The Devil's in the Details

To the Editor:

Physical therapy as a profession is beginning to develop an interest in pharmacology and in how pharmaceuticals interact with physical therapy interventions. In addition to or as a result of this interest, the journals of the physical therapy profession are beginning to publish articles related to the pharmacokinetics and pharmaco-dynamics of drugs.

I have just finished reading the research article titled "Phonophoresis Versus Topical Application of Ketoprofen: Comparison Between Tissue and Plasma Levels" by Cagnie et al (2003;83:707–712). The initial face validity of the article supports the widely held concept of localized drug delivery by modalities used in physical therapist practice. I have several questions regarding the article, many of which are related primarily to the methods; however, as a mentor of mine once taught me, in pharmacology research, the devil is in the details. I address the issues in order of concern.

No analytical methods were presented or referenced. This results in the majority of my subsequent concerns. As these methods are the basis for all of the results in the article, this is a major omission. Would the Journal have allowed force measurements to be reported in the results without requiring the authors to document the methods and devices utilized for these force measurements? I believe that the same standards should apply to pharmacologic measurements.

The samples were stated as having been "analyzed by a chromatography procedure." This is inaccurate. Chromatography describes a column packed with material for separating and/or concentrating compounds for subsequent detection. There are multiple types of chromatography: gas, high- and low-pressure liquid chromatography, and immunochromatography.

The method of detection was not stated. Analysis of the eluent from the chromatographic column may be by absorbance with a
spectrophotometer, fluorescence with a fluorometer, mass spectroscopy, or immunodetection with an enzyme-linked immunoassay.

Often when tissue is being analyzed for drug concentrations, several preparation steps are required. These steps may include homogenization, wet-wet extraction, or solid phase chromatography extractions. None of these were mentioned.

The level of detection for plasma (0.002 µg/mL) was stated, but no similar detection limits were stated for adipose or synovial tissues. In my research on the tissue detection of ketoprofen, I found that minimal drug detection concentrations vary as much as 100-fold, based on the tissue matrix.1–3

Within the results section, the plasma levels were stated in the nanogram per milliliter range. However, the tissue levels were in the microgram per gram range and that is approximate to microgram per milliliter range for most tissues. Were different extraction procedures used, or were different standard curves used?

Were dimerization or additional chemical reactions required to achieve these drug detection levels? Other studies1–3 have suggested that, to obtain this level of detection, some type of enhancement in spectrophotometric absorbance or fluorometric emission may be required. Additional procedural steps have the potential to result in erroneous (false positive) results if they are not controlled.

Were the samples with unknown drug concentrations compared with a standard curve of the drug in the same matrix? Again, if the standard curve for determining the concentration of the drug in a tissue is not derived from the same matrix, erroneous interpretation of the results may occur. In addition, if the unknowns were not analyzed in duplicate, were both intra-day and inter-day coefficients of determination conducted to examine the reproducibility of the results in the unknown samples?

With the previous concerns in mind, I also question the ketoprofen variation in the various tissues reported in the results. My own research on ketoprofen iontophoresis2,3 and that of other researchers in transcutaneous drug delivery4,5 indicates that the theoretical construct and experimental evidence support both the concept of the drug concentrations being greatest at the surface of the skin and the drug concentration in the tissue decreasing with increasing tissue depth.
Although Cagnie et al addressed this concern in their discussion section, I am uncertain whether they gave this pharmaco-kinetic construct the significance it deserves. The permeation of drugs from the surface of the skin to deeper tissue sites should result in an inverse drug concentration–tissue depth response curve. I agree that local vascular effects may result in penetration deeper than the integument without systemic vascular delivery; however, to date, there is no significant experimental pharmacokinetic evidence to support this theory.

In conclusion, although the article provides face validity for the capacity of phonophoresis to provide localized deep tissue drug penetration, due to major methods omissions the data are not reproducible, and the results should be viewed with some skepticism. I would discourage readers from using the article to document the value of phonophoresis in providing localized drug delivery.

Peter C Panus, PT, PhD  
Associate Professor  
Physical Therapy Department  
College of Public and Allied Health  
East Tennessee State University  
Johnson City, TN 37614-1709  
Panus@mail.etsu.edu

References


Author Response:

Dr Panus' main concerns are about the analytical method my colleagues and I used in our study. Therefore, in answer to his points 1 through 5 and 7 and 8, we would like to add a brief description of the analytical method we used. I hope that the additional information contributes to the report of our study. The concentration of ketoprofen in all samples were determined by gas chromatography-mass spectrometry after derivatization of ketoprofen. The following steps of the chemical analysis were done:

Sample preparation. Blood samples were collected in an EDTA tube and centrifuged within 1 hour. Plasma and tissue samples were frozen at –20°C and stored until analysis. At analysis, the tissues were defrosted, weighed, and transferred in polypropylene tubes for homogenization. Tissues were homogenized in 1mL HCl 0.1 N, glass pearls were added, and the homogenates were vortexed for 60 to 120 minutes at room temperature.

Extraction of ketoprofen. Ketoprofen was extracted using wet-wet extraction: 1 mL of plasma or tissue homogenate was mixed with 0.1 mL fenoprofen (internal standard), 0.1 mL phosphate buffer (1 M pH 2.0), 0.1 mL water, and 6 mL hexanethyl acetate. After 30 minutes of extraction at room temperature, the mixture was centrifuged, and the organic layer was separated and evaporated to dryness under nitrogen stream at 55°C. The residue was stored for derivatization.

Derivatization. The extracted ketoprofen and fenoprofen were derivatized with 0.5 g tetramethylammonium hydroxide in 10 mL dimethyl sulfoxide. Methylation was performed by adding 50 µL of methyl iodide. After 15 minutes of incubation, the reaction was stopped by adding 0.2 mL HCl 0.1 N. Methylesters were extracted in 2 mL isooctane and, after evaporation of the organic layer at 55°C, were resuspended in 30 µL ethyl acetate for gas chromatography-mass spectrometry.

Gas chromatography. 2 µL extract was injected into a 15-m capillary column (Chrompack CP SIL 8 CB MS) in the split-injection mode.
Helium was used as a carrier gas at a constant pressure of 5 psi. The starting temperature was 100°C, and, after 1 minute, the temperature was raised at 20°C/min to 310°C. This final temperature was maintained for 5.5 minutes. Injector and transfer line temperatures were 275°C. Under these chromatographic conditions, the retention times were 6.38 minutes for fenoprofen and 7.55 minutes for ketoprofen.

The compounds were detected from the column during elution using a mass spectrometer. The molecular ions of fenoprofen and ketoprofen were isolated and fragmented at a voltage of 1.25 V and a source temperature of 220°C. Fragment ions 197 for fenoprofen and 208 for ketoprofen were used for identification and quantification.

Quantification. A linear standard curve was obtained by dividing the ketoprofen- to-fenoprofen peak area ratios to the known concentrations of ketoprofen calibrators. The samples with unknown drug concentrations were compared with this curve.

With the described method, the detection limit was 2 ng/mL in plasma as well as in tissue extracts. Because no measures of ketoprofen in synovia as well as in adipose tissue were found below the detection limit, the detection limit was not mentioned in the article. We think that the above-mentioned analytical method is accurate and points out that the different procedural steps may result in a correct interpretation of the outcomes.

Dr Panus wonders (in point 6) if different extraction procedures or different standard curves were used. This was not the case. Therefore, as correctly remarked by Dr Panus, plasma and tissue levels could both be expressed in terms of nanograms per millimeter. Looking back, it would have been better to use the same units of measure.

We appreciate the opportunity to clarify certain shortcomings, and we hope the devil is a little bit exorcized.

Barbara Cagnie, PT
Department of Rehabilitation Sciences and Physiotherapy
Ghent University
Ghent, Belgium
barbara.cagnie@ugent.be