The release of biologically active substances from a phytopreparation when electrophoresis in the rehabilitation of patients with osteoarthritis

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SUMMARY

The kinetics of the release of flavonoids from a phytopreparation was studied during electrophoresis in model experiments. The main parameters of the processes are determined. The dependence of the rate of release of flavonoids on their initial concentration in the working solution of the phytopreparation has been established. The effect of the type of electric current and dimethyl sulfoxide on the release of biologically active substances from a phytopreparation was studied.

Key words: electrophoresis, release through the membrane,flavonoid, osteoarthritis.

RESUME

The kinetics of release of flavonoids from phytopreparation electrophoresis in model experiments is investigated. Key parameters of processes are defined. The dependence of speed release of flavonoids from the basic concentration in the working solution of the phytopreparation is established. Influence of a kind of an electric current and dimethyl sulfoxide on liberation of biologically active substances from a phytopreparation is studied.

Keywords: electrophoresis, release through the membrane, flavonoid, osteoarthrosis.

Introduction

Drug electrophoresis is an complicated an electropharmacotherapeutic method of treatment, in which both a medicinal substance and an electric current act on the patient's body [1]. These factors are capable of interference, so the response of the body will not be a simple sum of the effects of the drug and electric current, but more complex and specific, with the participation of the nervous and endocrine systems. For electrophoresis, only those medicinal substances can be used that are resistant to the action of electric current and retain their pharmacological activity, being released from the dosage form in therapeutically significant amounts [2].

In recent years, medicinal products are increasingly used for electrophoresis.

herbal products [3]. The phytopreparation offered by us for electrophoresis is a mixture of dry extracts of rhizomes with roots of marsh cinquefoil, alfalfa herb and common hop cones (2: 2: 1) [4]. Dry extracts are standardized, contain complexes of biologically active substances (BAS), due to which analgesic, anti-inflammatory, immunomodulating and other effects are realized, allowing them to be used comprehensively in medicine for inflammatory and degenerative diseases of the musculoskeletal system [5]. The main biologically active substances of phytopreparations are flavonoids. During electrophoresis, their release and penetration into the skin obeys Fick's law, according to which the flow of diffusing particles

(I) through the planeperpendicular to the direction of diffusion straight proportional to the concentration gradient (dc / dx):

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

where D is the diffusion coefficient.

Usually for analysis the majority diffusion experiments Fick's second law is used:

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

From equation (2) it follows that the change in concentration over time (dc / dt) tosome distance x from the initial plane is proportional to the rate of change of the concentration gradient in the x direction at the moment t. For practical using the equation (2) should be integrated with the appropriate boundary conditions [6, 7].

Currently, there are no systematic works in the literature devoted to the release of flavonoids from phytopreparations during electrophoresis. Hence, the relevance of these studies is obvious. The aim of the study was to study the kinetics of the release of biologically active substances (flavonoids) from a phytopreparation during electrophoresis in the rehabilitation of patients with osteoarthritis.

Materials and methods

In this work, a 5% working solution of a phytopreparation (1), a 10% solution (2) and a 15% solution (3) were used for electrophoresis. Determination of the content of flavonoids was carried out according to the method developed by us using the spectrophotometric method. The method is based on the reaction of complexation of flavonoids with aluminum chloride. The quantitative content of flavonoids was calculated for rutin, the predominant flavonoid of the herbal preparation. To obtain more reproducible results, the complexation reaction of rutin with aluminum chloride was carried out in the presence of acetic acid. In parallel, the optical density of a standard sample of rutin (R 5143, Sigma) prepared similar to the test solution was measured. The studies were carried out on a Titrtek MCC 1340 spectrophotometer (Finland) at a wavelength of 415 nm.

Preliminary, the absorption spectra of the phytopreparation and rutin were investigated on a Specord M40 with automatic registration of spectra and using the Scan Grap program. It was found that the phytopreparation does not displace

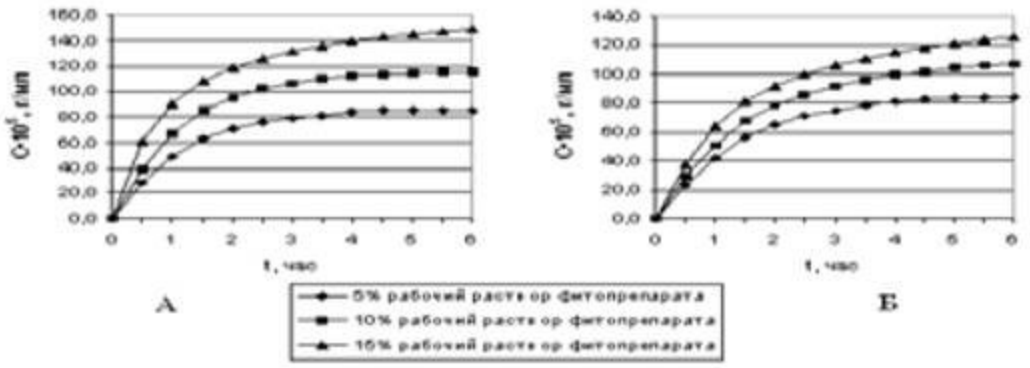
the maximum optical density of rutin, according to the intensity of which the photometry will be carried out. In addition, rutin had a similar differential absorption spectrum with differential spectrum absorption of phytopreparation flavonoids. Study of the release of flavonoids from a phytopreparation during electrophoresis in model experimentsin vitro carried out inmodified (for 3-chamber with electrodes) Franz diffusion cells (SES GmbHAnalysesysteme, Germany) through Carbosil-P membranes (TU 66-2-512-92) at a temperature of 36.0-C. Working solutions of the phytopreparation were placed in the central chamber, the side chambers were filled with a model medium - distilled water. Electrophoresis was carried out using various types of electric current: diadynamic (DDT) - full-wave continuous current with a frequency of 100 Hz - 1 minute, short-period modulated current with a modulation frequency of 1.5 s - 3 minutes, with a constant component of the apparatus; sinusoidal modulated currents (CMT) in rectified mode - with I and IV types of work, 5 minutes each, modulation frequency - 100 Hz, modulation depth - 75%, half-cycle duration - 2 and 3 s, current - 5 mA on the Combi device 500 "(Gymna Uniphy, Belgium Germany). Sampling was carried out at certain time intervals with a complete replacement of the model medium (this system in the first approximation can be considered as a flow system) and when taking samples of 4 ml with their subsequent return and adding the original medium to the required volume, if necessary (closed system). The latter technique is generally accepted, but it does not provide sufficiently accurate results of studying the kinetics of the release of biologically active substances from a phytopreparation at high degrees of extraction.

Previously, the stability of standard samples of the main flavonoids of the phytopreparation was established: rutin (R 5143, Sigma), apigenin (42251 Fluka), apigenin-7glucoside (44692, Fluka), biochanin A (14.563, Aldrich), hyperoside (00180585, Fluka), daidzein (D 7802, Sigma), isoquercitrin (00140585, Fluka), quercetin (Q 0125, Sigma), quercitrin (00740580, Fluka), as well as working solutions of phytopreparations 1, 2, 3 to the action of DDT and SMT. The absorption spectra of biologically active substances and working solutions were recorded before and after the action of an electric current. The quantitative content of flavonoids was determined spectrophotometrically at wavelengths: 360 nm (rutin), 339 nm (apigenin), 333 nm (apigenin-7-glucoside), 326 nm (biochanin A), 365 nm (hyperoside), 302 nm (daidzein) , 362 nm (isoquercitrin), 370 nm (quercetin), 363 nm (quercitrin) and 415 nm (the sum of flavonoids in terms of rutin with the addition of aluminum chloride in the presence of acetic acid in working solutions of the phytopreparation). It was also shown that the maximum electrophoretic mobility of the tested flavonoids of the phytopreparation is achieved when both the cathode and the anode are used as a working cathode in DDT and SMT.

Results and its discussion

In the study of the release of flavonoids from working solutions with different concentrations of phytopreparations in a closed system during electrophoresis

DDT and CMT showed that, during 6 hours of the experiment, approximately 47% of flavonoids diffused into the model medium from working solution 1, about 30–32% from solution 2, approximately 23–28% from solution 3. Equilibrium was established after 4– 6 o'clock. The rate of release of flavonoids from working solutions with different phytopreparation content at the beginning of the experiment was directly proportional to the initial concentrations of biologically active substances in the working solutions and sharply decreased by the time equilibrium was established (Fig. 1). Periodic replacement of the model medium (flow-through system) made it possible to obtain a more complete picture of the kinetics of the release of flavonoids from working solutions of the phytopreparation (Table 1). The maximum release rate of flavonoids from solution 1 during electrophoresis of DDT and SMT was achieved after 30 minutes of the experiment, from solutions 2 and 3 - after 20 minutes. The "lag time" was 1–2 minutes. In the first 20–30 minutes of the experiment (the approximate duration of the electrophoresis procedure), up to 16–18% of flavonoins from working solution 1, 12–15% from solution 2, 9–13% from solution 3, diffused into the model medium. When using DDT electrophoresis during the first two hours of the experiment, the rate and degree of extraction of flavonoids from working solutions of the phytopreparation were higher than during electrophoresis of the CMT. The time of complete release of flavonoids during DDT electrophoresis was reduced in comparison with the use of SMT electrophoresis (Table 1). When using DDT electrophoresis, during the first two hours of the experiment, the rate and degree of extraction of flavonoids from working solutions of the phytopreparation were higher than with SMT electrophoresis. The time of complete release of flavonoids during DDT electrophoresis was reduced in comparison with the use of SMT electrophoresis (Table 1). When using DDT electrophoresis, during the first two hours of the experiment, the rate and degree of extraction of flavonoids from working solutions of the phytopreparation were higher than with SMT electrophoresis. The time of complete release of flavonoids during DDT electrophoresis was reduced in comparison with the use of SMT electrophoresis (Table 1).



Rice. 1. Release of flavonoids from working solutions of phytopreparations indistilled water through Carbosil membranes when equilibrium is established in a closed system at 36-C during electrophoresis of DDT (A) and SMT (B) (t - time, C - concentration of flavonoids in the model environment).

Table 1

Release of flavonoids from working solutions of a phytopreparation into distilled water through Carbosil membranes in a flow-through system at 36-C for electrophoresis DDT and CMT

Время	Рабочий раствор фитопрепарата					
	1		2		3	
	Скорость высвобождения флавонидов (V) и процент высвобождения (В)					
	V × 105, г/млчас	В, %	V × 105, г/млчас	В, %	V × 105, г/млчас	В, %
Электрофорез ДДТ						
10 мин.	59,16 ± 0,02	5,48	101,92 ± 0,03	4,72	139,97 ± 0,02	4,32
20 мин.	68,82 ± 0,03	11,85	117,07 ± 0,04	10,14	152,93 ± 0,03	9,04
30 мин.	70,63 ± 0,04	18,57	113,40 ± 0,04	15,39	150,34 ± 0,04	13,68
40 мин.	69,55 ± 0,04	25,01	111,02 ± 0,04	20,53	145,48 ± 0,04	18,17
1 ч	66,26 ± 0,03	37,28	107,57 ± 0,04	30,49	143,05 ± 0,04	27,00
2 ч	39,20 ± 0,03	59,06	90,14 ± 0,04	55,53	110,92 ± 0,03	47,54
4 ч	16,53 ± 0,03	77,43	33,25 ± 0,03	74,00	55,30 ± 0,03	68,02
6 ч	7,16 ± 0,02	85,39	14,51 ± 0,03	82,06	26,87 ± 0,03	77,97
8 ч	0,23 ± 0,03	85,64	1,91 ± 0,03	83,12	11,72 ± 0,03	82,31
Электрофорез СМТ						
10 мин.	51,19 ± 0,02	4,74	82,94 ± 0,02	3,84	99,47 ± 0,02	3,07
20 мин.	60,16 ± 0,02	10,31	96,98 ± 0,02	8,33	108,22 ± 0,03	6,41
30 мин.	61,67 ± 0,01	16,02	96,98 ± 0,02	12,82	106,60 ± 0,02	9,70
40 мин.	58,00 ± 0,01	21,39	94,61 ± 0,01	17,20	101,09 ± 0,02	12,82
1 ч	53,73 ± 0,01	31,34	88,88 ± 0,01	25,43	97,20 ± 0,02	18,82
2 ч	36,13 ± 0,01	51,41	73,69 ± 0,01	45,90	89,86 ± 0,01	35,46
4 ч	18,10 ± 0,01	71,52	38,03 ± 0,01	67,03	55,54 ± 0,01	56,03
6 ч	8,96 ± 0,01	81,48	18,92 ± 0,01	77,54	33,62 ± 0,01	68,48
8 ч	4,13 ± 0,01	86,07	9,25 ± 0 ,01	82,68	22,49 ± 0,01	76,81
10 ч	0,24 ± 0,01	86,34	4,19 ± 0,01	85,01	11,61 ± 0,01	81,11

In practice, the efficiency of electrophoresis largely depends on the completeness and rate of release of biologically active substances in the first 20-30 minutes. In order to increase the rate of release of flavonoids during the first hour, additional dimethyl sulfoxide (DMSO) was added to the working solutions. The choice of this "carrier" of biologically active substances was also due to its anti-inflammatory, analgesic, antimicrobial action.

Studies of the release of flavonoids from working solutions of a phytopreparation containing DMSO were carried out in a flow-through system. It was previously established that DMSO does not shift the optical density maximum of rutin and does not affect the nature of the spectrum. Experimental data have shown that the introduction of DMSO into working solutions of a phytopreparation during electrophoresis of DDT and SMT has a significant effect on the release of flavonoids from solutions: with an increase in the concentration of DMSO in working solutions of a phytopreparation, the rate of extraction of flavonoids increases and

the time for complete release is reduced. Thus, from working solution 2, which did not contain DMSO, almost complete extraction of flavonoids using DDT was observed after 8 hours (Table 1); from a solution containing 10% DMSO - after 6 hours; from a solution containing 15% DMSO - after 5 hours (Table 2). The significant effect of DMSO was manifested in the first hours of the experiment. For example, after 20 minutes of the experiment, the rate of release of flavonoids from the working solution of the phytopreparation containing 15% DMSO under the action of DDT increased almost 2 times compared to the solution without DMSO, and reached its maximum value (Table 2).

conclusions

As a result of the study of the kinetics of the release of flavonoids from the phytopreparation during electrophoresis by diadynamic and sinusoidal modulated currents in model experiments, the main parameters of the processes were determined, the dependence of the rate of release of flavonoids on their initial concentration in the working solution of the phytopreparation was established. The influence of the type of electric current and dimethyl sulfoxide on the release of biologically active substances from the working solution of the phytopreparation was studied. It has been shown that the introduction of dimethyl sulfoxide into working solutions increases the rate of release of flavonoids from the phytopreparation, which is especially important in the first 20–30 minutes of electrophoresis. The results obtained provide a basis for further studies of the nature and mechanism of action of biologically active substances of the phytopreparation,

table 2

Release of flavonoids from working solution 2 of a phytopreparation containing DMSO into distilled water through Carbosil membranes flow system at 36-C for electrophoresis DDT and CMT

Время	Концентрация ДМСО в рабочей растворе, %					
	5		10		15	
	Скорость высвобождения флавонидов (V) и процент высвобождения (В)					
	$V \times 10^5$, г/мл·час	В, %	$V \times 10^5$, г/мл·час	В, %	$V \times 10^5$, г/мл·час	В, %
Электрофорез ДДТ						
10 мин.	139,61 ± 0,04	6,46	176,54 ± 0,05	8,17	222,76 ± 0,06	10,31
20 мин.	147,55 ± 0,05	13,29	210,74 ± 0,06	17,93	233,65 ± 0,08	21,13
30 мин.	142,50 ± 0,05	19,89	152,51 ± 0,05	24,99	191,50 ± 0,07	30,00
1 ч	120,80 ± 0,04	36,67	130,12 ± 0,05	43,06	137,08 ± 0,05	49,03
2 ч	82,52 ± 0,04	59,59	79,01 ± 0,04	65,01	76,70 ± 0,04	70,34
3 ч	46,03 ± 0,03	72,38	39,13 ± 0,04	75,88	33,32 ± 0,04	79,60
4 ч	23,29 ± 0,03	78,85	19,28 ± 0,03	81,23	13,89 ± 0,03	83,45
5 ч	13,29 ± 0,02	82,54	7,79 ± 0,02	83,40	0,25 ± 0,01	83,52
6 ч	2,29 ± 0,01	83,17	0,21 ± 0,01	83,45	–	–
Электрофорез СМТ						
10 мин.	118,42 ± 0,04	5,48	143,21 ± 0,04	6,63	171,46 ± 0,05	7,94
20 мин.	130,43 ± 0,05	11,52	177,04 ± 0,05	14,83	200,28 ± 0,06	17,21
30 мин.	109,76 ± 0,04	16,60	123,65 ± 0,05	20,55	142,40 ± 0,05	23,80
1 ч	91,32 ± 0,04	29,29	101,30 ± 0,04	34,62	112,32 ± 0,04	39,40
2 ч	77,21 ± 0,03	50,73	72,83 ± 0,03	54,85	72,38 ± 0,02	59,51
3 ч	52,84 ± 0,03	65,41	52,16 ± 0,03	69,34	52,21 ± 0,03	74,01
4 ч	36,53 ± 0,02	75,56	32,25 ± 0,03	78,30	29,44 ± 0,02	82,19
5 ч	22,19 ± 0,02	81,72	16,94 ± 0,02	83,00	10,96 ± 0,01	85,23
6 ч	2,63 ± 0,01	82,45	2,13 ± 0,01	83,59	0,21 ± 0,01	85,29

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