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● *Original Contribution*

DICLOFENAC PHONOPHORESIS IN HUMAN VOLUNTEERS

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Abstract—A quantitative study of sodium diclofenac (Voltaren Emulgel®, Novartis) phonophoresis was undertaken in humans. Fourteen healthy human volunteers were submitted to ultrasound irradiation on two 225-cm^2 areas on the dorsum (group A), followed by the application of the medication gel, and the plasma diclofenac mass was measured at 1, 2 and 3 h later by high performance liquid chromatography. The same procedure was repeated one month later with the same volunteers but with the ultrasound equipment switched off for the control group (group B). The plasma diclofenac mass was significantly higher in group A than in group B at 1 h (0.0987 μg/mL as opposed to 0.0389 μg/mL; $p = 0.01$) and 2 h (0.0724 μg/mL as opposed to 0.0529 μg/mL; $p = 0.01$), but not at 3 h (0.0864 μg/mL as opposed to 0.0683 μg/mL; $p = 0.16$). The authors conclude that previously applied therapeutic ultrasound irradiation enhances the percutaneous penetration of the topical diclofenac gel, although the mechanism remains unclear. (E-mail: chbarbie@fmrp.usp.br) © **2005 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Therapeutic ultrasound, Phonophoresis, Transdermal penetration, Sodium diclofenac, Plasma mass, High performance liquid chromatography.**

INTRODUCTION

Therapeutic ultrasound is widely used in the daily practice of physiotherapy and sports medicine for the treatment of a variety of acquired and traumatic conditions affecting the locomotor apparatus, its beneficial effects including the healing of varicose ulcers (Dyson and Suckling 1978), tendon repair (Enwemeka 1989; Jackson et al. 1991; Gan et al. 1995) and stretched skeletal muscle repair (Menezes et al. 1999) and the regeneration of peripheral nerves (Hong et al. 1988). It has also been used to enhance transdermal penetration of medications, the so-called phonophoresis, specifically with a corticosteroid (Byl et al. 1993), methyl-nicotinate (McElnay et al. 1993), protein (Mitragotri et al. 1995), indomethacin (Asano et al. 1997) and large size molecules as poly I-lysine (Weimann and Wu 2002).

Phonophoresis is defined as the enhancement of penetration of any medication through the normal skin by ultrasound irradiation. It can be obtained with the medication gel or cream either used as a coupling medium for the ultrasound head or with previous irradiation with any regular coupling gel, the actual medication gel or cream being applied on the irradiated area immediately thereafter. To use a medication gel or cream as coupling medium, it is necessary to determine that it actually is a good ultrasound conductor, otherwise the treatment will be ineffective (Low and Reed 2001). Actually, many variations have been found in the transmission properties of different medications, some of them having been demonstrated to be poor ultrasound transmitters (Benson and McElnay 1994).

The efficacy of phonophoresis of medication gels has been investigated in humans using the regular coupling gel for control (El-Hadidi and El-Garf 1991; Klaiman et al. 1998). However, the results are not entirely reliable, since the evaluation criteria include excessively subjective parameters, such as pain on pressure and motion, although measured with a pain analogical scale.

So far, no demonstration has been produced that phonophoresis results in satisfactory or effective blood

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concentration of diclofenac gel, one of the topical anti-inflammatory medications most extensively used in physiotherapy and sports medicine practice, also as a coupling gel for ultrasound irradiation. Therefore, it was the purpose of the present investigation to quantify in humans the transdermal absorption of sodium diclofenac as a topical gel following therapeutic ultrasound irradiation, by measuring its plasma mass by high performance liquid chromatography.

MATERIALS AND METHODS

The present investigation was approved by the Ethics in Research Committee of the University Hospital, Ribeirão Preto School of Medicine. All potential volunteers were adequately instructed about the nature and purpose of the investigation, warned about potential risks to their health and warranted freedom to quit at any time of their own free will. All were submitted to a questionnaire about their previous known health situation regarding possible heart, liver, kidney, lung, gastrointestinal, hematologic, neurologic problems and any hypersensitivity to medication, particularly antinflammatories; women were also questioned about a possible pregnancy and were submitted to a pregnancy test. Candidates with a single positive record of a previous health problem, smokers and suspected drug addicts were excluded. All candidates were examined for skin problems, particularly in the area to be treated (dorsum). Fourteen human volunteers were selected, including 10 women and 4 men aged on average 26.4 y (range: 19 to 50 y), with an average weight of 62 kg (range: 50 to 80 kg) and an average height of 1.7 m (range: 1.6 to 1.9 m). All selected volunteers signed an informed consent form and were warned not do drink any alcohol for at least one week before treatment.

Ultrasound equipment and irradiation: Diclofenac gel application

Conventional 1 MHz microprocessed therapeutic ultrasound equipment adjusted for continuous wave of 0.5 W/cm^2 intensity (SATA) was used (Pro Seven 977 to 2000 model, Quark Produtos Médicos, Brazil). It was certified that the equipment was in accordance with general IEC 601 and ultrasound specific IEC 601 to 2–5 safety rules.

The entire dorsum of the volunteers was carefully washed with neutral bath soap and a soft sponge and dried. Two 225 cm^2 (15 $\times$ 15 cm) square areas were then drawn, one on each side of the dorsum, and irradiated for 5 min, one immediately after the other, using a regular coupling gel. Immediately following irradiation of the second area, a total amount of 5 *g* of a commercially available diclofenac gel (Voltaren Emulgel®, Novartis) was applied to the irradiated areas (2.5 *g* on each area) and left to dry for at least 1 h before the volunteers were allowed to put their clothes back on. According to the producer, Voltaren Emulgel is an oil-based emulsion in an aqueous gel and contains 1*g* of sodium diclofenac per 100 *g* of the gel, so that each volunteer received a total dose of 0.05 *g* of the therapeutic agent.

To minimize possible errors due to different skin texture, all volunteers were submitted to the same treatment twice, the first with the ultrasound equipment switched on (effective irradiation, group A) and the second, one month later, with the equipment switched off (placebo effect, group B).

Blood samples (2 mL each) were collected from a forearm superficial vein of each volunteer with a sterile syringe and needle (Abbocath®, Abbot) and transferred into tubes with an anticoagulant (EDTA, 10 mg) immediately before and 1, 2 and 3 h after the application of the diclofenac gel to the irradiated areas. The samples were then centrifuged at 3000 rpm for 10 min to separate the plasma, which was stored in a freezer at -20°C until the day of the chromatographic analysis (one week on average), when it was taken from the freezer and defrosted at room temperature (23°C on average) for 6 h.

Chromatographic analysis

Plasma concentration (mass) of the absorbed diclofenac was measured by high performance liquid chromatography (HPLC) with an equipment (Shimadzu®) consisting of a pump (model LC-10AD), a 278 nm ultraviolet detector (UV 278 nm), a 20 μl injector, a 250 mm $\times$ 4.6 mm chromatographic separation column (C-18, 5 μm, Macherey-Nagel) and a recorder. The recorder was linked to an integrator able to supply the exact value of the area under the curve (AUC) and convert it into concentration (or mass, in μg min/mL) of the analyzed substance. Chromatographic extraction was done at a 1-mL/min flow using a mobile phase composed of a mixture of acetonitrile and sodium acetate (35:65, 0.1 mol/L) adjusted to pH 6.3 with glacial acetic acid. Under such conditions, extraction of sodium diclofenac took about 15 min.

Before analysis of the volunteers' plasma, the equipment was calibrated with plasma solutions of the sodium diclofenac of known concentrations, which were prepared according to the method proposed by Giagoudakis and Markantonis (1998), as follows:

1. Preparation of a 1 μg/mL stock solution (permanently kept in a refrigerator at 6°C) by the dilution of the sodium diclofenac analytical standard kindly donated by the producer (Novartis Pharma, Brazil) in ethanol;

2. Preparation of solutions of growing concentration (0.0125, 0.025, 0.05, 0.1, 0.2 and 0.3 μg/mL) by the addition of enough volume of the analytical standard