Joint Feedback Analysis Modeling of Nonesterified Fatty Acids in Obese Zucker Rats and Normal Sprague–Dawley Rats after Different Routes of Administration of Nicotinic Acid

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Received 2 April 2014; revised 12 June 2014; accepted 12 June 2014

Published online 1 July 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24077

ABSTRACT: Data were pooled from several studies on nicotinic acid (NiAc) intervention of fatty acid turnover in normal Sprague–Dawley and obese Zucker rats in order to perform a joint PKPD of data from more than 100 normal Sprague–Dawley and obese Zucker rats, exposed to several administration routes and rates. To describe the difference in pharmacodynamic parameters between obese and normal rats, we modified a previously published nonlinear mixed effects model describing tolerance and oscillatory rebound effects of NiAc on nonesterified fatty acids plasma concentrations. An important conclusion is that planning of experiments and dose scheduling cannot rely on pilot studies on normal animals alone. The obese rats have a less-pronounced concentration–response relationship and need higher doses to exhibit desired response. The relative level of fatty acid rebound after cessation of NiAc administration was also quantified in the two rat populations. Building joint normal-disease models with scaling parameter(s) to characterize the "degree of disease" can be a useful tool when designing informative experiments on diseased animals, particularly in the preclinical screen. Data were analyzed using nonlinear mixed effects modeling, for the optimization, we used an improved method for calculating the gradient than the usually adopted finite difference approximation. © 2014 The Authors. *Journal of Pharmaceutical Sciences* published by Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2571–2584, 2014

Keywords: pharmacodynamics; nonesterified fatty acids (NEFAs); nonlinear regression; disease modeling; feedback; mathematical models; monte carlo; disease state; sensitivity analysis

INTRODUCTION

Nicotinic acid (NiAc) is a lipid lowering agent that inhibits lipolysis in adipose tissue by activating the GPR109A receptor, resulting in a pronounced decrease in plasma nonesterified fatty acids (NEFAs) concentrations.[1] Current understanding of the mechanism is that activation of the G protein-coupled receptor GPR109A by NiAc inhibits adenylyl cyclase activity, leading to decreased formation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate. cAMP regulates lipolysis in adipocytes by activating protein kinase A that phosphorylates hormone-sensitive lipase (HSL). NiAc thus reduces the hydrolysis of triglycerides (TG) into NEFAs and glycerol, which is catalyzed by HSL.[2] A feedback model describing NiAc-induced changes in NEFA plasma concentrations has previously been published.[3–5] The characteristics described by the model are tolerance and oscillatory rebound effects, and depending on the parameter values, the model can be used for either Sprague–Dawley rats or obese Zucker rats.

Obese Zucker rats display insulin resistance and obesity, and are frequently used as a disease model. Obesity is frequently associated with insulin resistance and known to influence the distribution and clearance of compounds.[6–10] This suggests that insulin resistance, in an animal or a patient, can alter the pharmacokinetics (PK) and pharmacodynamics (PD) of the NiAc/NEFA system with changes in onset, duration and intensity of the drug effects. As a normal model, Sprague–Dawley rats are used. Their lipoprotein and lipid patterns are similar to those of the lean Zucker rat and therefore can serve as a normal model for comparison purposes.[11,12]

Analyzing all data collected during a preclinical screen allows us to better quantify the difference in PD between normal Sprague–Dawley rats and obese Zucker rats. A full analysis of all data available also allows us to better understand how

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This article contains supplementary material available from the authors upon request or via the Internet at http://onlinelibrary.wiley.com/.

The copyright line for this article was changed on August 14, 2014 after original online publication.

Journal of Pharmaceutical Sciences, Vol. 103, 2571–2584 (2014)

drug response in normal animals translates to the diseased animals. This is particularly valuable for decisions made in the preclinical phase, which are often based on selected data from a normal animal model. We therefore sought to determine how disease affects the PD and to quantify this effect. This report describes such a joint analysis that utilized data from 95 Sprague–Dawley rats and 19 obese Zucker rats simultaneously, to estimate the relative change in pivotal parameters across normal and diseased animals. Data were analyzed using nonlinear mixed effects modeling in which, for the optimization, we used a more exact method for calculating the gradient than the usually adopted finite difference approximation.[13]

We also compare our joint analysis to population PK/PD analysis carried out separately for normal and obese rats to investigate which PD parameters are affected by the disease. The combined analysis uses all available data simultaneously to maximize the information content from which inference about a pathophysiological system can be made. In a more general setting, this kind of modeling can be used to combine knowledge gained from previous, less information-rich studies. Accumulating information into models over the life-span of a discovery project, can in this way reduce the number of future measurements or studies but still keep the quantitative quality of results.

MATERIALS AND METHODS

The following material is mainly adapted from previous publications[3–5] but is repeated here for the convenience of the reader.

Chemicals

Nicotinic acid (pyridine-3-carboxylic acid) was obtained from Sigma–Aldrich (St. Louis, Missouri) and was dissolved in 0.9% NaCl. All solvents were of analytical grade and the water used in the experiments was obtained from a water purification system (Elgastat Maxima, ELGA, Lane End, UK).

Animals and Surgical Procedures

Male obese Zucker (fa/fa) and normal male Sprague–Dawley rats were purchased from Harlan Laboratories Nederlands B.V. (Horst, the Netherlands) at 7 and 11 weeks of age, respectively, and used at 16 weeks of age. The animals were housed in groups of 5–6 with free access to standard rodent chow (R3; Laktamin AB, Stockholm, Sweden) and tap water. They were kept in climate-controlled facilities at a room temperature of 20°C–22°C and relative humidity of 40%–60% under a 12:12-h light–dark cycle. The study was approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden (EA 100868). Surgery was performed under isoflurane (ForeneQR; Abbott Scandinavia AB, Solna, Sweden) anesthesia and body temperature was maintained at 37°C using a thermoregulated heating pad. Catheters were implanted in the left carotid artery for blood sampling and in the right external jugular vein for drug administration, as previously described.[14] Before cannulation, catheters (IntramedicQR, PE50; Becton, Dickinson and Company, Franklin Lakes, New Jersey) were filled with sterile sodium-citrate solution (20.6 mM sodium-citrate in sterile saline; Pharmaceutical and Analytical R&D, AstraZeneca, Mölndal, Sweden) to prevent clotting. After cannulation, the catheters were exteriorized at the nape of the neck and sealed. After surgery, the rats were housed individually and allowed 5 days to recover before the experiments began.

Experimental Design

The animals were fasted for 14 h before dosing and throughout the experiment to minimize the fluctuations in NEFA caused by food intake. They had free access to drinking water during the length of fast. On the day of experimentation, they were weighed, moved to clean cages, and the venous catheters were connected to infusion pumps (CMA 100; Carnegie Medicin AB, Stockholm, Sweden). Following a 30 min adaptation period, two consecutive arterial blood samples were collected 15 and 5 min before drug administration to determine predose baseline NEFA and NiAc concentrations. Normal Sprague–Dawley rats (weighing 220–367 g) were assigned to 12 groups. Groups 1–8 received an intravenous constant rate infusion for either 30 or 300 min. Four of the eight groups received vehicle (0.9% NaCl, $n = 10$), or 1 ($n = 4$), 5 ($n = 8$) or 20 ($n = 9$) μmol kg^{-1} NiAc over 30 min. The remaining four groups received vehicle ($n = 8$), or 5 ($n = 9$), 10 ($n = 8$) or 51 ($n = 7$) μmol kg^{-1} NiAc over 300 min. Groups 9–12 received oral doses by gavage with vehicle or 24.4, 81.2, or 812 μmol kg^{-1} NiAc ($n = 6$ per group). The concentrations of the dosing solutions were adjusted to give infusion volume flow rates in the range of 0.4–22 μL min^{-1} and oral dosing volume in the range of 1.4–1.6 mL, based on body weight. The dosing solutions were prepared within 30 min of administration by dissolving an appropriate amount of NiAc in saline solution. Among groups 9–12, two groups ($n = 5$ in each) received a constant infusion of NiAc of 5 μmol kg^{-1} for 30 min, followed by a stepwise decrease in infusion rate every 10 min. The last of the two groups was given a 5 μmol kg^{-1} for 30 min at 210 min. Control groups had the same schemes ($n = 1$ in each) but received vehicle. Obese Zucker rats (weighing 473–547 g) were assigned to four groups of which received an intravenous constant rate infusion for either 30 or 300 min. Two groups received vehicle (0.9% NaCl, $n = 2$) or 20 ($n = 8$) μmol kg^{-1} NiAc over 30 min. The other two groups received vehicle ($n = 2$) or 51 ($n = 7$) μmol kg^{-1} NiAc over 300 min.

Multiple arterial blood samples were drawn, 11–15 per rat, for both the 30 and 300 min infusion experiments for analysis of NiAc and NEFA plasma concentrations. The total blood volume removed did not exceed 1.5 mL, and was replaced with an equal volume of sterile sodium-citrate solution to maintain a constant circulatory volume. The control rats received the same volume of infusion solution (vehicle) as the NiAc groups, and all animals were subjected to similar sampling procedures. The blood samples (120 μL each) were collected in EDTA coated polyethylene tubes and kept on ice until centrifuged (10,000g, 5 min, 4°C). The plasma was stored at −20°C pending analysis. The start of infusion was taken as time zero (0 min).

Analytical Assays

Analysis and quantification of NiAc in plasma were performed using liquid chromatography—mass spectrometry. The HPLC system was an Agilent 1100 Series (Hewlett-Packard GmbH, Walbronn, Germany) coupled to an HTC PAL autosampler (CTC Analytics AG, Zwingen, Germany). Plasma samples (50 μL per sample) were precipitated with cold acetonitrile containing 0.2% formic acid (150 μL per sample). After vortex mixing and centrifugation at 4°C (4000g, 20 min), an aliquot of 100 μL of the supernatant was used for analysis. The mobile