Injectable Formulation of Disodium 1-[2-(Carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (PROLI/NO), an Ultrafast Nitric Oxide Donor Prodrug

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Received 27 January 2005; revised 20 May 2005; accepted 5 July 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20486

ABSTRACT: PROLI/NO is an agent of structure XN(O)=NONa (X = L-prolyl) whose 2-s half-life for nitric oxide (NO) release at physiological pH makes it an excellent prodrug for localizing NO's therapeutic effects at the site of application, but a difficult one to formulate and certify as pure. Despite its extraordinary thermal and hydrolytic instability, however, PROLI/NO could be formulated as an injectable drug by dissolving it in cold 0.1 M sodium hydroxide containing 5% D-mannitol, then quickly ultrafiltering and lyophilizing it in evacuated septum vials. No evidence for decomposition was seen in the contents of these evacuated vials when stored at −20°C over a 140-day observation period, as judged by quantifying NO release in simulated infusate solutions (10 mM carbonate/bicarbonate, pH 10.5). The only hydrolysis products detected were NO, nitrite ion, proline, and *N*-nitrosoproline, all products of normal human physiological processes.
 J Pharm Sci 95:108–115, 2006
Keywords: nitric oxide; analytical chemistry; lyophilization; stabilization; prodrugs; targeted drug delivery; solvate; kinetics; chirality; chemical stability

INTRODUCTION

PROLI/NO (structure shown in Scheme 1) has shown promise for treating a variety of vascular disorders thanks to the rapidity of its spontaneous hydrolytic conversion to the multifaceted bioeffector nitric oxide (NO) in the blood stream. With a half-life of less than 2 s at physiological temperature and pH, its vasodilatory and antithrombotic effects can be localized to the pulmonary vasculature,[1,2] a spastic cerebral artery,[3] or the site of a vascular surgery procedure[1,4] without inducing systemic hypotension. For the very reason that its high reactivity makes PROLI/NO a promising therapeutic agent, however, formulation for infusion or injection has been problematic. Proof-of-concept studies in animal models have generally been conducted by infusing the drug immediately after dissolving the powdered

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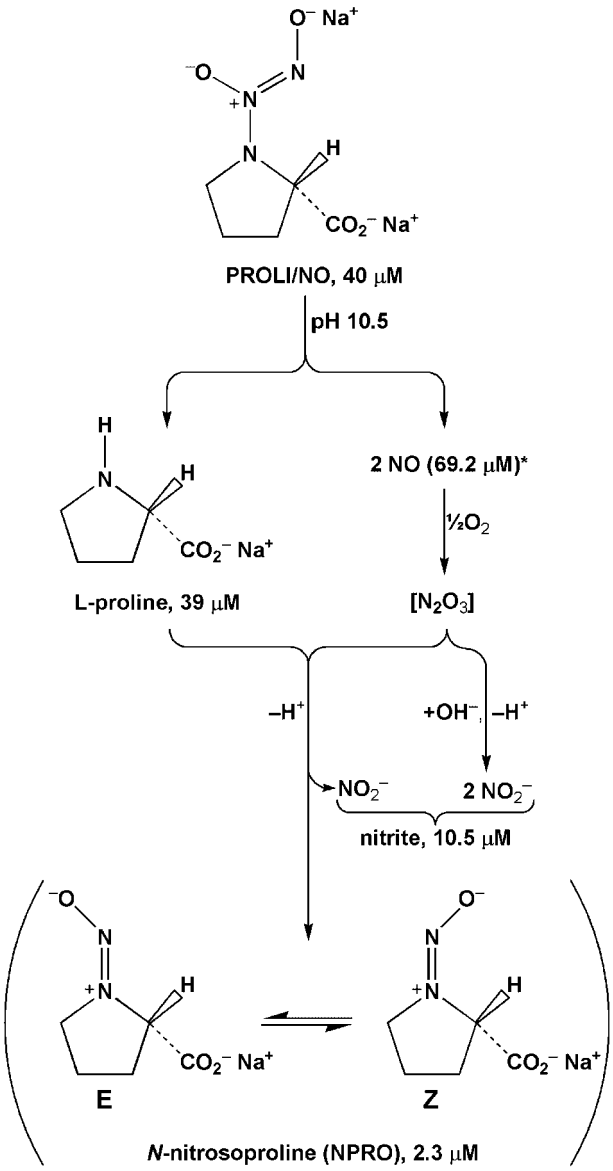
Journal of Pharmaceutical Sciences, Vol. 95, 108–115 (2006)



Scheme 1. Products of 40 μM PROLI/NO hydrolysis in pH 10.5 carbonate/bicarbonate buffer and mechanisms of their formation. *The "concentration" given for NO, 69.2 μM, is that which would have been seen if it had been retained in solution instead of being purged out of the system and into the chemiluminescence analyzer.

methanolate in media containing enough base to adequately slow its proton-induced dissociation[5] to NO. No toxic effects were reported even when 0.1 M sodium hydroxide was used as vehicle,[1] but the procedures used hardly seemed ideal for clinical application.

Here we describe procedures for successfully formulating, storing, and reliably infusing this sensitive NO prodrug.

EXPERIMENTAL

Samples of PROLI/NO monomethanolate[1] and *N*-nitrosoproline[6] (NPRO) were synthesized according to published procedures. *N*-Nitrosopyrrolidine was purchased from Sigma (St. Louis, MO). HPLC grade water was prepared using a High-Q 103S water still. HPLC or Omnisolve grade acetonitrile, formic acid, methanol, and dichloromethane were used as received from EM Science. L-Proline, sodium nitrite, and sodium nitrate were purchased from Aldrich Chemical Company (Milwaukee, WI) and used as received. Griess reagent derivatization solutions used for determining nitrite ion were prepared fresh daily according to published procedures.[7] Chinard's reagent[8] was prepared by mixing a solution of ninhydrin (2.0 g) in glacial acetic acid (50 mL) with 85% phosphoric acid (17.5 mL) in distilled water (50 mL) for use in determining proline.

HPLC studies were performed with a Rainin Dynamax Scale-up System connected to a Dynamax PDA-2 Diode Array Detector. A 5 μm Dynamax C18 250 × 4.6-mm column was used for quantifying NPRO (extinction coefficient 6.5/mM cm at 238 nm[6]), while an 8 μm Dynamax C18 250 × 21.4-mm column was used for semi-preparative collection of NPRO. The LC-MS system used to confirm the identities of the organic products proline and NPRO was a Finnigan LC Q DECA quadrupole ion trap mass spectrometer with electrospray ionization in the positive ion mode, heated capillary at 350°C (capillary voltage 20 V, sheath gas flow 70 U, auxiliary gas flow 15 U, tube lens offset −35); the separation was performed using a 3 μm LUNA C18(2) 150 × 2.0-mm column under isocratic conditions (70:30 water:methanol containing 0.1% formic acid in water, 150 μL/min). LC retention times and mass spectra of the observed products were identical to those of authentic standards: 2.80 min and $[M+H]^+$ m/z 116 for proline; NPRO showed LC peaks for the expected[6] E/Z isomers at 4.80 and 5.29 min, both of whose $[M+H]^+$ ions appeared at m/z 145. UV-visible spectra were recorded with a Hewlett–Packard Model 8452A spectrophotometer. Chemiluminescence studies were performed with a Thermo Electron Corp. Model 502A Thermal Energy Analyzer. Capillary electrophoresis studies were carried out with a Beckman Model 5510 CE at pH 3.0 (50 mM phosphate) in the reverse polarity mode (−14 kV) using an eCAP capillary column (50-cm effective length, 75 μm I.D.) at 22°C. CD studies were performed using an Aviv Model 202