Research & Development division[1], Krka d.d., Novo mesto, Slovenia, Department for Pharmaceutical Biology[2], Faculty of Pharmacy, University of Ljubljana, Slovenia

Oxidation of lovastatin in the solid state and its stabilization with natural antioxidants

S. Javernik[1], S. Kreft[2], B. Štrukelj[2] and F. Vrečer[1]

Oxidative instability of the hydroxy methylglutaryl Co-A reductase inhibitor lovastatin in the solid state and stabilization with natural antioxidants (ascorbic acid, rutin, gallic acid, quercetin and caffeic acid) was investigated. Lovastatin in the solid state and binary mixtures with 10% (w/w), 25% (n/n), 12.5% (n/n) and 6.3% (n/n) of each of the antioxidants were prepared. Oxidation experiments were performed on the scanning calorimeter using dynamic oxygen atmosphere. The amount of non-oxidized lovastatin was determined using HPLC. The results of the experiments have shown that lovastatin is unstable to oxidation under higher temperatures and in the presence of oxygen, and that some antioxidants markedly stabilize the drug. The most significant antioxidative effect was seen with caffeic acid and rutin, followed by gallic acid and quercetin. Ascorbic acid was only moderately effective. The results prove that flavonoids do have significant antioxidative potential. This phenomenon can be used to improve oxidative stability of drugs such as lovastatin which are sensitive to the presence of oxygen.

1. Introduction

Lovastatin, simvastatin, pravastatin and mevastatin are structurally similar cholesterol-lowering agents that competitively inhibit the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase [1–3]. The drugs are administered to patients as the prodrug lactones and are converted to their respective hydroxy acid forms *in vivo* [1].

Numerous literature reports describe the susceptibility of these drugs to oxidative degradation [3–6], and storage in inert (nitrogen) atmosphere is recommended [5, 7]. Only a few literature reports deal with the oxidation kinetics in solution [4, 6] and there are no reports on the oxidation kinetic of HMG-CoA reductase inhibitors in the solid state.

Flavonoids are polyphenolic compounds isolated from a wide variety of plants, with over 4000 individual compounds known. Individual differences arise in the various hydroxylation, methoxylation, glycosylation, and acylation patterns [8]. Phenolic compounds are of particular interest as protectors of biological systems against oxidative stress [9] and flavonoids play different roles in the ecology of plants and have long been used in medicinal practice. Today, studies of their strong antioxidant and metal chelation properties, and interaction with enzymes, adenosine receptors, and biomembranes are becoming more and more important [8].

The addition of butylated hydroxyanisole (BHA) [6] and butylated hydroxytoluene (BHT) has been found to stabilize drugs in the solid state (BHA is added as a preservative in commercially available tablets Mevacor®, Merck & SharpDohme [10]). Our aim was to find out how the polyphenolic natural antioxidants can stabilize substances susceptible to oxidation. They are known to have not only antioxidant and radical scavenging properties but also very interesting medicinal properties, such as anti-inflammatory, antispasmodic, antiallergic, antiviral, antibacterial, antitumor and anticancer activities [8, 11–13].

The HMG-CoA reductase inhibitor lovastatin was used as a model drug. Taking into consideration the oxidative decomposition of the drug and its lipophilicity, rutin, gallic acid, quercetin, caffeic acid and ascorbic acid [2] were chosen as antioxidants for the study.

2. Investigations, results and discussion

2.1. Chromatographic method

Chromatographic conditions were selected according to the lovastatin monograph of European Pharmacopoeia 1997, Supplement 2001 with minor adjustments [14]. The chromatograms show the binary mixture of lovastatin with rutin before and after oxidation at 155 °C for 15 min (Fig. 1).

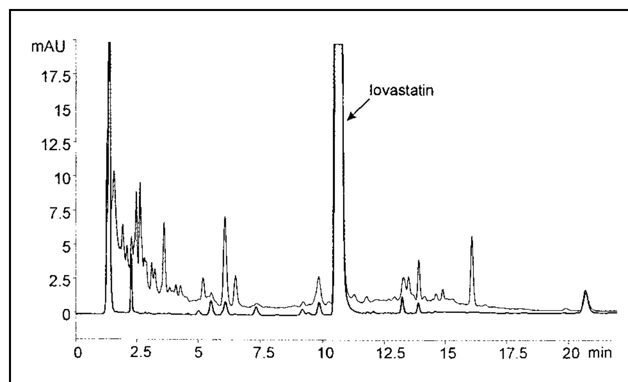

Fig. 1: Chromatogram of a mixture of lovastatin and rutin, before and after 15 minutes at 155 °C in an atmosphere of oxygen

2.2. Temperature and time dependent oxidation of the lovastatin and inhibition of oxidation process

The time dependence of lovastatin oxidation was followed in the solid state and in binary mixtures with 10% (w/w) of the antioxidants ascorbic acid, rutin, gallic acid, quercetin and caffeic acid by exposure to oxygen at different constant temperatures. The % remaining lovastatin was monitored as a function of time. The data are presented as % of non-decomposed lovastatin against time of the sample exposure to oxygen. No degradation was observed under nitrogen even at the highest temperature tested (155 °C) and the longest time of exposure (15 min). Comparable particle size was ensured by the sample preparation procedure in order to eliminate its possible influence (specific area of the sample) on oxidation kinetics. At

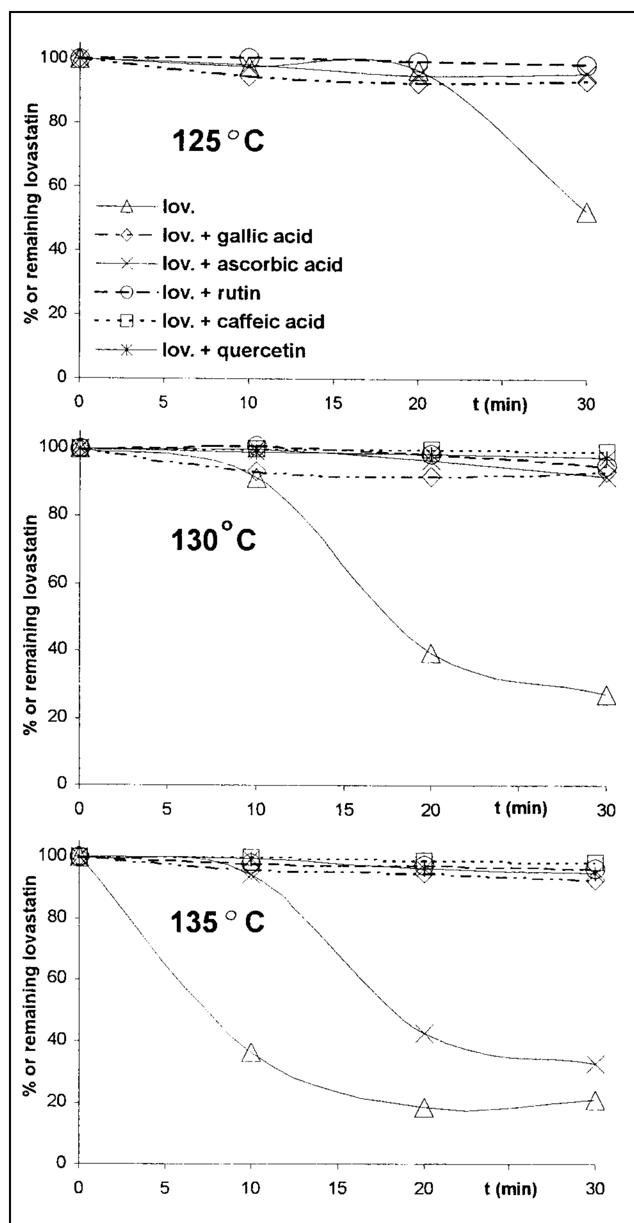

Fig 2: Time dependant course of lovastatin oxidation in the solid state and in 10% (w/w) binary mixtures

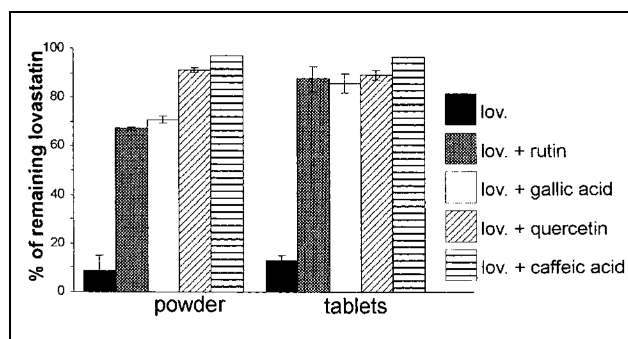

Fig 3: Comparison of non-oxidized lovastatin (in %) in the solid state and in binary mixtures, in powder and in tablet samples which were exposed for 15 min at 155 °C in oxygen atmosphere

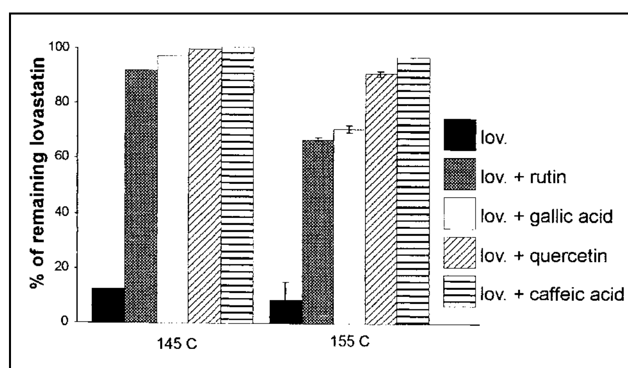

Fig. 4: Comparison of remaining lovastatin (in %) in the solid state and in binary mixtures (10% (w/w)) after exposing for 15 min at 145 °C and 155 °C in oxygen atmosphere

125 °C oxidation products were first observed about 20 min after the sample was exposed to oxygen and, within the next 10 min, the % of remaining lovastatin fell to 50% (Fig. 2). At 130 °C and 135 °C oxidation was faster and was observed earlier. Later on the oxidation of lovastatin was inhibited (oxidation rate decreased). All the antioxidants tested, apart from ascorbic acid, showed good antioxidant activity.
Chemical reaction of a solid and gas most frequently occurs according to one of the following mechanisms: A. reaction at immobile surface; B. nucleation with growth; C. barrier layer formation [15]. To test the possibility that the observed inhibition of oxidation was due to barrier layer formation the powder of lovastatin and its binary mixtures with antioxidants were compressed under 140×10^5 Pa pressure into tablets. The specific area was decreased substantially. In the case of barrier formation less oxidized lovastatin would be expected in the powder than in tablet samples. This was not observed in the experiment. In both tablet and powder forms, there were about 10% of non-oxidized lovastatin (Fig. 3).

To classify the selected natural antioxidants according to their antioxidant activity, their binary mixtures with lovastatin (10% (w/w)) were exposed to higher temperatures (145 °C or 155 °C) (Fig. 4). A strong antioxidative effect was observed for caffeic acid and less effect for quercetin, gallic acid and rutin.
The study of the thermochemical behaviour of lovastatin in oxygen shows an exotherm effect at about 150 °C influenced by oxidative reaction (Fig. 5). From the comparison of the DSC curve of pure lovastatin and those of binary mixtures (Fig. 5), it is evident that ascorbic acid has no effect on oxidative degradation (there is no additional endothermic effect for melting). A marked antioxidative effect was observed in case of mixing gallic acid, quercetin, rutin or caffeic acid with lovastatin. As can be seen from Fig. 5, the melting points of about 160 °C can be clearly

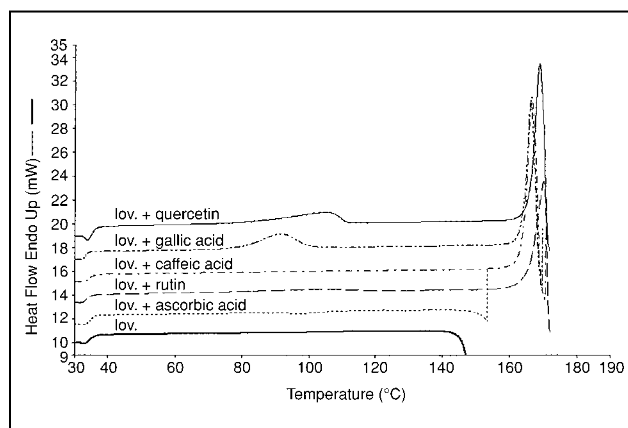

Fig. 5: DSC curves of lovastatin in the solid state and in binary mixtures in oxygen atmosphere

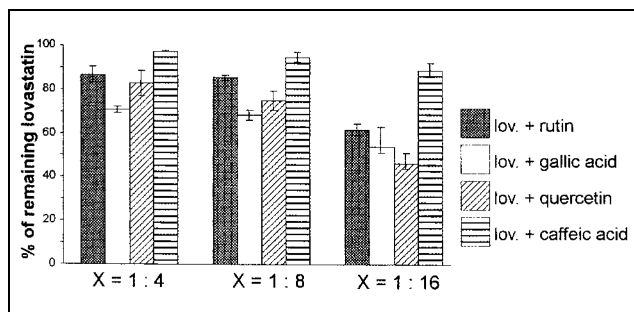

Fig. 6: Comparison of remaining lovastatin (in %) in binary mixtures with different lovastatin/antioxidant (n/n) ratios after exposing for 15 min at 155 °C in oxygen atmosphere

observed in case of mixture of lovastatin with antioxidants (except ascorbic acid). It was concluded that all antioxidants apart from ascorbic acid have marked influence on lovastatin stability. Classification according to the antioxidative effect cannot be determined because of the similarity of their DSC curves.

To further classify the antioxidants according to their inhibitory effect we prepared binary mixtures containing different molar ratios of antioxidant and lovastatin. In this order binary mixtures antioxidant : lovastatin in ratios: 1 : 4 (n/n), 1 : 8 (n/n) and 1 : 16 (n/n) were prepared. This experiment proved again that caffeic acid has a slightly higher antioxidation activity than the others, although the antioxidant activity of all four antioxidants tested is relative high even at a molar ratio antioxidant/lovastatin = 1 : 16 (Fig. 6).

The antioxidant activity of quercetin in aqueous phase has been found to be greater than that of gallic acid, which is greater than the antioxidant activity of rutin and caffeic acid [8]. In contrast to these results, caffeic acid resulted in the most significant stabilization under all the experimental conditions described. Rutin which, in the aqueous phase was the second to weakest, was in the solid state the second to strongest.

In conclusion, we estimate that the method is suitable for the study of antioxidant activity of substances in the solid state. The results of the experiments have clearly shown that lovastatin can be rapidly oxidized at higher temperatures in the presence of oxygen and that this is inhibited by some antioxidants. The most significant antioxidative effect was obtained with caffeic acid followed by rutin, quercetin and gallic acid and only a mild effect was obtained with ascorbic acid.

3. Experimental

3.1. Materials

Lovastatin was obtained from Krka, d.d., Novo mesto Research Laboratories and was at least 99% pure. Antioxidants were obtained from commercial sources and used without further purification: quercetin (for chromatography, $C_{15}H_{10}O_7 \cdot 2\,H_2O$, Kemika, Croatia), rutin (purum, $C_{27}H_{30}O_{16}$, Fluka AG, Germany), caffeic acid (for synthesis, $C_9H_8O_4$, Merck, Germany), gallic acid (for synthesis, $C_7H_6O_5 \cdot H_2O$, Kemika, Croatia), ascorbic acid (corresponds to Ph. Eur., USP quality, $C_6H_8O_6$, Fluka AG, Germany). All solvent and reagents were of analytical grade.

3.2. Methods

3.2.1. Oxidation procedure

Differential scanning calorimetry (DSC) was carried out using a Perkin Elmer DSC-7 calorimeter equipped with water cooling.

Experimental conditions: aluminium crucibles of 50 μl volume with four holes, atmosphere of dry nitrogen and oxygen with 40 ml/min flow rate, isothermal heating at 125 °C, 130 °C, 135 °C, 145 °C and 155 °C from 10 to 30 min. The calorimeter was calibrated with indium of 99.9% purity.

Lovastatin was examined in nitrogen and oxygen atmosphere, sample weights were 2.0–2.4 mg.

Binary mixtures of lovastatin with ascorbic acid, rutin, gallic acid, quercetin and caffeic acid, were prepared in the ratios 9 : 1 (w/w), 4 : 1 (n/n), 8 : 1 (n/n) and 16 : 1 (n/n). Samples of 2.0 to 2.4 mg were scanned. All experiments were performed at least twice. Oxidized samples were than analysed by HPLC.

3.2.2. Tablet preparation

Tablets were prepared using a Perkin Elmer hydraulic press with suitable mould and vacuum pump. A suitable quantity of lovastatin in alone or in a homogeneous binary mixture with antioxidant was transferred to the mould and compressed under 140×10^5 Pa.

3.2.3. Determination of lovastatin

The sample from an aluminium crucible was transferred quantitatively and dissolved in 2.0 ml of acetonitrile (J. T. Baker, HPLC grade). The standard solutions using for calibration purpose were prepared in the same way as described for the sample solutions.

Quantitative HPLC analysis were performed on a Hewlett-Packard 1100 VWD HPLC system using a Eurosphere C18 (endcapped octadecylsilane), 250 × 4.6 mm i.d. column with 5 μm particles equilibrated at 25 °C. Mobile phase A was acetonitrile (J. T. Baker, HPLC grade); mobile phase B was aqueous 0.1% (V/V) H_3PO_4 (Merck). The mobile phase flow rate was 1.5 ml/min, injection volume 5 μl and peak detection was at 238 nm. Analysis started with 40% B for 5 min and increased then to 65% B in 2 min. The gradient was then ramped up to 90% B in 5 min and remained the same for the next 10 min. After 22 min (stop time) the mobile phase B was decreased to 40% and the chromatographic system equilibrated to the starting condition for about 5 min.

Lovastatin eluted at approximately 10 min. The retention times of oxidized products range from 2 to 16 min.

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Sandra Javernik
Krka d.d. Novo mesto
Research & Development Division,
Šmarješka cesta 6
8501 Novo mesto
Slovenia
sandra.javernik@krka.si