



## PHARMACOKINETIC EVALUATION AFTER PERCUTANEOUS ADMINISTRATION OF SOME NON-STEROIDAL ANTIINFLAMMATORY DRUG PREPARATIONS USING ANIMAL EXPERIMENTS

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**Abstract.** Background and purpose The non-steroidal antiinflammatory drugs (NSAIDs) are used as elective medication in the therapy of various inflammatory diseases. This treatment ameliorates most of the clinical indices of inflammation, improving the exudative-congestive inflammatory process, without influencing the proliferative processes. They are used for the anti-inflammatory effect in the treatment of skin, joints and genital area located chronic inflammatory diseases. The aim of the study was to determine the plasma concentrations of some of the NSAIDs after single dose percutaneous administration in laboratory animals and to monitor their plasma concentrations within 24 hours. Experimental approach We used: male adult rabbits of 3 kg weight, 5% Diclac gel (HEXAL), Indometacin 4% cream (MARK PHARMACEUTICS), FINNIGAN - SURVEYOR liquid chromatograph (BDS - HYPERSIL C18 250X4,6 column, 5 micrometers particle size). For each NSAID, the experiment was simultaneously conducted on 5 rabbits. 24 hours before the experiment, the rabbits were kept fasting. During the experiment, they received water ad libitum. Each rabbit was weighed before the experiment. 24 hours before applying the investigated pharmaceutical formulas on the skin, the hair was removed on the dorsal area of 50 cm<sup>2</sup>, using a manual cutting mashine. 24 hours after the epilation, the control samples were collected (3 ml of blood). The collection was conducted from the marginal ear vein under the conditions of asepsie, using single use needles and syringes. 2 g of the NSAID pharmaceutical prepatation was applied on a 50 cm<sup>2</sup> area. The probes were collected at 1,2,3,6,12 and 24 h after the aplication. Each plasma sample was processed for chromatographic analysis. Samples were then analyzed using a FINNIGAN - SURVEYOR liquid chromatograph. During the procedures, the international rules concerning the experiments on laboratory animals were followed as recommended. Results In the first 12 hours, both Diclofenac and Indometacin had a similar progressive increasing of the plasma concentration, which then gradually decreased and maintained up to 24 hours after the percutaneous applicacion. Conclusions and implications Our results support the idea that the topical administered diclofenac and indometacin achieved plasma levels similar to the oral administration plasma levels, but later. The systemic absorption of the topical applied substances emphasizes the possibility of appearance of the corresponding side effects.

**Keywords:** NSAIDs, diclofenac, indometacin, percutaneous absorption

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## Introduction

The skin offers a number of special opportunities to the therapist. The general pharmacokinetic principles governing the use of drugs applied to the skin are the same as those involved in other routes of drug administration. However, human skin, though often depicted as a simple three layered structure, is a complex series of diffusion barriers. Quantization of the flow of drugs and drug vehicles through these barriers is the basis of pharmacokinetic analysis of dermatologic therapy; techniques for making such measurements are rapidly increasing in number and sensitivity. Major variables that determine pharmacologic response to drugs applied to the skin include the following:

- a. regional variation in drug penetration;
- b. concentration gradient;
- c. dosing schedule;
- d. vehicles and occlusion.[1]

The non-steroidal anti-inflammatory drugs (NSAIDs) are used as elective medication in the therapy of various inflammatory diseases, although the anti-inflammatory effect of this class of drugs is of moderate intensity. This treatment achieves therapeutic benefits for most of the clinical indices of inflammation. Starting with the first days of treatment, the pain and the inflammation in joints are diminished. Mainly, they are given in rheumatic diseases, improving an exudative-congestive inflammatory process without influencing the proliferative processes.

The prostaglandins are the active tissue substances that play an important role in the biochemical pathogeny of the inflammatory process. The non-steroidal anti-inflammatory drugs (NSAIDs) are usually defined as those agents that inhibit one or more reactions involved in the production of prostaglandins and thromboxanes, important mediators of the inflammatory process in many organs and tissues. The principal action of most NSAIDs is the inhibition of cyclooxygenase (COX), the first in a series of enzymes responsible for the conversion of arachidonic acid to prostaglandins.

Importantly, recent research has revealed that there are two forms of COX, the first which produces physiologic levels of prostaglandins in a constitutive manner (COX 1), and an inducible form of the enzyme (COX 2) which is responsible for the elevated levels of prostaglandins observed during inflammatory events in a variety of tissues. It appears that the constitutive activity of COX 1 is responsible for many of the homeostatic properties

ascribed to prostaglandins and the toxicity is predominately related to sustained COX 1 inhibition. Most currently available NSAIDs inhibit the activities of both COX isoforms, however the proportion of inhibition of COX 1 vs. COX 2 varies among compounds. Because of the inhibition of COX, another therapeutic effect is achieved: the formation of peroxides, free radicals and other reactive species of oxygen (involved in the biochemical pathogeny of inflammation) is inhibited.[2,3,4]

Another important anti-inflammatory effect is the inhibition of the neutrophil inflammatory stimulated activation, probably by precocious intervention on stimulus-answer coupling.[5]

Toxicity is correlated to plasma concentration of the drug and is largely a result of COX1 suppression and consequent diminution of the "physiologic" levels of prostaglandins. For example, prostaglandin E2 (PGE2) plays an important role in protecting the gastrointestinal tract from ulcers by a number of mechanisms and co-administration of synthetic PGE2 is protective for NSAIDs-induced ulcers.[6]

In order to have fewer side effects, the NSAIDs can be applied topically. The proportion of NSAID absorbed through the skin depends on the total dose, the time span of the contact, the covered area and the skin hydration.[7]

Considering the urinary excretion of the NSAIDs and their hydroxylated metabolites, the absorbed active substance after topical application represents 5-10% of the absorbed active substance after oral administration. The NSAIDs concentration in the synovial fluid was larger compared with the plasma concentration in rheumatic patients treated with Voltaren Emulgel. On the other hand, the plasma concentration of Diclofenac after Voltaren Emulgel topical application is 100 % smaller than that of oral administration.[8,9]

The aim of the study was to determine the plasma concentrations of some of the non-steroidal anti-inflammatory drugs after single dose percutaneous administration in laboratory animals and to monitor their plasma concentrations within 24 hours.

The study also proposed to determine if, after percutaneous administration:

- the NSAIDs are well absorbed;
- the plasma levels are similar to the therapeutic plasma levels described and,
- the single dose slow-release percutaneous preparations present a good efficiency and tolerability.

The proportion of NSAID absorbed through the skin depends on the total dose, the time span of the contact, the covered area and the skin hydration. The quantity of active substance absorbed through the skin was determined using plasma concentrations.

## Materials and methods

We used: male adult rabbits of 3kg weight, Diclac 5% gel HEXAL, Indometacin 4% cream Mark Pharmaceuticals, Finnigan - Surveyor liquid chromatograph (BDS - Hypersil C18 250X4,6 column, 5 micrometers particle size), injectable NaCl (90mg/10ml Zentiva, 3270607, expiry date: 05.2010), monopotasic phosphate (Merck), acetonitrile (Merck), chloroform (Merck), naproxene, cutting machine, syringe, single use needles, cotton wool, sterile test tubes.

For each NSAID, the experiment was simultaneously conducted on 5 rabbits.

During the procedures, the international (the European Council Directive 86/609/CEE 24.11.1986 and the Helsinki Declaration) and national rules (the Law No. 206 from 27.05.2004, the Romanian Government Decree No. 37 from 30.01.2002, the Decree No. 143/400 from 1.04.2002) concerning the experiments on laboratory animals were followed as recommended.

24 hours before the experiment, the rabbits were kept fasting. During the experiment, they received water ad libitum. Each rabbit was weighed before the experiment. 24 hours before applying the investigated pharmaceutical forms on the skin, the hair was removed on the dorsal area of 50 cm<sup>2</sup>, using a manual cutting machine.

Each rabbit from the first group of 5 rabbits received percutaneously 2g of 5% Diclac gel (Hexal). Each rabbit from the second 5 rabbits group received percutaneously 2g of 4% Indometacin cream (Mark Pharmaceuticals). The pharmaceutical formulations were applied on the previously shaved area. The surface did not show any trace of continuity lesions. During the experiment, the rabbits wore an occlusive dressing, in order to maintain the preparation at the application area.

24 hours after the depilation, the control samples were collected (3 ml of blood). The collection was conducted from the marginal ear vein under the conditions of asepsis, using single use needles and syringes. 2g of the NSAID preparation were applied on a 50 cm<sup>2</sup> area. The samples were collected at 2,

4, 6, 8, 12 and 24 hours after the application. Each plasma sample was processed for chromatographic analysis. Samples were then analyzed using a FINNIGAN - SURVEYOR liquid chromatograph.

The samples were maintained until plasma separation and then they were centrifuged for 10 minutes 4800rpm. From each rabbit sample, 500 microliters of serum were taken.

Each plasma sample has been processed as follows:

- each sample (500 microliters of serum) was treated with 100 microliters of monopotasic phosphate buffer 25 mM (KH<sub>2</sub>PO<sub>4</sub>), and then the internal standard 10 microliters Naproxen (5,01 microg/ml Naproxen in Acetonitril) was added;
- the probe was homogenized for 1 minute;
- 500 microliters of chloroform were added;
- the mixture was horizontally homogenized for 2 minutes and then it was centrifuged for 5 minutes at 4500 rpm;
- the organic layer was drawn and then dried up at 35°C;
- the residue was dissolved in 100 microliters of acetonitrile: phosphate buffer mixture (70:30)

The samples were analyzed using FINNIGAN - SURVEYOR liquid chromatograph (BDS - HYPER-SIL C18 250X4,6 column, 5 micrometers particle size) by isocratic dilution.[10]

Conditions: Mobile phase - acetonitril: monopotasic phosphate (25 mM) 70:30; 1 ml/min elution flow, manual injection (20μ microliters injected volume), diode detector (PDA) (280 nm for diclofenac, 254 nm for indometacin).

## Results and discussions

After chromatographic analysis, we obtained the following results (figures 1 and 2, tables I and II).

The peak plasma concentration for **Diclofenac** varied between 0,3621 – 0,4618 microg/ml (mean 0,35158) and was obtained 8-12 hours after the percutaneous administration of 2g 5% Diclofenac gel in single doses.

2 hours after administration, the Diclofenac plasma concentration was 0,108 – 0,0795 micro / ml.

4 hours after the topical administration of Diclofenac, the plasma concentration was 0,0955 – 0,195 microg/ml.

6 hours after the topical administration of

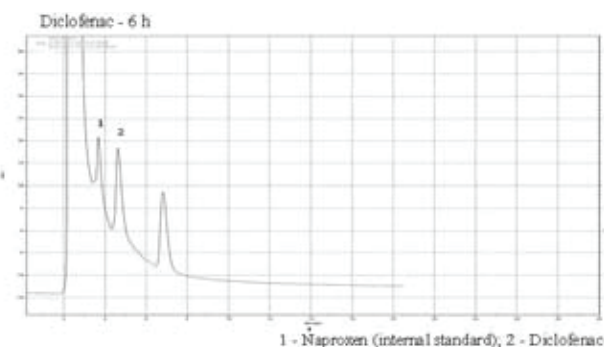


Figure 1. The Diclofenac chromatogram

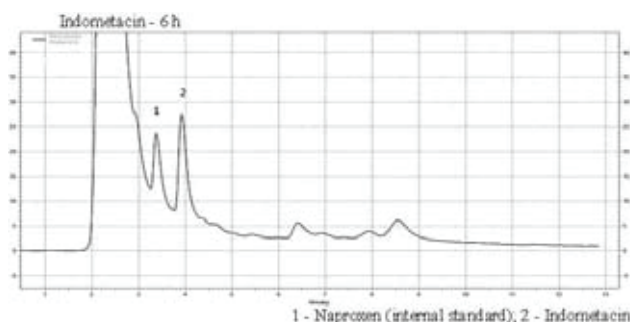


Figure 2. The Indometacin chromatogram

Diclofenac, the plasma concentration was 0,1576 – 0,4279 microg/ml.

8 hours after the topical administration of Diclofenac, the plasma concentration was 0,2218 – 0,4531 microg/ml.

10 hours after the topical administration of Diclofenac, the plasma concentration was 0,2579 – 0,462 microg/ml.

12 hours after the topical administration of Diclofenac, the plasma concentration was 0,2434 – 1,4618 microg/ml.

24 hours after the topical administration of Diclofenac, the plasma concentration was 0,1654

– 0,2164 microg/ml.

The peak plasma concentration for Indometacin varied between 0,335 – 0,427 microg/ml (mean 0,35404) and was obtained 8 – 12 hours after the percutaneous administration of 2g 4% Indometacin cream in single doses.

2 hours after administration, the Indometacin plasma concentration was 0,098- 0,047 microg/ml.

4 hours after the topical administration of Indometacin, the plasma concentration was 0,180 – 0,2383 microg/ml.

6 hours after the topical administration of In-

Plasma concentration of Diclofenac (microg/ml)

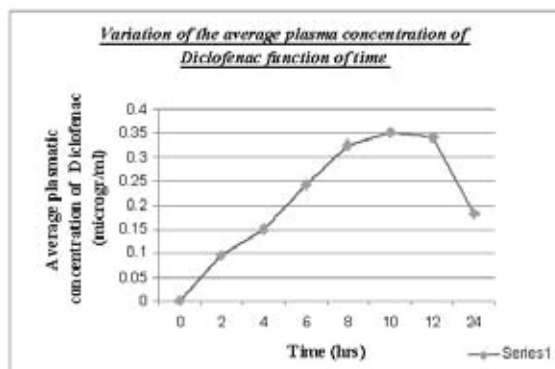
Time (hrs)	Diclofenac 1 Rabbit	Diclofenac 2 Rabbit	Diclofenac 3 Rabbit	Diclofenac 4 Rabbit	Diclofenac 5 Rabbit	Average concentration
0	0	0	0	0	0	0
2	0.1023	0.0795	0.109	0.0832	0.103	0.0954
4	0.195	0.0955	0.189	0.1241	0.1436	0.14944
6	0.4279	0.1579	0.225	0.1576	0.2501	0.2437
8	0.4531	0.2218	0.304	0.2815	0.3621	0.3245
10	0.462	0.2579	0.361	0.336	0.341	0.35158
12	0.4618	0.2434	0.331	0.335	0.339	0.34204
24	0.1999	0.1654	0.1526	0.2164	0.1753	0.18192

Table I. The plasma concentration of Diclofenac

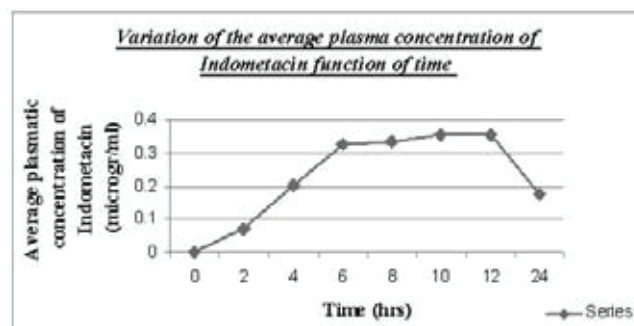
Plasma concentration of Indometacin (microg/ml)

Time (hrs)	Indometacin 1 Rabbit	Indometacin 2 Rabbit	Indometacin 3 Rabbit	Indometacin 4 Rabbit	Indometacin 5 Rabbit	Average concentration
0	0	0	0	0	0	0
2	0.0827	0.0987	0.0477	0.0684	0.0576	0.07102
4	0.2383	0.1901	0.2086	0.1859	0.1802	0.20062
6	0.2562	0.3271	0.3942	0.2903	0.3582	0.3252
8	0.2845	0.3302	0.3869	0.3274	0.3469	0.33518
10	0.336	0.3352	0.437	0.337	0.335	0.35404
12	0.408	0.3349	0.3481	0.3498	0.3183	0.35182
24	0.2156	0.1719	0.1441	0.1589	0.1743	0.17296

Table II. The plasma concentration of Indometacin



**Figure 3.** The variation of the average plasma concentration of Diclofenac function of time



**Figure 4.** The variation of the average plasma concentration of Indometacin function of time (hours)

dometacin, the plasma concentration was 0,256 – 0,3942 microg/ml.

8 hours after the topical administration of Indometacin, the plasma concentration was 0,2845 – 0,3869 microg/ml.

10 hours after the topical administration of Indometacin, the plasma concentration was 0,335 – 0,427 microg/ml.

12 hours after the topical administration of Indometacin, the plasma concentration was 0,3183 – 0,408 microg/ml.

24 hours after the topical administration of Indometacin, the plasma concentration was 0,1719 – 0,2156 microg/ml.

After 24 hours, the plasma concentration reached half of the maximum concentration for both Diclofenac and Indometacin.

In our study, we determined the bioavailability and the pharmacokinetics on Diclofenac and Indometacin after their topical administration in rabbit.

In the first 12 hours, both Diclofenac and Indometacin had similar plasma concentration progressive increasing, which then gradually decreased and maintained up to 24 hours after the percutaneous application. Both substances have achieved similar concentrations in plasma. (figures 3 and 4)

Considering that the rabbits had the same sex, weight, and the same way of administration was used, the plasma level of the active substance was not influenced by the absorption fluctuation or by the first passage effect.

The skin of the rabbits did not have excoriations that might influence the absorption by tissue interruption.

At the end of the study, the general condition of the experimental animals was good. The animals did not have any kind of infections or haematoma.

During the experiment, there were not registered side effects (percutaneous eruption, digestive effects or anaphylactic reactions).

Comparing with the literature data obtained after oral administration of Diclofenac and Indometacin, our maximum plasma concentration after percutaneous administration has been smaller (0,35158 micrograms/ml for Diclofenac and 0,35404 micrograms/ml for Indometacin compared to the therapeutic oral literature data). In the field literature, the maximum plasma concentration for Diclofenac patches was: 376, 1562, 2953, 2902, 2864, and 2948 ng/ml, after 2, 4, 8, 24, 30, and 48 h from patches each containing 50 mg of diclofenac diethylamine respectively, and the mean concentrations of the drug in plasma after the oral administration of marketed tablet containing 50 mg diclofenac sodium were 383.7, 2569, 3693.5, 162.5, and 55.3 ng/ml at 2, 4, 8, 24, and 30 h after oral administration. The values of C<sub>max</sub> were 3693.5 after oral administration and 2953.8 ng/ml in the case of transdermal application. The maximum plasma concentration was obtained after larger time intervals (our maximum concentration was obtained 12 hours after the topical application, in relation to studies where the oral administration concentration peak was obtained at 4 hours after the administration of 100mg of NSAID). [11,12,13]

S. Liu et al. (2001) used rats as experimental animals. Using Diclofenac as the active substance and chromatographic method of detection, they had the maximum plasma concentration 12 hours after dermal application of the NSAID, which corresponds with our results on rabbits for both Diclofenac and Indometacin.[14]

In the Magnette JL et al. (2004) study, the plasma concentrations in human were higher after topical administration of Indometacin on almost 15% of

the body surface in the UV induced erythema. The topical application of NSAIDs on a limited area of the skin does not produce high plasma concentration in human.[15,16]

## Conclusions

Our results support the idea that the administered NSAIDs (Diclofenac, Indometacin) achieved levels close to the oral administration plasma levels, but later in time. They can be used for the anti-inflammatory effect in the treatment of skin, joints and genital area located chronic inflammatory diseases.

Because of the NSAIDs mechanism of action, even for the topical applied NSAIDs, the digestive side effects must be mentioned and gastric protective means must be taken, because the plasma concentration can lead to systemic therapeutic effects and side effects.

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