Method of detection of dexamethasone in biological tissues and its application to assess the local kinetics of this drug

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Abstract

Introduction: The study of the pharmacokinetics of glucocorticosteroids is often required to solve fundamental and applied tasks of pharmacology. HPLC methods based on ultraviolet detection are attractive due to their availability, but their sensitivity is low enough to study in vivo kinetics. In this study, we propose a method for the determination of dexamethasone in biological objects, based on the use of HPLC with UV detection and having sufficient sensitivity to determine the drug in biological media (blood and periarticular tissues).

Materials and Methods: Extraction of dexamethasone from biosamples was carried out by liquid-liquid extraction with acetone in an acidic medium using atenolol as an internal standard. The analysis was carried out on a Kromasil-100 C18 column. A mixture of methanol with phosphate buffer in the ratio 50÷50, pH=5.6 was used as the mobile phase. Detector – UV, wavelength - 254 nm. The LLOQ of the method was 50 ng/mL; the calibration curve demonstrated linearity in the concentration range of 50-1000 ng/mL. The method was used to detect the medicinal product in peri-synovial tissues of rats with an autoimmune arthritis model.

Results and Discussion: This study demonstrated that intraarticular injection of the liposomal form of dexamethasone, compared with its water-soluble form, allows maintaining the active concentration of the product in the joint and periarticular tissues for a longer time, which creates prerequisites for enhancing its therapeutic effect.

Conclusion: The proposed method provides a sensitive and specific approach for measuring dexamethasone in biological samples, such as blood and periarticular tissues. Preliminary findings indicate that the liposomal form of dexamethasone may exhibit better pharmacokinetic properties than the water-soluble form, which could lead to improved therapeutic outcomes.


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Introduction

Currently, a promising direction in the treatment of autoimmune arthritis is local therapy with prolonged nanostructured forms of glucocorticosteroids (GCSS). Due to the creation of a high local concentration, such forms contribute to more effective compensation of the inflammatory process in the joint and periarticular tissues (Badokin 2013). Previously, we developed a liposomal extended-release form of dexamethasone and showed its greater efficacy compared to the water-soluble form in animals with an autoimmune arthritis model (Kulikov et al. 2021). The most likely explanation for the improvement in the pharmacodynamics of nanostructured forms of GCSSs may be a change in their pharmacokinetic profile (Raguzin et al. 2022); however, when using each of the newly synthesized compositions, it is necessary to prove this provision by assessing the local and systemic kinetics of the medicinal product. The solution of this problem became one of the objectives of this work.

We should note that for a pharmacokinetic study of glucocorticosteroids there are currently quite a large number of analytical procedures. In many cases, high performance liquid chromatography (HPLC) with mass spectrometric detection is used (Li et al. 2013; Jóhannesson et al. 2014; Prieto et al. 2017), but this approach is characterized by high cost and low availability. HPLC with ultraviolet detection can also be utilized (Duarah et al. 2021; Brugnera et al. 2022), but its major limitation is its insufficient sensitivity, which does not allow the assessment of tissue concentrations. In this study, we propose a method for the determination of dexamethasone in biological objects based on the application of HPLC with UV detection and having sufficient sensitivity to determine the medicinal product in biological media (blood and periarticular tissues). The method was applied to assess the dexamethasone kinetics during its intraarticular injection in the water-soluble and liposomal form in rats with an autoimmune arthritis model.

Keywords

intraarticular injection, dexamethasone, liposomes, HPLC-UV detection, pharmacokinetics

Materials and Methods

Chemical substances and reagents

Dexamethasone substance, Merck (USA); lecithin (phosphatidylcholine) EPCS 10 8018-1/130, Lipoid (Germany); cholesterol, Avanti Polar Lipids, Inc. (USA); chloroform (trichloromethane) stabilized (c.p.), Khimmed (Russia); purified deionized water, PM 42-2619-98; sodium hydroxide, sodium chloride, Ekonomkemikal LLC (Russia); methanol (c.p.), Vekton (Russia); acetone, Vekton (Russia).

Medicinal products

We used: dexamethasone (aqueous solution for injection 4 mg/mL, KRKA Novo mesto (Slovenia); a liposomal form of dexamethasone that we obtained according to the previously described procedure (Kulikov et al. 2021) and having the following characteristics: the concentration of...
**Dexamethasone** in suspension is 2.99 mg/mL, the size of the vesicles is 86±5 nm; the ratio of **dexamethasone**:lecithin is 1:12.5.

**Animals**

The pharmacokinetic study was performed on male Wistar rats weighing 200-250 g with a collagen-induced arthritis model, which is pathogenetically similar to human rheumatoid arthritis. All manipulations with the animals were carried out in compliance with the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The study was approved by the local Ethics Committee at National Research Mordovia State University, minutes No. 110 dated October 31, 2022. Arthritis was modeled according to the procedure described by Su et al. (2019).

**Modeling of arthritis**

To model arthritis in rats, bovine collagen type II (BC) (Sigma-Aldrich, Germany) was dissolved in 0.05 n. acetic acid solution to a concentration of 2 mg/mL, then emulsified in a 1:1 ratio with complete Freund’s adjuvant (CFA) (Sigma-Aldrich, Germany). The first immunization of animals was carried out by subcutaneous injection of 0.1 mL of an emulsion containing 0.1 mg of BC. The second immunization was carried out 7 days after the first using a similar emulsion by subcutaneous injection into the plantar region of the right hind paw. Arthritis manifested 2 days after the second injection of the BC/CFA emulsion.

**Experiment design**

The therapy was initiated at the peak of inflammatory manifestations in the joint (on the third day after repeated immunization). The animals were divided into 2 groups of 7 animals each. The first received liposomal dexamethasone, and the second – an aqueous solution of dexamethasone. The products were injected into the joint cavity twice with an interval of 72 hours. The dose of dexamethasone was 0.15 mg/kg. The volume of the injected solution was 0.05 mL per injection. The animals were slaughtered 6 days after the therapy initiation. The ankle joints were frozen and used to study the distribution of dexamethasone in tissues.

The **dexamethasone** plasma concentration was determined 24 hours after the first injection at the end of the experiment, and in perisynovial tissues only at the end of the experiment (on the 11th day after the product first injection).

**Chromatographic analysis**

**Extraction**

Dexamethasone was extracted from biological samples (plasma, periarticular tissues) by liquid-liquid extraction with acetone in an acidic medium using atenolol as an internal standard. 0.5 mL of plasma or an aqueous suspension of organ homogenate was taken into a 10 mL glass vial, 0.1 mL of 1% hydrochloric acid solution and 0.01 mL of atenolol solution with a concentration of 50 µg/mL were added. The resulting solution was stirred for 10 minutes, then 5 mL of acetone was added and stirred for another 10 minutes. The supernatant was separated by centrifugation at 5000 rpm for 10 minutes, then evaporated in a vacuum drying cabinet at a temperature of 40°C. The resulting dry residue was dissolved in 0.5 mL of the mobile phase.

**Chromatographic analysis**

The analysis was performed using the Gilson 155 UV/VIS chromatographic system, Gilson Inc. (USA). Chromatographic parameters: wavelength: 241 nm; Kromasil 100 C18 column (250×4.6 mm, 5 µm); mobile phase: methanol-phosphate buffer 50:50, pH=5.6; flow rate – 0.7 mL/min; internal standard – atenolol.

Since the development of the dexamethasone detection method was the objective of this work, the validation parameters of the method are described in detail in the results section.

**Statistics**

For all the obtained values, mean, standard deviation (SD), coefficient of variation (CV) were calculated. The significance of differences was assessed using the Student’s test for normal distribution or using the Shapiro-Wilk’s test and the W Wilcoxon’s test for distribution other than normal. Differences were considered significant if p<0.05.

**Results**

**Method for the determination of dexamethasone in biological media**

The following validation parameters of the method were determined: limit of detection (LOD), lower limit of quantification (LLOQ), linearity. Short-term-, long-term- and freeze-thaw stability

**Limit of detection, lower limit of quantification**

The limit of detection was determined as the concentration of the analyte creating a peak with a response 3 times higher than the response of the zero sample. The LOD of the developed procedure was 25 ng/mL; at this concentration, the response of the test sample was 3.4±0.4 times higher than the response of the zero sample.

The lower limit of quantification was determined as the concentration of the analyte creating a peak with a response 5 times higher than the response of the zero sample (Mironov et al. 2013). The LLOQ of this procedure was 50 ng/mL; at this concentration, the response of the control sample was 8.1±1.6 times higher than the response of the zero sample.

**Calibration curve and linearity**

The calibration curve was plotted and the dexamethasone plasma concentration was estimated using an internal standard, for which atenolol was selected. To plot the dependence of dexamethasone concentration on the ratio of the dexamethasone peak area (S_{dexam}) to the atenolol peak area (S_{atenol}) plasma samples containing 50, 100, 200, 400, 600, 800, 1000 ng/mL of dexamethasone were prepared. 500 ng of atenolol was also added to each plasma sample. Three samples were prepared for each concentration. The data for plotting the calibration graph are given in Table 1.
The equation of the calibration curve according to the mean was as follows:

$$C = 121.7X + 4.5$$

where $C$ is the dexamethasone plasma concentration, $X = \frac{S_{dex}}{S_{sat}}$.

A calibration graph of $S_{dex} / S_{sat}$ dependence on dexamethasone concentration was plotted (Fig. 1).

The value of $R^2$ was 0.9994, which is higher than 0.99 and meets the linearity requirements. Figure 2 shows samples of dexamethasone chromatograms in concentrations used to plot the calibration curve.

The deviation of the calculated dexamethasone concentration in calibration solutions from the nominal one was determined (Table 2). For all solutions, it did not exceed 15%, which corresponds to the accepted validation standards (Mironov et al. 2013).

<table>
<thead>
<tr>
<th>Nominal concentration, ng/mL</th>
<th>Calculated concentration, ng/mL</th>
<th>Deviation, %</th>
<th>Acceptable deviation values</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>46.9</td>
<td>6.2</td>
<td>±20%</td>
</tr>
<tr>
<td>100</td>
<td>103.9</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>186.5</td>
<td>6.8</td>
<td>±15%</td>
</tr>
<tr>
<td>400</td>
<td>409.7</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>802.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>1010.7</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Chromatogram of dexamethasone in plasma in concentrations used to plot the calibration curve: A – 50 ng/mL; B – 100 ng/mL; C – 200 ng/mL; D – 400 ng/mL; E – 800 ng/mL; F – 1000 ng/mL.
To determine accuracy and precision, quality control (QC) samples were used at the following concentrations: LLOQ (50 ng/mL); low QC (LQ, 200 ng/mL); average QC (MQ, 400 ng/mL); and high QC (HQ, 800 ng/mL). Table 3 shows the intraday and interday precision for rat plasma.

Table 3. Intraday and interday accuracy and precision of determination of dexamethasone in blood plasma

<table>
<thead>
<tr>
<th>Nominal concentration, ng/mL</th>
<th>Found concentration, ng/mL</th>
<th>Accuracy, %</th>
<th>Precision, %</th>
<th>Acceptable deviation values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday accuracy and precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>47.3</td>
<td>5.4</td>
<td>14.3</td>
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<tr>
<td>200</td>
<td>183.2</td>
<td>8.4</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>408.4</td>
<td>2.1</td>
<td>5.7</td>
<td>±15%</td>
</tr>
<tr>
<td>800</td>
<td>821.4</td>
<td>2.7</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Interday accuracy and precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>52.4</td>
<td>4.8</td>
<td>12.7</td>
<td>±20%</td>
</tr>
<tr>
<td>200</td>
<td>212.4</td>
<td>6.2</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>389.1</td>
<td>2.7</td>
<td>6.1</td>
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<tr>
<td>800</td>
<td>778.9</td>
<td>2.6</td>
<td>7.9</td>
<td></td>
</tr>
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</table>

Table 4. Short-term, long-term and freeze-thaw stability of dexamethasone in plasma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nominal concentration, ng/mL</th>
<th>Found concentration, ng/mL</th>
<th>Relative mean error (RME), %</th>
<th>Acceptable deviation values</th>
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<tr>
<td>Short-term stability</td>
<td>50</td>
<td>43.4</td>
<td>13.2</td>
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<td>200</td>
<td>224.3</td>
<td>12.2</td>
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<tr>
<td>400</td>
<td>376.9</td>
<td>5.8</td>
<td>±15%</td>
<td></td>
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<tr>
<td>800</td>
<td>834.7</td>
<td>4.3</td>
<td></td>
<td></td>
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<tr>
<td>Long-term and freeze-thaw (30 days)</td>
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<td>54.7</td>
<td>9.4</td>
<td>±20%</td>
</tr>
<tr>
<td>200</td>
<td>187.9</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>377.4</td>
<td>5.7</td>
<td>±15%</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>821.9</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze-thaw (3 cycles)</td>
<td>50</td>
<td>54.1</td>
<td>8.2</td>
<td>±20%</td>
</tr>
<tr>
<td>200</td>
<td>184.5</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>425.8</td>
<td>6.5</td>
<td>±15%</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>768.4</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Some parameters of pharmacokinetics and tissue distribution of liposomal dexamethasone

The developed method was used to determine the medicinal product in plasma and in perisynovial tissues of rats with an autoimmune arthritis model. The results of this study are given in Figure 3.

Figure 3. Dexamethasone concentrations in biological media during intraarticular injection in water-soluble (WD) and liposomal (LD) forms. WD – water-soluble dexamethasone; LD – liposomal dexamethasone. In plasma, measurements were made 24 hours and 11 days after administration of the drug. In perisynovial tissue, the concentration was determined 11 days after drug administration.

We can see that after the first injection in the water-soluble dexamethasone group, its concentration in the blood plasma was 3.1 times higher than when the liposomal product was injected. At the end of the study, the product was not detected in plasma in the group receiving water-soluble dexamethasone. In the group receiving liposomal dexamethasone, dexamethasone was detected in concentrations exceeding the LLOQ in five out of seven animals. Trace amounts of dexamethasone were found in two animals. The average concentration was 105±22 ng/mL. The results of the determination of dexamethasone in perisynovial tissue were opposite to...
those in plasma. In the group receiving the water-soluble form of dexamethasone, its concentration exceeded the LLOQ in only two animals, in four it was higher than the LOD but lower than the LLOQ, and it could not be found at all in one animal. The average value in the group was 74.4±37.0 ng/mL. In the liposomal dexamethasone group, the product was found in all animals; its average concentration in perisynovial tissue was 854±148 ng/mL, which is more than 11 times higher than in the water-soluble dexamethasone group.

Discussion

One of the key problems that has to be solved when developing a method for detection of analytes in biological media is extraction losses due to the binding of the analyte to proteins or its destruction. As a rule, when using nonpolar solvents as extractants, the analyte is converted into a molecular (non-ionized) form by creating an appropriate pH. For dexamethasone, an extraction method using a water-acetonitrile-methanol (43:32:25) mixture is described (Duran et al. 2021). We tested this method, but its sensitivity was insufficient for conducting pharmacokinetic studies in vivo: the LLOQ was 500 ng/mL. We have developed a method of extraction of dexamethasone with acetone with preliminary acidification of the medium to a pH=5.6, which significantly increased the sensitivity of the method (LLOQ=50 ng/mL). The increase in extraction efficiency is probably due to the fact that dexamethasone at low pH becomes more soluble in organic solvents, while its binding to blood proteins, due to a decrease in charge, weakens, which eventually leads to an increase in the extracted substance proportion passing into the organic phase.

According to the literature, intravenous injection of dexamethasone in plasma creates concentrations in the range of 100-2000 ng/mL, while the upper limit depends on the dose and is substantially unlimited (Song et al. 2020). With intraarticular injection, plasma concentrations also exceed 50 ng/mL (Earp et al. 2008). Thus, the sensitivity of the developed method is sufficient for the detection of dexamethasone in blood and tissues in the time range up to 3 T1/2, capturing more than 90% AUC. This allows the method to be used for pharmacokinetic studies in vivo.

Conclusion

Thus, a relatively simple and affordable method for detecting dexamethasone in biological media has been developed. The LLOQ of the method was 50 ng/mL; the calibration curve demonstrated linearity in the concentration range of 50-1000 ng/mL. The method was used to detect the medicinal product in perisynovial tissues of rats with an autoimmune arthritis model. This study demonstrated that intraarticular injection of the liposomal form of dexamethasone, compared with its water-soluble form, allows maintaining the active concentration of the product in the joint and periarticular tissues for a longer time, which creates prerequisites for enhancing its therapeutic effect.

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Conflict of interest

The authors have declared that no competing interests exist.

References


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