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Control of the Transdermal Delivery Process of Active Substances of the Phytocomplex during Phonophoresis in Model Experiments

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Abstract

BACKGROUND: The scientific substantiation for the selection of therapeutically significant dosage of phytocomplex in the dosage form for phonophoresis, control over the delivery of active substances into the body, and what affects this process require the study of the kinetics of phytocomplex flavonoids delivery during phonophoresis.

AIM: The aim was to study the possibilities of controlling the process of transdermal delivery of phytocomplex active substances (flavonoids) during phonophoresis in vitro model experiments.

METHODS: Working compositions with different concentrations of phytocomplex for phonophoresis were used. The content of flavonoids in the compositions was determined using the spectrophotometric method and was calculated equivalent to quercetin, the flavonoid prevailing in the phytocomplex. The study of the kinetics of flavonoids delivery from working compositions was carried out using Franz diffusion cells and Carbosyl-P membranes. The authors determined the main parameters of the process and established the dependence of the delivery rate of flavonoids on their initial concentration in the working composition. The authors studied the effect of dimethyl sulfoxide and the base-forming substances of the working composition on the kinetics of phytocomplex flavonoid delivery during phonophoresis.

RESULTS: The authors recorded an increase in the rate of delivery of the active substances from working compositions containing dimethyl sulfoxide into the model medium by almost 1.5-2 times during the first ten minutes of the experiment (approximate duration of the phonophoresis procedure). The authors proposed technological techniques for improvement of the phonophoresis method for the phytocomplex. The possibilities of control over the process of transdermal delivery of the phytocomplex active ingredients during phonophoresis in vitro model experiments were shown.

CONCLUSION: The obtained results provide information for further pharmacological studies of the nature and mechanism of the effect of phytocomplex flavonoids during phonophoresis in the rehabilitation of patients with osteoarthrosis.

Introduction

One of the promising trends in modern physiotherapy is the creation of new, more effective pharmaco-physiotherapeutic methods for the rehabilitation of patients, including the use of herbal drug products [1], [2], [3], [4], [5], [6]. When developing new methods, ensuring control over the delivery of active substances into the body and the ability to influence and control this process is crucial [7], [8], [9], [10], [11], [12]. Duration of physiotherapeutic procedures is limited; therefore, it is necessary to quickly and fully utilise the resources of the drug for its rational use and maximum therapeutic efficacy.

This paper shows the possibility of regulating the process of transdermal delivery of active substances by the example of phonophoresis of phytocomplex in the rehabilitation of patients with osteoarthrosis. Previous studies have shown the need for improved technology and new, more effective phytocomplex dosage forms for phonophoresis [13], [14]. The study of the transdermal delivery of the phytocomplex active substances is also needed for the possible industrial production of a new drug based on the phytocomplex in the form of an ointment or gel suitable for phonophoresis. This allows for wider use of a new pharmaco-physiotherapeutic method.

The proposed phytocomplex is a dry extract

from grass and roots of marsh cinquefoil, grass or alfalfa, and multiple fruits, or cones, of common hop (TOR 9375-021-00003938-11 “Marsh cinquefoil, alfalfa, and common hop dry extract (phytocomplex)”) [14]. It contains flavonoids, polysaccharides, coumestans, tannins, essential oils, phenol carboxylic acids, macro- and microelements, and vitamins [15]. The main active ingredients of the phytocomplex are flavonoids, which have anti-inflammatory, analgesic, antioxidant and other effects. This stipulates the possible use of the phytocomplex in medicine for inflammatory and degenerative diseases of the musculoskeletal system, including osteoarthritis.

Currently, there are no systematic works in the literature devoted to the penetration of flavonoids from the medications through the skin during phonophoresis.

The study aimed to study the possibilities of controlling the process of transdermal delivery of the active ingredients (flavonoids) of the phytocomplex during phonophoresis in *vitro* model experiments.

Material and Methods

Phytocomplex working compositions with concentrations of 5% (W1), 10% (W2) and 15% (W3) were used for phonophoresis in the study. The following substances were used as base-forming substances for the preparation of 10% working composition: Carbopol 974P (Carbomer 974P, BufferGel) (C974P, Noveon Inc.) (W2) [16], [17]; ointment composition with 48.5 g of vaseline (FS.2.2.003.15, State Standard 3582-84), 15.0 g of anhydrous lanolin (TOR 9154-015-00333865-05), 1.5 g of distilled monoglycerides (TOR 9145-357-00336444-2005), and up to 100.0 g of purified water (FS.2.2.0020.15, State Standard 6709-72) (W4); ultrasound gel – Ecoultragel (Pirrone & Co. S.p.A., Italy) (W5). Analysis of the rheological characteristics of the working compositions was carried out using a Rheotest rotary viscometer (RN 4.1 modification, RHEOTEST Messgerate Medingen GmbH, Germany).

Previously it was established that the main active ingredients of the phytocomplex are flavonoids, and the working compositions are resistant to exposure to ultrasound with an intensity of 0.1-1.0 W/cm^2.

The content of flavonoids in working compositions was determined using the spectrophotometric method [15] and calculated equivalent to quercetin (Q 0125, Sigma), the flavonoid prevailing in the phytocomplex. To do this, an exact weight of the working composition (about 1 g) was placed in a flask with a capacity of 150 ml, 30 ml of 70% ethanol was added, the flask was connected to a reflux condenser and heated in a boiling water bath for 30 minutes. Then the flask was cooled to room temperature, and its contents were filtered through a filter paper into a 100 ml volumetric flask. The extraction was repeated twice, with 70% ethanol for 30 minutes. The extracts were filtered into the same volumetric flask, the filter was washed with 70% ethanol, and the filtrate was brought to the mark with a solution of 70% ethanol (solution A). In a volumetric flask with a capacity of 25 ml, 2 ml of solution A was added and brought to the volume of the solution to the mark with 70% ethanol (solution B). Studies were performed using a Titrtek MCC 1340 spectrophotometer (Finland) at a wavelength of 370 nm. The absorption spectra of the working compositions and quercetin were previously investigated. It was established that the base-forming substances do not shift the maximum optical density of quercetin, based on the intensity of which photometry was carried out.

The content of flavonoids was 0.35 ± 0.01% in W1, 0.70 ± 0.02% in W2, 1.04 ± 0.03% in W3, 0.72 ± 0.03% in W4, and 0.69 ± 0.02% in W5.

The study of the kinetics of the transdermal delivery of flavonoids from working compositions during phonophoresis performed by diffusion through a semipermeable membrane in Franz diffusion cells (# 4G-01-00-09-05, SES GmbH-Analysesysteme, Germany) in a V6-SFCS system using Carbosyl-P membranes (TOR 66-2-512-92). During phonophoresis, penetration of flavonoids through the skin follows the Fick's law, according to which the flux of the particles *(I)* diffusing through the plane perpendicular to the direction of diffusion is directly proportional to concentration gradient *(dc/DX)*:

$$I = -D(dc/dx), \quad (1)$$

where *D* is the diffusion coefficient.

The second Fick's law is usually used for analysing the majority of diffusion experiments:

$$dc/dt = D(d^2c/dx^2) \quad (2)$$

From equation (2) it follows that the change in the concentration over time *(dc/dt)* at a distance *x* from the initial plane is proportional to the rate of changing the concentration gradient towards *x* at moment *t*. For the practical use, equation (2) should be integrated under appropriate boundary conditions [18], [19].

Sodium hydroxide solution, 0.1 n. (quercetin is very poorly soluble in water) And 0.9% sodium chloride solution (physiological solution) were used as model media. Phonophoresis was carried out using the labile contact technique in a continuous mode with ultrasound intensity of 0.6 W/cm^2 using the UZT-1.07F device (Maloyaroslavets instrument factory, Russia). Samples were taken at certain time intervals with a complete replacement of the model medium (this system can be considered as a flow system at a first

approximation) and with taking 4 ml samples with their subsequent return and addition of the original medium to maintain required volume if necessary (closed system).

Statistical processing of the results was carried out using SPSS Statistics v17 Multilingual-EQUiNOX (SPSS Inc.) software.

Results

We studied the influence of the base-forming substances in the working composition on the delivery of flavonoids into 0.1 n. Sodium hydroxide solution in a flow system during phonophoresis. It was found that, during the first ten minutes of the experiment (the approximate duration of the phonophoresis procedure), more than 5% of flavonoids diffused into the model medium from the working composition W2, about 4% from the composition W4 and less than 3% from the composition W5. According to the results of the organoleptic control, the drying-up testing, the pH value determination and the thermal stability, the compositions with the best properties were W2 and W4. Analysis of the rheological characteristics of the working compositions demonstrated that the working composition W2 is optimal for the use in ultrasound therapy, it can be used with the prolonged application to the skin, it is sufficiently stable over time, which facilitates the preservation of the good quality of the drug during storage. Therefore, we used a basis with Carbopol to prepare the working compositions W1, W2, and W3 for further research.

The study of the kinetics of the flavonoids delivery from working compositions with different phytocomplex concentrations into 0.1 n. Sodium hydroxide solution in a closed system during phonophoresis showed that within four hours of the experiment approximately 43% of flavonoids diffused into the model medium from the working composition W1, about 26% – from composition W2, and about 20% – from composition W3 (Figure 1).

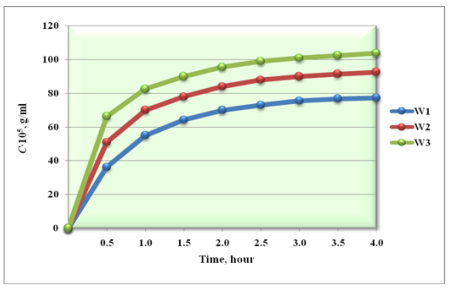

Figure 1: Kinetics of flavonoids delivery from the phytocomplex working compositions into 0.1 n. sodium hydroxide solution through Carbosil-P membranes when the equilibrium was reached in a closed system at 23°C during phonophoresis (C is the concentration of flavonoids in the model medium)

The equilibrium was reached in 3.5-4 hours. “Time lag” was 2.5-3 minutes. The flavonoids delivery rate from working compositions with different phytocomplex concentrations was directly proportional to the initial active substances concentrations in the compositions at the beginning of the experiment and sharply decreased by the time the equilibrium was reached.

It is known that the time lag has a significant effect on the diffusion process of substances into the model medium through a membrane. The diffusion coefficient (D) is calculated using the formula:

$$D = l^2 / 6\Theta,$$

where l is the film thickness, Θ is the time lag.

For comparison, we determined the time lag for the flavonoid's delivery from the composition W2 into 0.1 n. Sodium hydroxide solution without exposure to ultrasound – it was 3.5 minutes. Increasing the temperature of the applied working composition to 42°C reduced the time lag to three minutes. For rational use of the phytocomplex and increased therapeutic efficacy of the method, it is advisable to apply warm (40-42°C) working composition to the skin surface in the affected joint area, leave it for three minutes and then expose to ultrasound.

Periodic replacement of the model medium (flow system) allowed us to obtain a broader picture of the kinetics of the flavonoids delivery from working compositions with phytocomplex during phonophoresis — the maximum rate of flavonoids delivery from the composition W1 into 0.1 n. Sodium hydroxide solution was achieved after 30 minutes of the experiment, from compositions W2 and W3 – after 20 minutes (Table 1).

Table 1: Kinetics of flavonoids delivery from phytocomplex working compositions into 0.1 n. sodium hydroxide solution through Carbosil-P membranes inflow system at 42°C during phonophoresis

Time	Rate (V) and proportion (P) of flavonoids delivery					
	5% phytocomplex working composition (W1)		10% phytocomplex working composition (W2)		15% phytocomplex working composition (W3)	
	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %
10 min	60.9 ± 0.2	5.6	114.7 ± 0.3	5.3	147.9 ± 0.4	4.7
20 min	81.8 ± 0.3	13.2	141.1 ± 0.6	11.8	178.2 ± 0.5	10.5
30 min	94.0 ± 0.3	21.9	136.1 ± 0.4	18.1	154.8 ± 0.6	15.4
40 min	71.1 ± 0.2	28.5	130.9 ± 0.5	24.2	145.1 ± 0.5	20.1
1 h	55.3 ± 0.2	38.7	106.2 ± 0.5	34.0	125.6 ± 0.5	28.1
2 h	39.9 ± 0.2	60.9	78.8 ± 0.3	55.9	113.9 ± 0.4	50.0
4 h	18.1 ± 0.1	81.0	38.0 ± 0.2	77.0	62.3 ± 0.4	74.0
6 h	9.2 ± 0.1	91.3	19.2 ± 0.1	87.7	28.7 ± 0.2	85.0
8 h	4.4 ± 0.1	96.2	9.3 ± 0.1	92.9	14.1 ± 0.1	90.5

To increase the delivery rate of flavonoids, during the first ten minutes of the experiment, dimethyl sulfoxide (DMSO) was added into the working composition W2 with 10% and 15% DMSO concentrations were chosen also due to anti-inflammatory, analgesic and antimicrobial effects. A significant effect of DMSO on the delivery of flavonoids during the first several minutes of the experiment was recorded. For example, after ten

minutes of the experiment, the rate of flavonoids delivery from the working composition W2 with 10% of DMSO increased by more than 1.5 times, and 15% DMSO concentration resulted in an almost twofold increase (Table 2). The time lag was 2-2.5 minutes.

Table 2: Kinetics of delivery of flavonoids from working compositions containing DMSO, in 0.1 n. sodium hydroxide solution through membranes Carbosil-P inflow system at 42°C with phonophoresis

Time	Rate (*V*) and proportion (*P*) of flavonoids delivery					
	W2 + 10% of DMSO		W2 + 15% of DMSO		W2 + 10% of DMSO*	
	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %
10 min	173.3 ± 0.5	8.0	273.0 ± 0.7	10.5	216.0 ± 0.5	10.0
20 min	222.0 ± 0.6	18.3	264.8 ± 0.6	24.9	110.2 ± 0.4	15.1
30 min	190.1 ± 0.5	27.1	233.3 ± 0.5	35.7	67.0 ± 0.3	18.2
40 min	159.8 ± 0.4	34.5	183.6 ± 0.5	44.2	45.4 ± 0.2	20.3
1 h	145.8 ± 0.4	48.0	126.4 ± 0.4	55.9	40.0 ± 0.2	24.0
2 h	78.5 ± 0.3	69.8	66.6 ± 0.2	74.4	36.4 ± 0.2	34.1
4 h	31.1 ± 0.2	87.1	28.4 ± 0.2	90.2	26.8 ± 0.2	49.0
6 h	12.8 ± 0.1	94.2	9.5 ± 0.1	95.5	16.9 ± 0.1	58.4
8 h	4.1 ± 0.1	96.5	-	-	5.2 ± 0.1	61.3

*The working composition was exposed to ultrasound (beginning of the experiment) three minutes after it was applied warm (42°C) on the Carbosyl-P membrane. Ultrasound exposure time was ten minutes.

The results for the flavonoids delivery from working compositions based on W2 with 10% and 15% of DMSO into a 0.9% sodium chloride solution also indicated an increase in the phytocomplex active ingredients delivery rate.

An analysis of the rheological properties of working compositions with DMSO showed that composition W2 with 10% of DMSO had optimal plastic viscosity and yield value for ultrasound therapy.

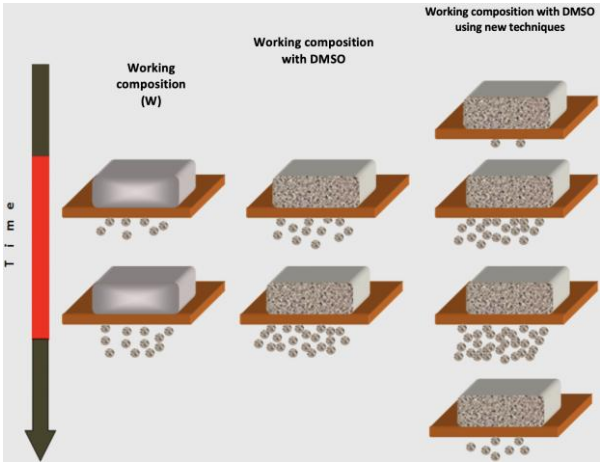

Figure 2: Control of the transdermal flavonoids delivery process from the phytocomplex working compositions during phonophoresis (red colour indicates the time of the phonophoresis procedure)

In order to increase pharmacokinetics measuring parameters of the process and make the use of the phytocomplex more rational, the following techniques were proposed: application of a thin layer of warm (40-42°C) working composition locally to the skin surface in the affected joint area followed by two or three minutes of wait time before the ultrasound exposure using contact labile method with an intensity of 0.6-0.8 W/cm^2 in a continuous mode lasting ten minutes, with working composition subsequently left on the skin for up to 6-8 hours (as an ointment) (Table 2, Figure 2). It should be noted that with the use of this technology, the flavonoids delivery rate from the working composition W2 with 10% of DMSO immediately after exposure to ultrasound is similar to that of composition W2 with 15% of DMSO, and the proportion of active substances delivery at the end of the experiment is almost eight times higher compared to traditional phonophoresis procedure.

Discussion

We presented pharmacokinetics measuring parameters of the transdermal flavonoids delivery process from the phytocomplex working compositions during phonophoresis in *vitro* model experiments: the maximum concentration of flavonoids in model media, the time of achievement of maximum concentration in model media, the rate and degree of release of flavonoids from the dosage form. The optimal dosage of the phytocomplex in working compositions was established – 10%. The main parameters of the process were determined; the dependence of the flavonoids delivery rate on their initial concentration in the working composition was established. The effect of dimethyl sulfoxide and the base-forming substances in the working composition on the delivery of phytocomplex flavonoids during phonophoresis was studied. An almost 1.5-2 times increase in the delivery rate of the active substances from working compositions containing dimethyl sulfoxide into the model medium during the first ten minutes of the experiment (approximate duration of the phonophoresis procedure) were recorded. Technological methods for the increase of the efficiency of the phonophoresis method and rational use of the phytocomplex were proposed. The obtained results give grounds for further pharmacological studies of the nature and mechanism of the effect of phytocomplex flavonoids during phonophoresis in the rehabilitation of patients with osteoarthrosis.

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