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Osteogenic potential of a chalcone in a critical-size defect in rat calvaria bone

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ABSTRACT

Background: This study describes the bone formation stimulated by the application of a type of chalcone to critical-size defects in rat calvarial bone.
Material and methods: Sixty female Wistar rats were divided into 6 groups of 10 animals per group: control (no treatment), vehicle (vaseline) and the chalcone (1-phenyl-3-(4-chlorophenyl)-2-propen-1-one) suspended in vaseline at 10%. A critical-size defect of 5 mm was prepared using a trephine in the calvarial bone, after which the treatment was applied, in a single dose, according to the experimental group. The samples were evaluated macroscopically using ImageJ software, and histologically 30 and 45 days after surgery.
Results: At 30 days after surgery, there was significant bone formation ($p < 0.05$) in the groups treated with chalcone, compared with the other groups. Many active osteoblasts were observed adjacent to the borders of the newly formed bone tissue. 45 days after surgery in the chalcone group, the surgical defects showed complete bone closure.
Conclusion: The results of this study suggest that chalcone has significant potential to induce the formation of new bone.

1. Introduction

Diseases such as osteoporosis, traumatic injuries, orthopaedic surgery, and resection of primary tumours can result in bone defects, which become critical defect wounds when there is no spontaneous repair, and which require replacement materials to fill the injured tissue (Mauney et al., 2005).

Some widely-used techniques used to treat musculoskeletal diseases are autologous, allogeneic or xenotransplants. However, these methods require rigorous control of the transmission of infectious agents (Laurencin and El-Amin, 2008; Heneghan and McCabe, 2009; Putzier et al., 2009; Bayat et al., 2010; Lee et al., 2012; Zwitser et al., 2012).

Tissue engineering has developed the use of extracellular matrices, as support for organ function, allowing cell proliferation, migration and differentiation in the process of bone regeneration (Elsalanty and Genecov, 2009; Chan et al., 2009; Tuzlakoglu and Reis, 2009; Mieszawska and Kaplan, 2010; Lee et al., 2012; Stockmann et al., 2012); and also the development of an intelligent matrix, i.e. without the addition of molecules from the TGF-beta family, which can initiate the cascade of cellular differentiation to produce new bone (Srouji et al., 2005; Ripamonti, 2010).

Many investigations of active principles derived from medicinal plants have been reported in relation to the repair of non-mineralized tissue (Sasidharan et al., 2010; Mezadri et al., 2012; Manoj and Murugan, 2012). However, little is known about their use in the repair of bone tissue.

Chalcones, molecules that are found abundantly in several plant species, have shown intense pharmaceutical activity, including antinociceptive (Campos-Buzzi et al., 2006), antifungals (Konduru

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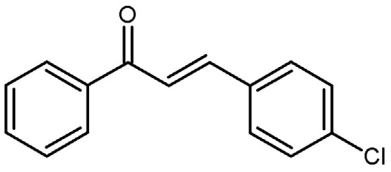

Fig. 1. Chemical structure of synthetic chalcone 1-phenyl-3-(4-chlorophenyl)-2-propen-1-one.

et al., 2013), antimicrobial (Nowakowska et al., 2008) and anti-inflammatory effects (Tran et al., 2009).

Studies have reported synthetic chalcones exhibiting promising properties in the inhibition of inflammatory enzymes and have also shown inhibitory action on the activation of mast cells, neutrophils, and macrophages, important mediators of inflammation, interfering in the formation of bone tissue (Oliveira et al., 2010).

Recently, Tames et al. (2010), based on reports of Corrêa et al. (2008), Batovska and Todorova (2010), Han et al. (2010); Vogel et al. (2010) describing the biological and pharmacological properties of chalcones, have suggested inducing bone repair using the chalcone 1-phenyl-3-(4-chlorophenyl)-2-propen-1-one in a critical-size defect model in rats.

Carpeggiani et al. (2010) also found potential to stimulate repair of the pulp–dentin complex in a model to study pulp capping in rat molars, using the same molecule.

Continuing the studies of Tames et al. (2010), this study aims to evaluate the osteogenic potential of the synthetic chalcone 1-phenyl-3-(4-chlorophenyl)-2-propen-1-one at 30 and 45 days after treatment.

2. Material and methods

The chalcone 1-phenyl-3-(4-chlorophenyl)-2-propen-1-one (Fig. 1) was synthesized by an adaptation of the general method of Claisen–Schmidt condensation (Corrêa et al., 2001; Campos-Buzzi et al., 2007). The method uses an equimolar mixture of 4-chlorobenzaldehyde and acetophenone dissolved in water and ethanol, in the presence of 10% sodium hydroxide. The solution was stirred mechanically for 24 h. In order to confirm the end of the reaction, formation of the product was analyzed by thin layer chromatography. The product was percolated and washed several times with cold distilled water, until the wash waters presented a neutral pH value. The isolated product was dried under reduced pressure in the presence of phosphorus pentoxide. All the solids obtained were purified by recrystallization with ethanol 95% and characterized by their melting point using a Microquimica APF-300 apparatus (Microquímica, Florianopolis, SC, Brazil). Microanalysis (CHN) was performed with a Perkin Elmer PE 2400 Series KK CHNS/O analyzer (Perkin–Elmer, Norwalk, CT, USA). The IR spectra was recorded with a BOMEM-MB 100 spectrometer (BOMEM, St. Jean Baptiste, QB, Canada) with KBr disks, and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy using a Bruker AC-300 apparatus (Bruker, Karlsruhe, Germany). The compounds were dissolved in DMSO deuterated with tetramethylsilane (TMS) as the internal standard.

1-Phenyl-3-(4-chlorophenyl)-2-propen-1-one: yield: 74.0%; mp: 114.0–116.0 °C (from ethanol); IR (KBr, ν_{max} cm^{-1}): 1660 (CO), 1605 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.17–8.12 (m, 2H, Ar), 7.82–7.77 and 7.41–7.36 (2d, 2H, CH=CH, J = 15.57 Hz), 7.70–7.57 (m, 3H, Ar), 7.53–7.48 (m, 2H, Ar), 7.45–7.40 (m, 2H, Ar); ^{13}C NMR (300 MHz, DMSO-d_6, δ ppm): 122.32, 128.48, 129.13, 129.54, 132.79, 133.43, 136.65, 137.98, 143.09, 189.76. $C_{15}H_{11}ClO$ requires: C (74.23%), H (4.57%).

The study protocol and experimental designs were approved by the Animal Ethics Committee of Univali.

60 female Wistar rats, with 45 days old, were used, divided into 6 groups: control (no treatment), vehicle group (vaseline – 34 mg/wound/single application) and chalcone (1-phenyl-3-(4-chlorophenyl)-2-propen-1-one) 10% (34 mg/wound/single application) suspended in vaseline (vehicle), over two experimental periods; 30 and 45 days.

The animals were anesthetized with ketamine (10%), administered intramuscularly. After shaving and antiseptic preparation of the surgical site, the skin of the skull was incised, and a periosteal excision was made. A defect of 5 mm in diameter (corresponding to a wound area of 19.635 mm^2) was made the right side of the calvarium with a trephine at low-speed under continuous irrigation with sterile saline (Fig. 2). After the treatments (application of vaseline and chalcone) the periosteum and the soft tissues were then repositioned and sutured with absorbable suture. Ketoprofen 1% 5 mg/kg (0.25 ml/100 g) was given subcutaneously as analgesia, in the first 72 h, in all six groups.

After 30 days and 45 days the animals were sacrificed by anaesthetic overdose. The material collected was fixed by immersion in 4% paraformaldehyde in phosphate buffer, pH 7.4, for 72 h, followed by demineralization with 10% EDTA in phosphate buffer. In this step, the samples were photographed to calculate the remaining wound areas in mm^2, using the ImageJ software. The values obtained were analyzed by ANOVA followed by the Tukey test.

Continuing the histological technique, we performed dehydration of the material with alcohol in increasing concentrations, clearance with xylene embedded in paraffin to obtain semi-serial sections 1:10 with thickness of 7 μm, and staining with haematoxylin and eosin (HE).

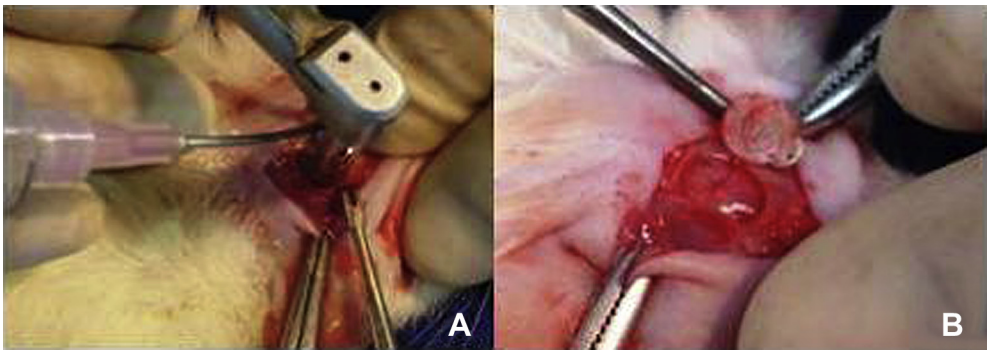

Fig. 2. A – Trephine (5 mm) used to made a critical wound. B – Bone fragment resulting wound.