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Long-Term Stability Study of L-Adrenaline Injections: Kinetics of Sulfonation and Racemization Pathways of Drug Degradation

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ABSTRACT: Injectable formulations of L-adrenaline are commonly used in emergency medicine. Despite numerous studies, the comparative contribution and kinetics of the L-adrenaline inactivation pathways during storage have not been conclusively evaluated. We examined the kinetics of L-adrenaline degradation in a prospective study and determined the extent of drug inactivation by different pathways during and beyond the stipulated product shelf-life in 42 batches of adrenaline ampules stored under controlled conditions. The content of L-adrenaline and degradation products was determined with a chiral high-performance liquid chromatography (HPLC) assay, and the degradation products were identified by mass spectrometric detection as D-adrenaline and L- and D-adrenaline sulfonate. The kinetics of the content change with storage was analyzed simultaneously for L-adrenaline and the degradation products using kinetic modeling. The lower acceptable level of adrenaline content in the formulation stated by US Pharmacopoeia (90% as a sum of L- and D-isomers) was attained after 2.0 years of storage, at which time the content of the therapeutically active L-isomer amounted to as low as 85%. The modeling revealed significant differences in the degradation kinetics in the formulations produced before and after 1997, whose cause remained unidentified in this study. J Pharm Sci 93:969–980, 2004

Keywords: adrenaline; stability; chirality; HPLC (high-performance liquid chromatography)

INTRODUCTION

Adrenaline is a catecholamine compound that is commonly applied by intravenous injection in emergency medicine due to its effects on the cardiovascular system. In accordance with the United States Pharmacopeia (USP), the injections contain an aqueous solution of L-adrenaline (as a bitartrate salt) that is several-fold more potent than its optical isomer.[1]

Adrenaline in solution is subject to degradation; therefore, numerous studies addressed the effect of formulation variables on the drug inactivation kinetics, and attempts have been made to improve the formulation stability.[2–9] The results of these studies indicate that L-adrenaline in solution is inactivated by racemization and oxidation or to reaction with auxiliary formulation components (e.g., sodium metabisulfite) employed as an antioxidant (Fig. 1). The products of these reactions (including D-adrenaline and adrenaline sulfonate) possess little or no pharmacological activity compared with the parent compound.[6,10] The reversibility of reactions involved in L-adrenaline degradation should be taken into account for

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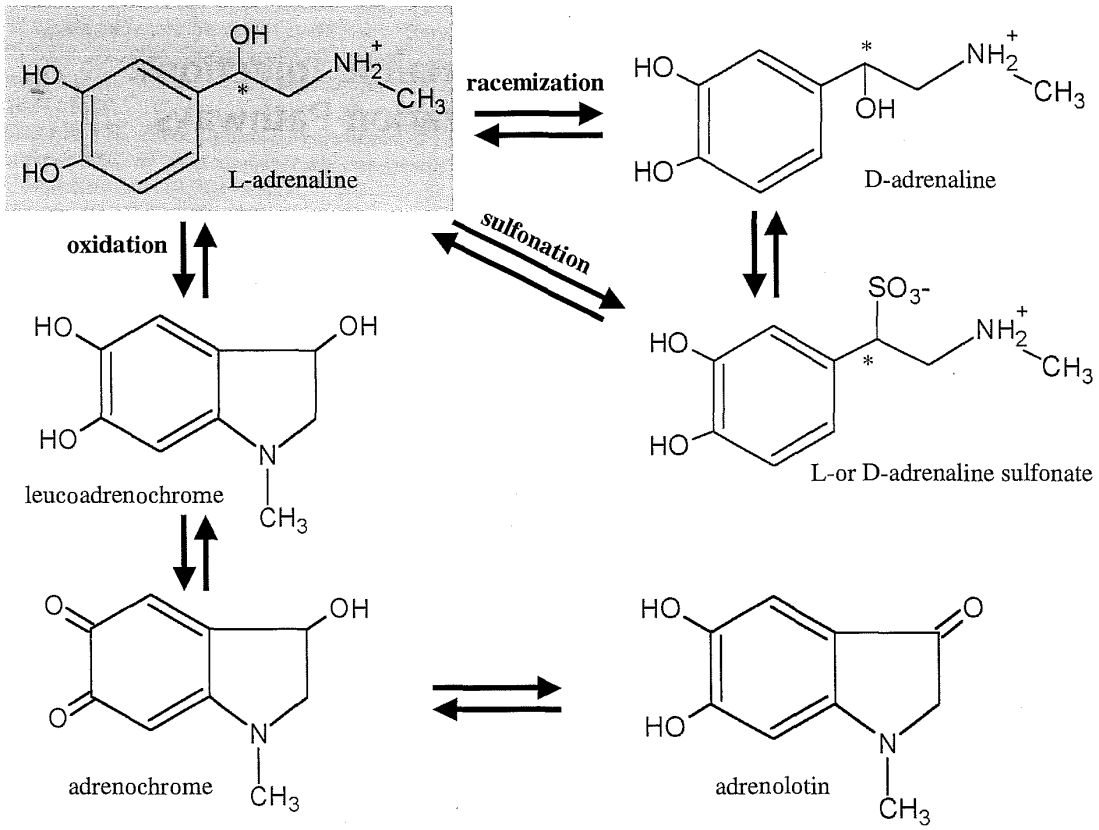

Figure 1. Degradation reactions of L-adrenaline.

long-term stability studies. For instance, D-adrenaline, which is formed from L-adrenaline by the racemization process, degrades by racemization (to produce L-adrenaline isomer), bisulfite addition, and oxidation reactions.

Despite the obvious significance of L-adrenaline optical isomerization in the overall drug inactivation process, the USP assay for adrenaline injections does not provide quantification of the optical isomers in the formulation.[11] Moreover, most of the adrenaline injections stability data available in the literature were obtained using comparatively nonspecific colorimetric, fluorimetric, or bioassay techniques that do not allow for accurate determination of the pharmacologically active drug isomer. The conclusive evaluation of the comparative contribution and kinetics of the drug degradation pathways requires well-characterized analytical methods that provide enantiomeric separation and reliable quantification. Examples of such methods based on chiral liquid chromatography have recently been published.[8,12,13]

The objective of this research was to examine in a prospective study the kinetics of the different pathways of L-adrenaline degradation in commercially available preparations acquired by Israel Defense Forces (IDF), and determine the extent of the drug inactivation therein during and beyond the stipulated product shelf-life. The analysis was conducted using a chiral high-performance liquid chromatographic (HPLC) method with ultraviolet–visible (UV–vis) detection, and the degradation products were identified by mass spectrometry (MS-MS).

EXPERIMENTAL

Samples of Adrenaline Injections

Adrenaline injections (1 mg of adrenaline base per milliliter) were produced by manufacturer A (Teva Ltd, Israel; 28 batches, manufactured 1985–1998) and manufacturer B (Biogal Ltd., Hungary; 14 batches, manufactured 1998–2002)

using a similar preparation process, except for a higher minimal initial content of adrenaline starting from 1997. The minimal initial drug amount in the formulation was changed from 90 to 103% of the declared content, corresponding to 0.90 and 1.03 mg/mL adrenaline base, respectively, because of concerns regarding the limited stability of the preparations. The additional components of the formulation were 1 mg/mL of sodium metabisulfite, 8 mg/mL of sodium chloride, and water for injection.

All the studied samples were received shortly (1–2 months) after the production of the corresponding batch. Following receipt, all batches were stored under controlled conditions recommended by the manufacturers. On the date of analysis, the storage period was 5.8–17.3 years for the batches produced by manufacturer A and 0.2–5.4 years for the batches produced by manufacturer B.

Chemicals and Reagents

L-Adrenaline bitartrate and potassium chloride were from Sigma (St. Louis, MO). Glacial acetic acid and HPLC-grade acetonitrile was from J.T. Baker (Deventer, Holland). Water was purified with a tandem RiOs (reverse osmosis)/Milli-Q Gradient A-10 system (Millipore, Molsheim, France). All other chemicals used in this study were of analytical or HPLC grade.

Chiral HPLC Assay

The chiral assay was a modification of the method provided by Showa Denko K.K., Tokyo, Japan. The mobile phase used for the chiral HPLC assay was 0.2 M potassium chloride in water: 0.2 M potassium chloride and 0.4% (v/v) acetic acid in water: acetonitrile (96:1:3, v/v). The mobile phase was filtered under vacuum through 0.45-μm nylon filters (Millipore, Bedford, MA).

The chromatographic system consisted of an HPLC model HP 1100 (Hewlett Packard, Palo Alto, CA) interfaced to an HP ChemStation, and a chiral Shodex ODS 5 μm column, 150 × 4.6 mm (Showa Denko K.K., Tokyo, Japan). The volume of injection was 50 μL, the column temperature was 10°C, the flow rate was 0.7 mL/min, and the run time was 20 min. The mobile phase was deaerated by on-line degasser, and detection was performed by UV–vis photodiode-array detector at 280 nm. A stock solution of L-adrenaline bitartrate, obtained by dissolving 21.84 mg of the compound in 10.0 mL of water, was further diluted in the mobile phase to prepare calibration standards in the 10–120% nominal drug concentration range.

Samples for analysis were prepared by diluting 1.0 mL of adrenaline injection solution with the stipulated content 1.8 mg/mL of adrenaline bitartrate (equivalent to 1 mg/mL of adrenaline base) with mobile phase to 20 mL.

HPLC-MS-MS Assay

A non-chiral HPLC method applying volatile mobile phase was developed for identification of the degradation products in adrenaline injections. The mobile phase was ammonium acetate buffer (5 mM, pH 7.0)/acetonitrile/formic acid (89.03:10:0.07, v/v/v) pumped at a flow rate 1.0 mL/min. The HPLC-MS-MS system consisted of Thermo Separation Products HPLC system (Egelsbach, Germany), Millipore Solvent delivery system (Millipore Corp., Milford, MA), MS-MS detector (Micromass Ltd., UK), UV detector (HPLC detector 432, Kontron Instruments, Switzerland), and 5 μm Luna Phenyl-Hexyl HPLC column (250 × 4.6 mm; Phenomenex, Inc., Torrance, CA).

Samples of aged adrenaline batches were prepared for analysis by diluting 1.0 mL of the adrenaline injection solution with mobile phase to 20 mL, and 20 μL of the obtained solution was injected into HPLC-MS-MS system.

After the HPLC column, the eluent was split to obtain the flow rate of ~0.25 mL/min and was modified by continuous injection of 0.65% formic acid in acetonitrile at a rate of 25 μL/min. The UV detector was set to 280 nm. HPLC-MS-MS was performed using electrospray ionization (EI) in the positive ion mode, with nitrogen as the nebulizer and drying gas. The mass range was 50–500 amu, and the dwell time was 0.1 s. The molecular ion masses of the degradation product and adrenaline were identified, and daughter scans of $m/z = 248$ (degradation product) and $m/z = 184$ (adrenaline) were measured with the collision energies of 11 and 18 eV.

pH Measurements

The pH values of the studied samples were determined using Metröhm Titroprocessor (model 796, Herisau, Switzerland) with a combined glass electrode.

Determination of Aluminum Concentrations

Aluminum concentrations in the samples of adrenaline batches were determined by furnace

atomic absorption analysis using Analyst 300 apparatus (Perkin Elmer, Norwalk, CT) following dilution 1:100 with 0.2% nitric acid. The analysis was performed versus standard solutions at 309.3 nm applying the recommended pretreatment and atomization conditions.[14]

Analysis of Degradation Kinetics of Adrenaline

Exponential Regression

Data analysis by exponential regression and calculation of 95% confidence intervals was performed according to the method described by Zar.[15]

Modeling

The goal of modeling was identification of the most parsimonious model that could appropriately describe the experimental outcomes. The structure of the proposed models was based on available data concerning adrenaline degradation pathways in injections (see Discussion),[6] and models with different kinetic order of underlying chemical reactions have been studied.

Analysis of the content versus storage period data was performed with ADAPT II Pharmacokinetic/Pharmacodynamic Systems Analysis Software (Biomedical Simulations Resource, Los Angeles, CA) applying the generalized least-square function.[16] The variance was described by the linear model: Var $R = (a + b \cdot R)^2$, where a and b are the variance parameters. Goodness of fit for the individual model was assessed from the graphs of the predicted and observed data, the coefficients of variation of the resulting parameters, and the values of the Akaike and Schwartz criteria.[17] The modeling was performed separately for the batches produced before and after 1997 (see Results), and for each period, the four sets of data (content of L-adrenaline, D-adrenaline, L-adrenaline sulfonate, and D-adrenaline sulfonate in the injections) were fitted simultaneously.

RESULTS

The Chiral HPLC Method and Its Validation

The chiral assay applied in this study enabled quantification of the optical isomers of adrenaline and its degradant in injectable preparations. The chromatograms of aged formulations obtained by this method (see Fig. 2) show four peaks corresponding to L-adrenaline ($t = 15.0$ min), D-adrenaline ($t = 16.3$ min), and two degradation products ($t = 3.4$ and 3.6 min, respectively).

The number of theoretical plates obtained for the L-adrenaline, D-adrenaline, and L- and D-degradation products were 6966, 4695, 3510, and

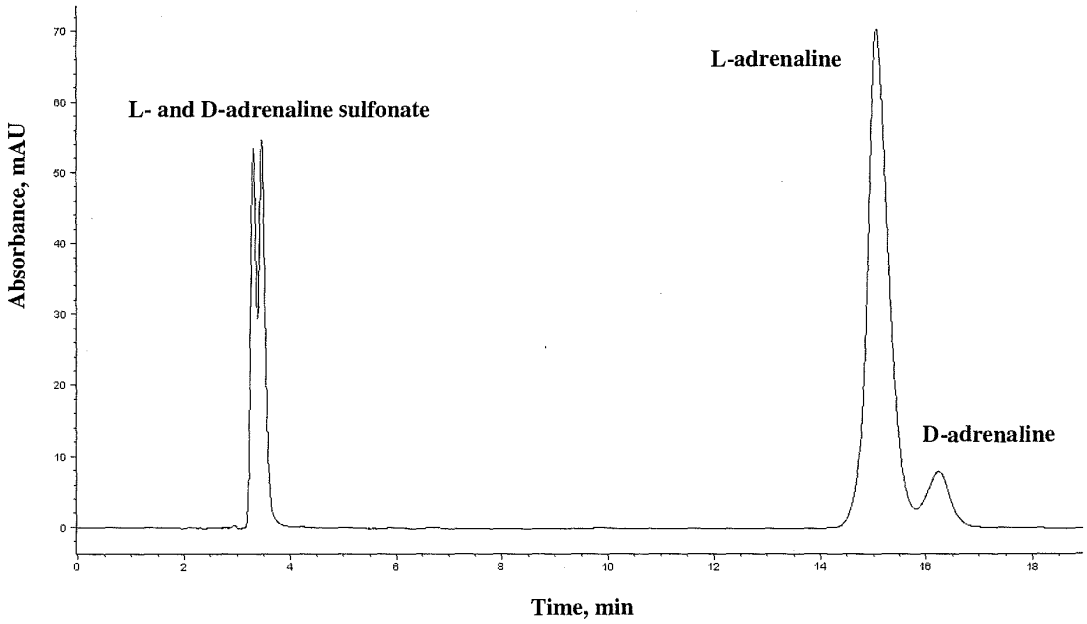

Figure 2. Chromatogram of aged adrenaline injection.

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