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The effect of different drugs on the preparation and biological outcomes of plasma rich in growth factors

Eduardo Anitua [a,b], María Troya [b], Mari Mar Zalduendo [b], Gorka Orive [b,*]

[a] Private Practice in Implantology and Oral Rehabilitation in Vitoria, Spain
[b] Eduardo Anitua Foundation, c/José María Cagigal 19, Vitoria 01007, Spain

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ABSTRACT

Chronic diseases are the major contributors to the global burden of disease and involve prodigious consumption of various drugs that usually affect platelet function. The autologous technology of plasma rich in growth factors (PRGF) provides a biological approach using autologous platelets as a reservoir and local delivery of proteins to promote tissue healing. The purpose of this study was to evaluate the effect of the consumption of acetylsalicylic acid, acenocoumarol and glucosamine sulfate on the preparation as well as on the biological properties of the PRGF technology. Clotting time and platelet activation of PRGF was evaluated. The latter was performed by flow cytometry. PRGF growth factor content and the release of various biomolecules by gingival fibroblasts were quantified by enzyme-linked immunosorbent assay. Cell proliferation was evaluated by means of a fluorescence-based method and cell migration was performed on culture inserts. None of the parameters evaluated was modified by the consumption of any of the three drugs tested; only the plasma of patients who had consumed acetylsalicylic acid and acenocoumarol expressed greater gingival fibroblast migration compared to plasma control. The intake of acetylsalicylic acid, acenocoumarol and glucosamine sulfate does not alter the preparation and biological properties of the autologous technology of PRGF.

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1. Introduction

It is well known that platelets play a major role in haemostasis and wound healing (Agren et al., 2013; Ciciliano et al., 2014). After tissue injury, a coordinated response involving several processes such as coagulation, inflammation and tissue remodeling takes place to restore the normal function of the tissue (Brass et al., 2007; Mercer and Chambers, 2013). Coagulation involves a tightly controlled sequence of activation of proenzymes giving rise to the active forms by the upstream activated clotting factor. The ultimate goal of this process is to convert pro-thrombin to thrombin, which in turn converts fibrinogen to fibrin leading to the formation of a stable clot (Adams and Bird, 2009; Mercer and Chambers, 2013; Versteeg et al., 2013).

Chronic diseases are now the major contributors to the global burden of disease. Cardiovascular and rheumatic pathologies are amongst the most prevalent diseases in developed countries (Laslett et al., 2012; Sangha, 2000; Seymour et al., 2003). These pathological conditions involve a chronic intake of several drugs that usually affect platelet function and which are commonly classified under the following headings: antiaggregants, anticoagulants and non-steroidal anti-inflammatory drugs.

Acetylsalicylic acid (ASA) is the main antiaggregant therapy used for cardiovascular disorders. This compound irreversibly inhibits cyclooxygenase-1 and blocks the synthesis of thromboxane A2 (Aframian et al., 2007; Awtry and Loscalzo, 2007). Coumarin derivative anticoagulants are the most commonly used worldwide. Warfarin and acenocoumarol are among this type of oral anticoagulants (Bauersachs, 2012). The mechanism of action of these types of drugs is based on the inhibition of the vitamin K conversion cycle (Aframian et al., 2007; Cervera and Chamorro, 2010; Hirsh et al., 2001).

On the other hand, glucosamine sulfate is a slow acting drug for the symptoms of osteoarthritis, and therefore is one of the most commonly used drugs for rheumatic diseases due to its anti-inflammatory effects. It is a biological compound, which plays a key role in the construction of cartilage (Selvan et al., 2012; Tat et al., 2007).

Besides their role in haemostasis and their procoagulant effects, platelets are a source of growth factors and constitute the basis of platelet rich plasma products (Albanese et al., 2013; Anitua, 1999; Boswell et al., 2012). The latter represent a biological approach to promote and accelerate tissue regeneration by mean of those

* Corresponding author at: San Antonio 15, 3° 01005 Vitoria, Spain. Tel.: +34 663027696; fax: +34 945155095.
E-mail address: gorka.orive@ehu.es (G. Orive).

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growth factors that promote cell proliferation, migration, angiogenesis, differentiation and survival (Chen et al., 2010; Kao et al., 2009; Rodrigues et al., 2010). This technology is widely used in many different fields, especially in dentistry and oral implantology but also in orthopaedics and sports medicine among others (Albanese et al., 2013; Anitua et al., 2007; Chen et al., 2010; Lopez-Plandolit et al., 2011).

The technology of plasma rich in growth factors (PRGF) is the pioneering technology that uses plasma and platelet derived growth factors as therapeutics for tissue regeneration and wound healing (Anitua et al., 2012a, 2013a,b).

Assuming the high prevalence of chronic diseases and the use of drugs that affect platelet function, and given the extended use of platelet rich plasma products used in dentistry, it is necessary to evaluate the possible interaction of these drugs on the biological function of these autologous preparations. In the present study, we explore the potential effects of the consumption of three different types of drugs: acetylsalicylic acid, acenocoumarol and glucosamine sulfate, all of them commonly prescribed for chronic diseases, on the preparation as well as on the biological outcomes of plasma rich in growth factors (PRGF).

2. Material and methods

2.1. Patient selection

Three groups of drugs were selected for the study: antiaggregants, anticoagulants and non-steroidal anti-inflammatory drugs (NSAIDs). Acetylsalicylic acid, acenocoumarol and glucosamine sulfate were chosen as a respective representative of each of those groups according to the active principles. Each study group consisted of 3 patients. The last were selected in an age range of 55–85 years. In all cases, patients had been taking the medication for at least one year. Patients without medication were used as control (Table 1).

The study was performed following the principles of the Declaration of Helsinki.

2.2. Preparation of plasma rich in growth factors (PRGF)

Blood from the above mentioned donors was collected in 9-ml tubes with 3.8% (wt/v) sodium citrate, after written informed consent was provided. Samples were centrifuged at $580 \times g$ for 8 min (Endoret Dentistry, BTI Biotechnology Institute, S.L., Miñano, Álava, Spain) at room temperature. The 2 ml plasma fraction (F2) just above the buffy coat was separated in each donor. Platelets and leukocytes counts were performed with a hematology analyzer (Micros 60, Horiba ABX, Montpelier, France). Plasma preparations were incubated with the activator (at a proportion of 50 μl of 456 mM $CaCl_2$ activator to 1 ml of plasma) (Endoret Dentistry) at 37 °C for 1 h. The PRGF supernatants were collected by aspiration after centrifugation at $3000 \times g$ for 15 min at 4 °C. Finally, the supernatant obtained from each donor was aliquoted, filtered and stored at −80 °C until its use to quantify the content of growth factors as well as to evaluate its biological effect.

2.3. Characterization of PRGF

2.3.1. Determination of clot formation time

Part of the F2 obtained above was intended to determine the time of plasma coagulation. In order to achieve that, plasma samples of each donor were divided into 3 aliquots and incubated with the activator (Endoret Dentistry) at 37 °C. Coagulation time was written down once the clot was completely formed.

2.3.2. Platelet activation by flow cytometry

Two antibodies were employed to analyze the platelet activation: CD41 and CD62b (P Selectin). CD41 recognizes the platelet membrane glycoprotein GpIIb and a component of the α-granule accessible after platelet activation can be identified by CD62b.

For each donor, 7 tubes were used, distributed as follows: one for the blank, two for CD41 control (in rest and stimulated), two for CD62 control (in rest and stimulated) and the last two for each sample (in rest and stimulated). In all the tubes, 10 μl of F2 without activator, was added. Five microliters of the antibody CD41 and 5 μl of the antibody CD62 (BD Biosciences, San Jose, CA) were added to the CD41 tubes and CD62 tubes, respectively. Both antibodies were dispensed into the samples tubes as well. After that, 10 μl of the stimulating mixture (U46619 prostaglandin (Cayman Chemical, Tallinn, Estonia) + Adenosine 5′-diphosphate (ADP) (Sigma–Aldrich, St. Louis, MO) in phosphate-buffered saline (PBS)) was added to the tubes labeled as “stimulated”. For the blank and for the tubes labeled as “rest”, the stimulating mixture was replaced by PBS.

The remaining volume up to 110 μl was completed with phosphate-buffered saline (PBS).

All tubes were incubated for 15 min in the dark at room temperature. Then, the samples were fixed by the addition of 400 μl of formaldehyde (1.25% (v/v)) to prevent further activation. A flow cytometer (Beckman Coulter, High Wycombe, UK) was used to analyze events.

2.4. Quantification of growth factors in PRGF

Concentrations of insulin-like growth factor 1 (IGF-1), platelet-derived growth factor AB (PDGF-AB) and vascular endothelial growth factor (VEGF) were determined on the plasma supernatant obtained from each patient. Those growth factors were quantified using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA).

Table 1
Characteristics of the patients of each study group.

Study groups	Patient	Age (years)	Sex	Active principle	Dose	Drug consumption time
Antiaggregants	1	65	Male	Acetylsalicylic acid	100 mg/day	2 years
	2	61	Male			7 years
	3	62	Male			9 years
Anticoagulants	1	79	Male	Acenocoumarol	Individualized for each patient[a]	1 year
	2	70	Male			10 years
	3	60	Male			10 years
Nonsteroidal anti-inflammatory drugs (NSAIDs)	1	84	Female	Glucosamine sulfate	1500 mg/day	5 years
	2	67	Male			5 years

[a] requires frequent international normalized ratio (INR) monitoring and adjustment.

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