EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Determination of Plasma Concentration of the Cardioprotective Preparation Histochrome

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A method for measuring the plasma concentration of histochrome is described. Echinochrome, a naturally occurring polyhydroxynaphthoquinone with antioxidant and cardioprotective activities, is the active ingredient of histochrome. Plasma histochrome concentration is measured in patients with acute myocardial infarction. This method can be used for pharmacokinetic analysis.

Key Words: *free-radical pathology; antioxidants; polyhydroxynaphthoquinones; histochrome; pharmacokinetics*

Antioxidants have been widely used in the treatment of various diseases [12]. Histochrome is a new antioxidant drug [1,4,5] exhibiting cardioprotective activity in experimental reperfusion-induced ischemia [5,6,9]. Clinical trials showed that in combination with thrombolytics this drug effectively prevents the reperfusion-induced damage to the myocardium after acute infarction. Monitoring of histochrome concentration in biological fluids is necessary for the optimization of therapeutic scheme and evaluation of the drug activity.

Our objective was to develop a method for determination of plasma concentration of histochrome after its injection.

MATERIALS AND METHODS

Healthy donor plasma was used as a model. Histochrome was added to it to a final concentration of 1-40 μg/ml.

Plasma prepared from heparinized blood of healthy donors and patients with acute myocardial infarction was stored in liquid nitrogen. The specimens were thawed immediately before experiments, and chylomicrons were sedimented by centrifugation (80,000*g*, 30 min, 6°C, a Beckman centrifuge) [9]. All other procedures were performed at room temperature. Plasma concentration of histochrome was measured in a Shimadzu UV-260 spectrophotometer.

The preparation, histochrome for injections (1% water solution), was produced at the Experimental Plant of Biomedical Preparations (Cardiology Research Center) using materials and technology from the Pacific Institute of Bioorganic Chemistry (Russian Academy of Sciences). Russian-manufactured chloroform for anesthesia and distilled ethanol were used in experiments.

RESULTS

Echinochrome (2,3,5,7,8-pentahydroxynaphthoquinone), a quinoid pigment of sea-urchins, is the active ingredient of 1% histochrome for injections [3,13]. The preparation is a dark red liquid. After its intravenous injection in a dose of 1-2 mg/kg body weight, biologic fluids were stained red.

Individual variations of the plasma pigment levels and overlapping of histochrome and plasma pigment

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TABLE 1. Amount of Histochrome Extracted From Model Plasma

Parameter	Sample No.							
	1	2	3	4	5	6	7	8
Histochrome content, mg	1	2	4	6	10	20	30	40
Extracted histochrome, mg	1.0±0.1	1.9±0.3	3.8±0.2	5.1±0.4	9.0±0.1	17.6±0.8	25.8±0.6	34.2±1.0
Yield, %	100	96	96	86	91	88	86	86

spectra hamper the determination of plasma histochrome concentration by direct spectrometry.

Based on the results of experiments with healthy donors' plasma, we have chosen the method of Folch — chloroform:ethanol (1:2) [2,10] — for the extraction of histochrome from plasma.

Before extraction (histochrome plasma content varied from 0 to 40 μg/ml), plasma pH was adjusted to 1.2-1.4 with 5 N HCl (100 μl/ml plasma) to protonize histochrome and increase the efficiency of the procedure. Chloroform:ethanol mixture (1:2, 4 ml) was added to plasma (1 ml), centrifuged for 10 min at 2500 rpm, and the supernatant was collected. The procedure was repeated (2 ml of the extraction mixture was added to the pellet). The extracts were pooled, water (1 ml) and chloroform (1 ml) were added, and the phases were separated by centrifugation (1000*g*, 5-10 min). The chloroform phase (3.6-4.0 ml) was aspirated and used for spectral analysis. The reference cuvette contained a chloroform:ethanol (2:1) mixture.

The absorbance spectrum of histochrome in acidified ethanol has maxima at 342 and 470 nm and poorly resolvable maxima at 485 and 525 nm [4]. Analysis of the spectra for histochrome in acidified ethanol (100 ml ethanol+1 ml 1 N HCl) and in chloroform with acidified ethanol (1.6 ml chloroform+0.9 ml acidified ethanol) showed that histochrome can be quantitated not only by the absorbance peak (λ=470 nm) but also by the λ=525 nm shoulder. Absorbance spectra for extracts from histochrome-free plasma have no maximum at λ=525.

Figure 1 shows the absorbance spectra for histochrome in a chloroform-ethanol mixture. The dependence of $\Delta D_{525\text{-}600}$ on histochrome concentration was used for construction of the calibration curve. The histochrome concentration in acidified ethanol was determined by the method [5] with E_{341}=10,650 $M^{-1}cm^{-1}$.

The amount of extracted histochrome was calculated from the following formula: $47 \times V \times (\Delta D_{525\text{-}600} - 0.006)$, where $\Delta D_{525\text{-}600}=(D_{525}-D_{600})$, i.e., the difference between light absorbances at 525 and 600 nm at an optic pathway length of 1 cm, and V is the volume of extract. This equation was deduced assuming that plasma absorbance at 525 and 600 nm is 0.006.

As Table 1 shows, the effectiveness of the chosen extraction method is 86-96%. This method was employed for extraction of histochrome from the plasma of patients with myocardial infarction (Table 2). Histochrome was injected in two bolus doses (100 mg) before and 1 h after thrombolytic therapy.

This method can be employed for determination of plasma concentration of histochrome and for pharmacokinetic studies. Its sensitivity, specificity, and selectivity are sufficiently high to analyze small plasma samples (0.5 ml), rapid, and reliable. The method requires no expensive reagents and sophisticated equipment.

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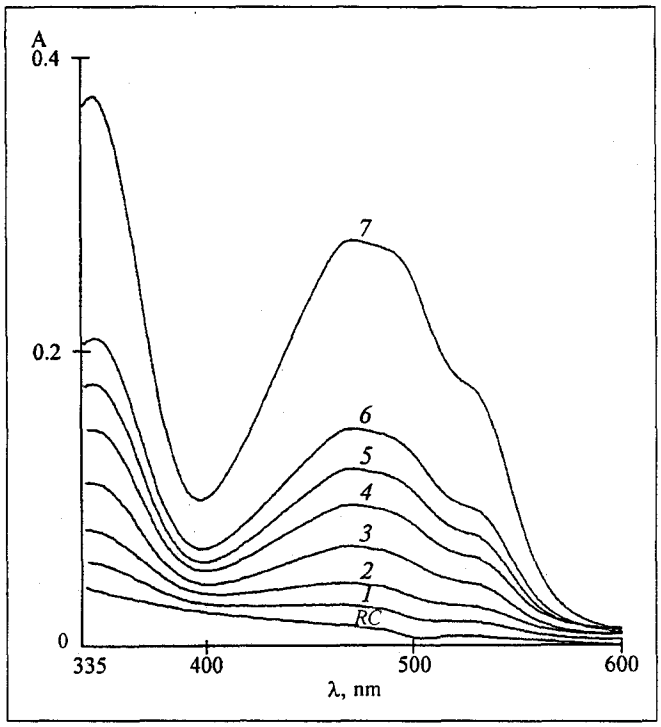

Fig. 1. Absorbance spectrum of histochrome in a chloroform: acidified ethanol mixture (1.6:0.9). *1*) 0.75; *2*) 1.66; *3*) 3.41; *4*) 5.16; *5*) 7.08; *6*) 8.91, *7*) 17.8 μg/2.5 ml. *RC*: reference cuvette (2.5 μl chloroform—acidified ethanol).

TABLE 2. Changes in Histochrome Concentration (mg/ml) in the Plasma of Patients with Myocardial Infarction

Patient No.	Time after the first injection						
	5 min	3 h	6 h	12 h	24 h	48 h	72 h
1st	-	22.5	hemolysis	11.75	10.00	7.20	7.00
2nd	19.38	23.00	20.70	hemolysis	15.19	8.60	7.50
3rd	11.0	16.19	16.50	13.03	13.68	10.56	9.16

Note. The preparation was injected two times at 1-h interval.

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