we handle in autologous serum. In our experience, the Durapore® filter is more liable to become saturated and obstructed. The Millipore ExpressT filter has a sulphate polyester membrane which makes it particularly useful for filtering these biological products. Most authors utilize physiological serum for diluting serum. However, authors such as Liu et al find in epithelial cell cultures that the cell proliferation is greater when the dilution is made with BSS (39). The utilization of a 20% concentration of autologous serum is empirical, in fact no significant differences have been found between the migration of epithelial cells of in vitro cultures with concentration between 10% and 20% (2). Some authors like Poom et al (26) believe that a greater concentration would have more effect on the eye surface. These authors utilize concentration s of 50% and 100%, like Noble et al who utilize a 50% concentration (33). Even so, the most standardized utilization of autologous serum is at a concentration of 20% (7). This concentration seems sufficient and avoids the irritations derived from the higher viscosity of highly concentrated preparations and considerably reduces the number of blood extraction (42).

A flask of autologous serum eye drops adequately identified and wrapped in aluminum paper to protect it against the light is given to the patient. Previously and after signing an informed consent, the patient is informed about the correct use and handling of the preparation, mainly in what concerns preservation and hygiene in application as well as manipulation by other individuals. We must not forget this is a parenteral fluid and could transmit infectious diseases. In this sense and even though we do not routinely carry out a screening of blood samples, it is recommendable to know whether the patient has any parenteral transmission disease excluding the use of this preparation or educate him about the potential dangers inadequate use may have on other individuals. It is important for the eye drops to be kept in the refrigerator away from light because some of its components such as vitamin A degrade quickly with light. On the other hand, the correct utilization of the eye drops noticeably reduces the risk of contamination. Although the sample can become contaminated at any time during preparation, the most frequent cause is erroneous manipulation by the patient. For this reason, the autologous serum is most frequently contaminated by S. Epidermidis. Sauer et al (43) found that 7.5% of flasks kept at 7°C become contaminated with S. Epidermidis after 7 days. Similarly, Lagnado et al (30) did not find important infectious complications in these patients. Some authors like Poom et al (26) utilize autologous serum with antibiotics such as chloramphenicol at 0.5%. However, others like Nakamura et al state that the use of antibiotics reduces the effects on the autologous serum epithelium (44).

The application and dispensation of these eye drops varies with each author. The number of applications varies from once every hour to three times a day. In what concerns dispensation, some authors use a flask per day while others use the same flask up to ten days (7). In our case, we recommend instillations every 3 hours and utilize the same flask for one week. The patient is instructed to bring back the utilized flasks in each checkup visit to deposit them in the biocontaminated material container. We haven’t had any case of conjunctivitis or corneal infection due to the application of autologous serum.

**INDICATIONS**

Autologous serum has been utilized with varying success for managing numerous processes involving the corneal surface. Its application has been notable in the treatment of:
Fig. 1: D: After separating the autologous serum, the preparation process can continue or it can be frozen. E: Two cc of autologous serum are separated in laminar flow chamber and diluted with 8cc of physiological serum. F and G: Final mixture at a concentration of 20%. H: Final appearance of the container as provided to the patient with name data. Notice the container is protected against the light by an aluminum layer.
Persistent epithelial defects (PED) produced by several etiologies (2,14,45). PED can be defined as an epithelial defect with a diameter over 2 mm and a duration of over two weeks without responding to conventional therapy with tears, contact lens, etc. Many therapies have been tried with PED including the provision of tears (46), therapeutic contact lenses (47), tarsorrhaphy (48), amniotic membrane transplant (49), limbus transplant (50), topical fibronectine 851, tetracycline (52) or reconstruction with cultured epithelial cells (53). In these patients, autologous serum therapy noticeably reduced the duration of PED. Accordingly, 63% of PED treated with autologous serum healed in under a month against the 7.2 months average time elapsed for healing of PEDs treated with conventional therapy (26). In another paper authored by us and awaiting publication, we found that autologous serum treatment produced improvements in 100% of the PEDs of our series, achieving complete healing in 70% of cases after two months of treatment.

Severe dry eye. This was the first utilization of autologous serum in ophthalmology (1), subsequently utilized as well by other authors (3,26). Nearly all authors report considerable subjective improvement of symptoms and tincture with Bengal rose, fluorescein and Schirmer test and a progressive worsening 4 weeks after suspending application (3).

Similar results are referred by authors like Poom et al (26). In dry eye cases it has been speculated that the effects could be dosage-dependent, with more benefits being found in dosage patterns applying drops 8 times a day than 4 times a day. A study we carried out in dry eye patients with varying severity we found that the application of autologous serum improved on average 3 mm the Schirmer test and 4 seconds the BUT. Tincture with fluorescein and fluorescein Bengal rose also improved as well as the stability of the lachrymal film.

Management of neutrophic keratopathy (54,55). In the treatment of this disease authors like Matsumoto et al (2004) found that the epithelial defect healed completely in 17.1 SD 8.0 days, improving corneal sensitivity determined by a Cochet-Bonnet stesiometer, going from 11.8 SD 11.6 mm before treatment to 30.0 SD 22.9 mm after treatment (55).

Recurring corneal erosions. In these patients the application of autologous serum reduced the recurrence rates (56). In a study we carried out it was found that the corneal erosion recurrence rates reduced 87.5% in patients treated with autologous serum vis-à-vis the same patients treated with conventional therapy using artificial tears.

In some occasions, the application of autologous serum was associated to ocular surface reconstruction surgery such as limbus transplant, amniotic membrane transplant, penetrating keratopathy in patients with Stevens-Johnson syndrome or cicatricial ocular pemphigoid, contributing to improve the corneal epithelium stability (57).

It has also been utilized for managing dry eye associated to graft against host disease (2,58). In these patients a quick subjective clinical improvement is found, with signs such as dotted keratopathy disappearing at a slower rate (59). In addition, it has been utilized as associated therapy for treating Mooren’s ulcer (1) and superior limbal keratoconjunctivitis, in which some authors have found an adequate response to treatment in over 80% of patients (60).

Autologous serum has also been utilized in clinical environments for application over leak points in filtration blebs (61), in the treatment of full width macular holes (applied over the hole or added to the irrigation serum for cleaning the indocyanine green used for visualizing the internal limiting membrane in macular hole surgery) (62,63), retinal ruptures (64), in association with platelet concentrates in retinal cicatrization experimental models (65).

We are utilizing autologous serum for managing calcium keratopathy as support therapy for calcium chelators. Although we have few cases in our study, it seems that topical application reduces the intensity of the calcium layer, with more translucent spaces appearing over it and an unmistakable regression in some patients. We have also utilized it in postop situations after the surgical removal of calcium plates. Similarly, we are utilizing autologous serum in postop of patients with pterigion. In these patients we have found that the application of autologous serum for a month after removal of the pterigion reduces the rate of relapses and patients refer important improvements of their symptoms with it use. In addition, we have utilized autologous serum in the treatment of keratopathy associated to aniridia. Ninety-five percent of patients exhibited significant clinical improvements after treatment with autologous serum in relation to previous therapies with artificial tears. Squamous metaplasia improved about two degrees (average) in all severity levels and regardless of the keratopathy severity.
Autologous serum enhanced epithelium formation and stability of the lachrymal film in 92% of patients, exhibiting few effects on the regression of neovessels and corneal transparency. These characteristics make treatments with autologous serum particularly useful in slight and moderate keratopathy cases. In severe cases of keratopathy associated to aniridia, we can utilize it as a supplement of other therapeutic procedures such as limbus transplant.

**DISCUSSION**

We can conclude that treatment with autologous serum is an efficient method for stimulating the feasibility of corneal and conjunctival epithelium cells by supplying a number of growth factors which have been reduced by the dry eye syndrome which accompanies most processes which develop epithelial defects or disorders of corneal epithelial formation. In this regard and even though some authors do not find statistically significant differences when comparing this treatment with conventional therapy (31), for most authors the application of autologous serum broadly exceeds substitute therapy with artificial tears (33). Moreover, both treatments can supplement each other, thus reducing artificial tear dependency (26).

On the other hand, the preparation of autologous serum does not entail technical difficulties and is relatively cost-effective because hospitals usually have the necessary technology for its preparation (centrifuge, laminar flow device, etc.) (7). In addition, the fact that autologous serum treatment is well tolerated by patients further encourages its utilization.

The main drawback of autologous serum treatments are the frequent blood extractions, mainly in the groups requiring large concentrations. In these cases, we must be careful because not all patients can be treated due to systemic difficulties and on some occasions it is even necessary to provide an iron sulphate supplement to prevent anemia (33).

Although the literature describes some complications such as the deposit of immunoglobulines in the cornea and the presence of corneal peripheral infiltrates in a rheumatoid arthritis patient (66,26), treatment with autologous serum is usually very well tolerated. The literature does not describe either adverse effects when utilized for long periods of time (30). Even though uncommon, some patients may experience increased discomfort, slight epiteliopathy, bacterian conjunctivitis or eyelid eczema, according to some descriptions (58,59). However, in such cases, complications need not be caused by the treatment. Although rare, Eberle et al (67) describe the case of an HIV infection transmitted by autologous serum eye drops. In this regard, we must emphasize that this preparation is a body fluid and therefore liable to transmit infections. Accordingly, we must screen all patients for infectious diseases and insist on the correct use and manipulation of these eye drops by patients as well as anybody who is in contact with them.

The challenge for the future, with the goal of avoiding frequent blood extractions, is to research in several fields such as the utilization of new molecules to carry autologous serum and enhance the bioavailability of the product, the use of filters or the development of an enriched universal tear. Some attempts have been made to develop tear substitutes, but these were focused on the ionic and physical composition of the tear and not on supplying components which are particularly important for the balance of the eye surface epithelium (68,69). The use of isolated components such as fibronectine, vitamins or EGF (51,70) has so far failed to produce satisfactory results.

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