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Retinol palmitate as a model substance to test antioxidant properties *in vitro* on the example of captopril

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In the test of accelerated ageing it has been shown that captopril inhibits autooxidation of retinol palmitate. The results of the test have confirmed that retinol palmitate can be used as a model substance to estimate the effectiveness of antioxidant properties of different agents. The study was performed for different temperatures and concentrations of captopril. In order to estimate the strength of the antioxidant effect of captopril its activity was compared with that of the well known antioxidant octyl gallate. The thermodynamic parameters and frequency index of the decomposition of retinol palmitate were determined.

1. Introduction

It has been shown that captopril significantly inhibits the autooxidation process in sunflower oil, which is manifested as an increase in the induction time [1] and protective influence on unsaturated fatty acids, as proved in gas chromatography experiments [2]. Chopra et al. [3] have proved that Captopril shows antioxidant effect because of the presence of the sulfhydryl (SH) group. A comparative study performed with several ACE inhibitors indicated that only captopril shows antioxidant properties, while ramiprilat and lisinopril devoid of the SH group do not [4–7].

The aim of the study was to check if, and if yes to which degree, captopril inhibits autooxidation of retinol palmitate. The method applied was the test of accelerated ageing. It is known that retinol and its esters easily undergo decomposition under the influence of oxygen, which is clearly evident from the UV spectra. The decomposition of retinol palmitate is a pseudo-zero and first order reaction [8, 9]. Dobrucki and Jakubiec [10] applied retinol palmitate as a model substance to test antioxidant properties of different substances and proved (*in vitro*) the antioxidant activity of disopiramide, cinnarizine and dimenhydrinate.

2. Investigations, results and discussion

The antioxidant properties of captopril *in vivo* have been discussed in many papers [11–13]. Apart from its antihypertensive activity, captopril was shown to inhibit the oxidation processes at cell membranes, which improves their function and ion transport. It has been found that due to its ability to capture free radicals, captopril can prevent inactivation of enzymes, affect the DNA synthesis and support cell membrane functions including ion transport. Moreover, captopril is an indicator of microsomal lipid peroxidation [5, 12]. The mechanism of the antioxidant activity of captopril has been established as

$$R\bullet + Cp\ SH \rightarrow RH + CpS\bullet \quad [14]$$

As a result of free radical capturing captopril is converted to an inactive compound and free radical of thienyl. The

Table 1: Rate constants of retinol palmitate decomposition in non-stabilised and stabilised samples

Substance	Concentration of the substance (% w/w)	Temperature (°C)	No of measurements	Correlation coefficient	Rate constants $k \pm \Delta k$ (mol l^{-1} s^{-1})
Retinol palmitate	–	20	10	0.998	$2.56 \cdot 10^{-13} \pm 2.03 \cdot 10^{-14}$
	–	40	11	0.999	$1.41 \cdot 10^{-12} \pm 4.38 \cdot 10^{-13}$
	–	60	13	0.987	$3.77 \cdot 10^{-11} \pm 2.36 \cdot 10^{-11}$
Retinol palmitate + captopril	0.05	20	14	0.998	$1.28 \cdot 10^{-13} \pm 1.56 \cdot 10^{-14}$
		40	15	0.987	$8.56 \cdot 10^{-13} \pm 1.67 \cdot 10^{-14}$
		60	14	0.998	$2.65 \cdot 10^{-11} \pm 6.46 \cdot 10^{-12}$
	0.1	20	14	0.997	$1.22 \cdot 10^{-13} \pm 1.92 \cdot 10^{-15}$
		40	14	0.988	$7.69 \cdot 10^{-13} \pm 2.12 \cdot 10^{-13}$
		60	14	0.978	$2.29 \cdot 10^{-11} \pm 7.79 \cdot 10^{-12}$
	0.2	20	15	0.989	$7.29 \cdot 10^{-14} \pm 9.31 \cdot 10^{-15}$
		40	15	0.989	$7.57 \cdot 10^{-13} \pm 1.67 \cdot 10^{-13}$
		60	14	0.998	$1.81 \cdot 10^{-11} \pm 5.13 \cdot 10^{-12}$
Retinol palmitate + octyl gallate	0.05	20	15	0.989	$1.67 \cdot 10^{-13} \pm 2.19 \cdot 10^{-14}$
		40	14	0.999	$9.60 \cdot 10^{-13} \pm 1.35 \cdot 10^{-13}$
		60	15	0.988	$2.81 \cdot 10^{-11} \pm 5.89 \cdot 10^{-12}$
	0.1	20	13	0.988	$1.42 \cdot 10^{-13} \pm 1.16 \cdot 10^{-14}$
		40	14	0.998	$9.32 \cdot 10^{-13} \pm 1.61 \cdot 10^{-13}$
		60	15	0.988	$2.44 \cdot 10^{-11} \pm 5.82 \cdot 10^{-12}$
	0.2	20	14	0.988	$7.29 \cdot 10^{-14} \pm 7.42 \cdot 10^{-15}$
		40	14	0.998	$8.41 \cdot 10^{-13} \pm 1.02 \cdot 10^{-13}$
		60	14	0.998	$2.27 \cdot 10^{-11} \pm 7.51 \cdot 10^{-12}$

Table 2: Thermodynamical parameters of decomposition of retinol palmitate stabilised by captopril and octyl gallate

Substance	Concentration of the substance (% w/w)	ln A	Ea (kJ mol^{-1})	$\Delta H \neq$ (kJ mol^{-1})	$\Delta S \neq$ (JK-1 mol^{-1})
Retinol palmitate	0.0	12.36 ± 17.56	101.54 ± 45.67	101.51 ± 45.67	−104.18 ± 90.38
Retinol palmitate + captopril	0.05	15.09 ± 18.34	109.84 ± 47.96	107.40 ± 47.96	−119.34 ± 152.45
	0.1	16.59 ± 14.03	114.60 ± 36.70	112.16 ± 36.70	−106.85 ± 116.67
	0.2	25.24 ± 21.13	137.98 ± 52.14	135.54 ± 52.14	−138.85 ± 125.16
Retinol palmitate + octyl gallate	0.05	12.84 ± 17.83	103.78 ± 46.63	103.57 ± 46.63	−117.55 ± 108.57
	0.1	12.64 ± 15.55	103.61 ± 40.68	103.40 ± 40.67	−119.22 ± 152.45
	0.2	17.25 ± 11.29	116.30 ± 29.54	116.10 ± 29.54	−80.80 ± 76.54

(ln A – frequency coefficient Ea – activation energy; $\Delta H \neq$ – enthalpy; $\Delta S \neq$ – activation enthropy)

latter can react with the sulphur anion leading to the disulphide anion balance/equilibrium:

$$CpS\bullet + CpS\text{-} \rightarrow [CpS\bullet^{\cdot}\bullet SCp]^{-}$$

The aim of our study was to check if captopril also inhibits autooxidation of vitamin A in vitro. A model system applied was a paraffin solution of retinol palmitate. Vitamin A having a system of unsaturated bonds easily undergoes oxidation. The method used was the test of accelerated ageing, performed at 20, 40 and 60 °C. Octyl gallate, a commonly used antioxidant served as reference system. Both substances were applied in concentrations of 0.05%, 0.1% and 0.2%. The molecular mass of octyl gallate is 282.3, that of captopril 217.2. The experiment was carried out until total decomposition of the substance occurred according to pseudo-zero or first order kinetics [8, 9]. Table 1 presents the rate constants of retinol palmitate decomposition in two non-stabilised samples, one sample stabilised with captopril and one with octyl gallate. The thermodynamic parameters and frequency coefficients of the retinol palmitate decomposition are presented in Table 2. The course of the reaction of retinol palmitate decomposition in the stabilised and non-stabilised samples is illustrated in Figs. 1, 2, 3. The measurements of retinol palmitate concentration were performed by direct spectrophotometry according to USP 23.

For all samples with antioxidants, captopril or octyl gallate, the rate constant of the decomposition reaction decreases with increasing concentration of the antioxidant. With increasing temperature of incubation the reaction rate constant increases. At 60 °C and for antioxidant concentrations of 0.2%, the rate constant was the lowest for the sample of retinol palmitate stabilised with captopril, which means that the antioxidant properties of the latter are stronger than those of octyl gallate. On the basis of the linear plots of the reaction rate constants as a function of inverse temperature, the thermodynamical parameters of the accelerated ageing of retinol palmitate in liquid paraffin at different temperatures were calculated. The values of entropy, enthalpy and activation energy were determined (Table 2).

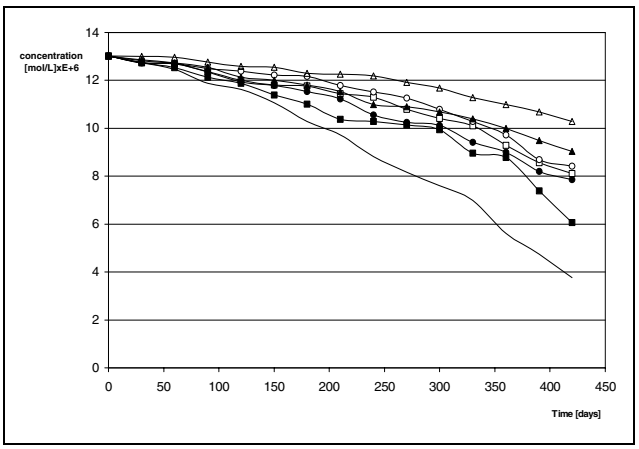

Fig. 1: Time changes in retinol palmitate concentration in the paraffin solution in the samples stabilised with captopril, octyl gallate and non-stabilised, measured at 20 °C. — retinol palmitate, —□— retinol palm. + captopril 0.05%, —○— retinol palmit. + captopril 0.1%, —△— retinol palm. + captopril 0.2%, —■— retinol palm. + octyl gallate 0.05%, —●— retinol palm. + octyl gallate 0.1%, —▲— retinol palm. + octyl gallate 0.2%

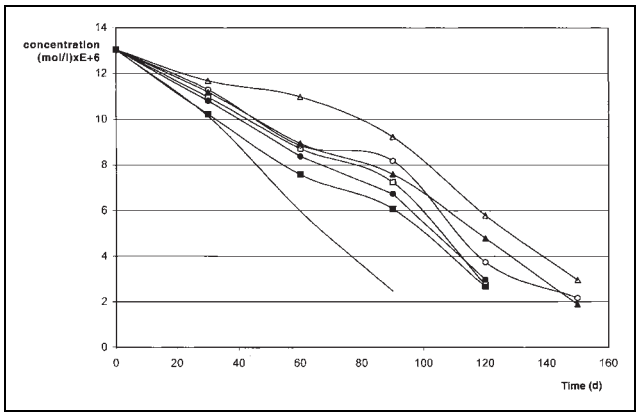

Fig. 2: Time changes in retinol palmitate concentration in the paraffin solution in the samples stabilised with captopril, octyl gallate and non-stabilised, measured at 40 °C. — retinol palmitate, —□— retinol palm. + captopril 0.05%, —○— retinol palmit. + captopril 0.1%, —△— retinol palm. + captopril 0.2%, —■— retinol palm. + octyl gallate 0.05%, —●— retinol palm. + octyl gallate 0.1%, —▲— retinol palm. + octyl gallate 0.2%

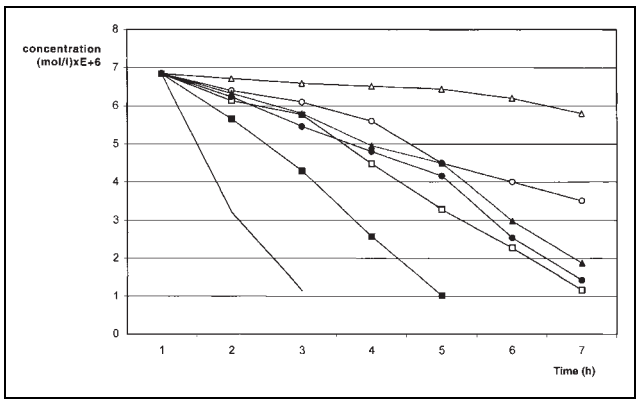

Fig. 3: Time changes in retinol palmitate concentration in the paraffin solution in the samples stabilised with captopril, octyl gallate and non-stabilised, measured at 60 °C. — retinol palmitate, —□— retinol palm. + captopril 0.05%, —○— retinol palm. + captopril 0.1%, —△— retinol palm. + captopril 0.2%, —■— retinol palm. + octyl gallate 0.05%, —●— retinol palm. + octyl gallate 0.1%, —▲— retinol palm. + octyl gallate 0.2%

The value of entropy was negative for all samples, which indicates that the reactions are bimolecular. At all temperatures for the samples with captopril the reaction rate constant was the lowest, which means that its antioxidant properties were higher than those of octyl gallate. The greatest antioxidant effect of captopril was observed at its concentration of 0.2%, whereas at a concentration of 0.05% the antioxidant effect of captopril proved insufficient. The values of thermodynamical parameters describing the retinol palmitate decomposition in the presence of the antioxidants studied and the concentration changes of retinol palmitate during the reaction indicate that the antioxidant effect of captopril is stronger than that of the commonly used antioxidant octyl gallate. The stronger effect of captopril was observed at all temperatures and concentrations used.

The results of the study have shown that the paraffin solution of retinol palmitate is a good model system for determination of the properties of an antioxidant.

3. Experimental

3.1. Materials

The paraffin solution of retinol palmitate of a concentration of 49 000 j.m./g, was prepared from the retinol palmitate concentrate containing 1 700 000 j.m./g, purchased at the Hoffman La Roche (Basel, Switzerland). Captopril 1-[(2,S)-3-merkapto-2metylopropionylo]-L-prolina, of purity meeting the requirements of USP 23 was purchased at the Jelfa S.A., (Jelenia Góra, Polska). Octyl gallate-an octyl ester of 3,4,5-trihydroxy – benzoesic acid, was purchased at the GlaxoWellcome (Poznań, Poland).

All other chemicals were analytical grade and were used as received.

3.2. Methods

The model system applied was a paraffin solution of retinol palmitate of a concentration of 49 000 j.m./g. Portions of 15.0 g of the solution were placed in open glass vessels of 10 cm in diameter. Three series of samples were prepared: with non-stabilised retinol palmitate, with retinol palmitate stabilised with captopril and octyl gallate, the stabilisers were used at the concentrations of 0.05%, 0.1% and 0.2%. The samples were incubated at 20, 40 and 60 °C.

Changes in the concentration of retinol palmitate were measured by direct spectrophotometry against isopropyl alcohol, according to the recommendation of USP 23, at $\lambda = 328$ nm.

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