VERSITA

Central European Journal of Medicine
DOI: 10.2478/s11536-006-0035-6
Research article
CEJMed 1(4) 2006 348–355

Hypolipidemic effect of a pro-drug containing nicotinic acid in rats

Cristiana Filip[1*], Elena Albu[4], Nastasia Gheorghita[1], Nicolae Ghitler[2], Georgeta Mocanu[3], Mihai Nechifor[1]

[1] *Department of Biochemistry, University of Medicine and Pharmacy, Iasi, Romania*

[2] *Institute of Public Health "M. Ciuca", Iasi, Romania*

[3] *Institute of Macromolecular Chemistry "P. Poni", Iasi, Romania*

[4] *Department of Pharmacology, University of Medicine and Pharmacy, Iasi, Romania*

Received 12 April 2006; accepted 2 October 2006

Abstract: We studied the release of nicotinic acid from a macromolecular pro-drug containing niacin bound to a polymeric support of dextran. Using a modified High-performance Liquid Chromatography (HPLC) method to provide an improved separation between nicotinic acid and its metabolites, we compared the plasma levels of nicotinic acid 24 h after administration of the pro-drug or a similar dose of unbound nicotinic acid to rats. Nicotinic acid exerts a number of pharmacological activities among which is the hypolipidemic effect. To determine the hypolipidemic effect of the pro-drug, we measured the triglyceride levels and examined the correlation with the plasma levels of nicotinic acid.Starting 6 h after administration of the pro-drug, the plasma levels of nicotinic acid were high enough to cause a decrease in the triglyceride level. These results suggest that nicotinic acid was gradually released from the polymeric support, leading to the sustained presence of the active substance and, therefore, a reduction in the level of triglycerides.

Keywords: Nicotinic acid, pro-drug, plasma level, hypolipidemic effects

* E-mail: cfilip2000@yahoo.com

1 Introduction

Hyperlipidemias are an important medical problem and a major cause of atherosclerosis. Because cardiovascular diseases (i.e., heart failure and stroke) are currently the main cause of death in the world, increased attention has been focused on their treatment.

Nicotinic acid (niacin) is used alone or in combination with other drugs for the treatment of atherosclerotic cardiovascular disease [1, 2]. Niacin acid is an important vitamin in humans and animals, and an inadequate dietary supply of it leads to pellagra, a disease involving the skin, gastrointestinal tract, and central nervous system [3]. Nicotinic acid also exerts a number of pharmacological activities at doses higher than the normal daily requirement. Of clinical relevance are its antihypertensive effects at medium doses (100-150 mg/kg weight) and its hypolipidemic effects at high doses (300 mg - 6 g/kg weight) [4]. Because niacin rapidly disappears from the blood, it is administered as a sustained or controlled release formulation. This avoids peak concentrations that can induce side-effects, such as flushing, pruritus, and gastric dyspepsia [5, 6].

We have previously studied a macromolecular pro-drug of nicotinic acid bound to a polymeric support of dextran [7]. In the current study, we examined the release of the active substance (nicotinic acid) from the polymeric support of dextran. We also determined whether the plasma levels of nicotinic acid is sufficient for a hypolipidemic effect. Using HPLC, we compared the plasma levels of nicotinic acid 24 h after administration of the pro-drug and a similar dose of unbound nicotinic acid. To study the hypolipidemic effect of the pro-drug administration, we measured the triglyceride levels and their correlation with the plasma levels of nicotinic acid. We focused on triglycerides because, amongst the lipids, they respond the most rapidly to metabolic changes and because they are considered by many to be an independent risk factor for coronary artery disease [8–11].

2 Statistical methods and Experimental Procedures

2.1 Materials

Nicotinic acid, methanol, and ammonia were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of reagent or HPLC grade. Reagents for the enzymatic determination of triglycerides were obtained from Dow Chemicals (Indianapolis, USA).

2.2 HPLC

We used a Beckman Module 126 HPLC with a Model 166 UV detector and an ODS column (inner diameter, 250 x 4.6 mm; particle size, 5 μm). The mobile phase was a mixture of CH_3OH:NH_3 (100:2) with deionized distilled water (50:50) delivered at a flow rate of 0.8 ml/min and 25°C. Eluted compounds were detected at 220 nm.

2.3 Animals

We used two groups of six adult Wistar male rats (180-220 g). Animals received no food 24 h before drug administration but had free access to water. All experiments were performed according to the standards of the European Legislation for animal in scientific procedure, concerning the care and use of experimental animals.

2.4 Sample preparation

A 0.5 ml sample of plasma was added to 3 ml of 9:1 acetone/HCl in a test tube and shaken for 2 min with a vortex mixer. After centrifugation at 1500xg for 10 min, 3 ml of the supernatant was transferred to a screw-capped test tube containing 3 ml of chloroform and shaken 10 min on the vortex mixer. After centrifugation at 750xg for 5 min, 0.35 ml of the supernatant was acidified with 0.1 ml of 0.1 N HCl [12] and dried. The dried residue was resuspended in 1 ml of absolute methanol. A 0.95-ml aliquot of the resuspended sample was dried under vacuum. The resulting residue was resuspended in 1 ml acetone, and a 0.95 ml sample was collected and dried under vacuum. This residue was dissolved in 200 μl mobile phase and injected into the column.

2.5 Determination of the plasma concentration of nicotinic acid

The plasma concentration of nicotinic acid was measured in two groups of rats.

Group I received free nicotinic acid (300 mg/kg weight) via an endogastric catheter. Group II received an equivalent dose of nicotinic acid as the macromolecular pro-drug. Blood samples were taken from the retroorbitary plexus before the experiment and 1, 2, 3, 4, 6, 12, and 24 h after administration. Plasma levels of nicotinic acid were determined using the above chromatographic method (see “sample preparation” and “HPLC”).

2.6 Determination of triglyceride concentrations

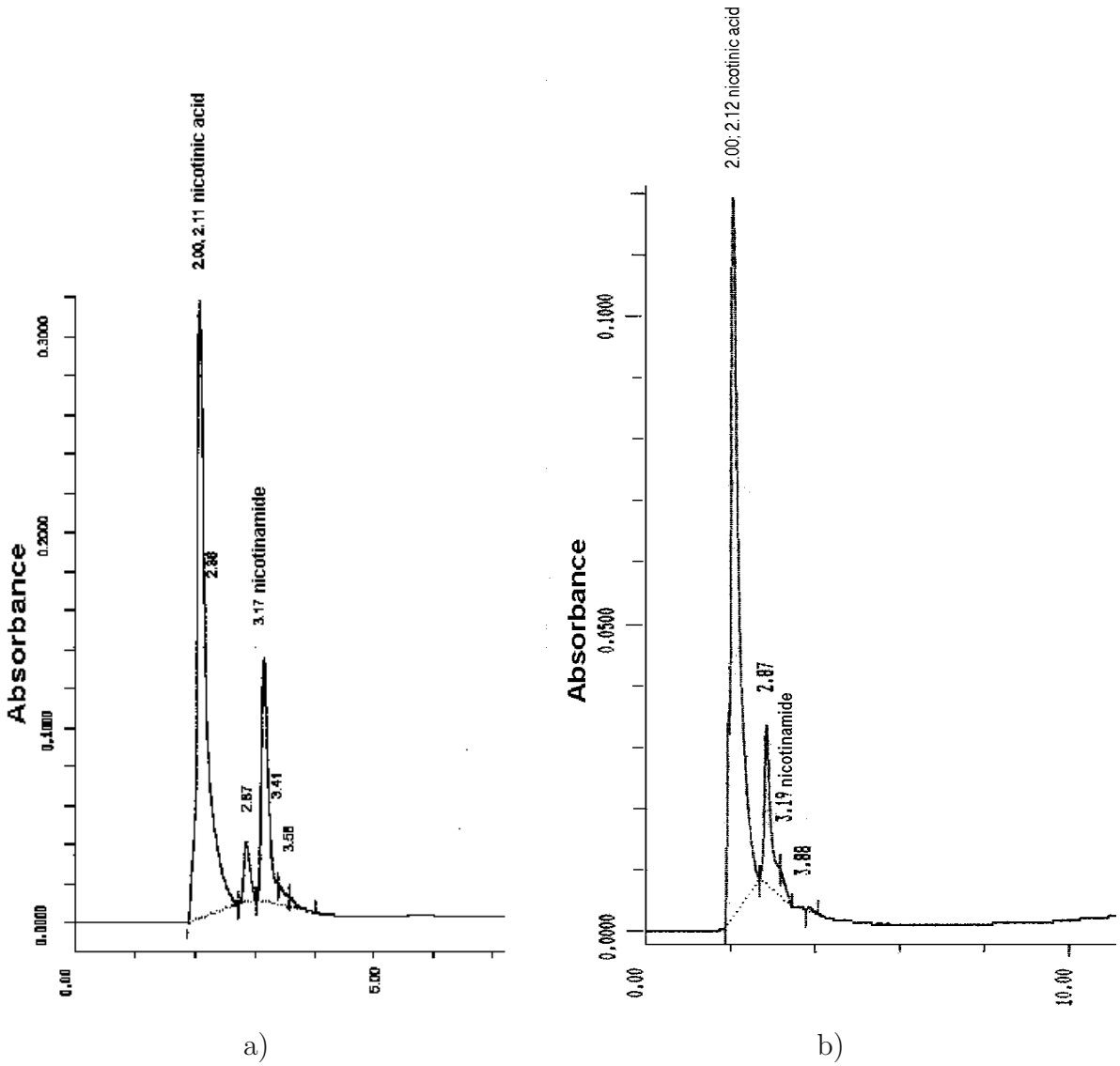
Triglycerides were mobilized by fasting animals for 24 h [13]. Plasma levels were determined initially, after 24 h of fasting and before drug administration, and 24 h after drug administration.

The statistical analysis was performed using SPSS for Windows 13.0.

The triglycerides plasma concentrations for the treatment groups were compared by analysis of variance (ANOVA one-way), and $P<0.05$ was considered to indicate a statistically significant difference.

3 Results

We used HPLC to measure the nicotinic acid concentrations following the administration of both free nicotinic acid and the macromolecular pro-drug in rats (Fig. 1).



a) b)

Fig. 1 Chromatograms of plasma samples obtained from rats that received free nicotinic acid (a) or the macromolecular pro-drug (b).

Nicotinic acid was effectively separated from nicotinamide and other interfering compounds by the HPLC method. The chromatograms for samples obtained from rats that received free nicotinic acid indicate the presence of three compounds: nicotinic acid, nicotinamide, and nicotinuric acid. In contrast, the chromatograms for samples obtained from rats receiving the macromolecular pro-drug indicate the presence of only nicotinic acid and nicotinamide. These results are similar to previous reports showing that the ratio of these compounds depend on the administered dose. Specifically, under basal conditions (i.e., nicotinic acid obtained from food), the main metabolites are N-methyl nicotinamide, N-methyl,4-oxopyridine-3-carboxamide (4-pyridone), and N methyl,6-oxopyridine-3-carboxamide (2-pyridone), and nicotinuric acid is present only in trace amounts. In contrast, under treatment conditions (i.e., continuous administration),

nicotinamide and nicotinuric acid are the main metabolites [14].

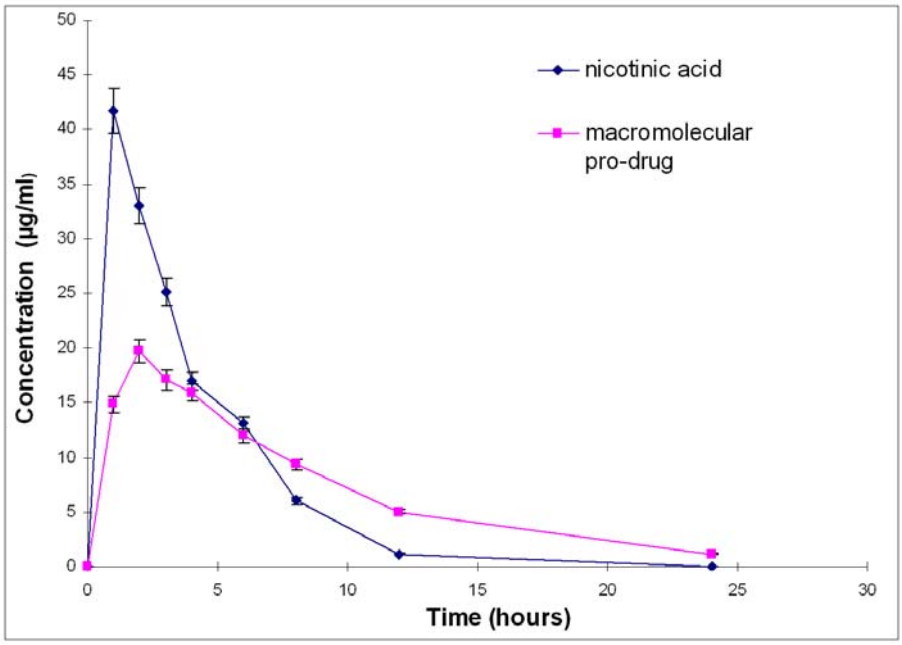


Fig. 2 Time course of nicotinic acid levels in plasma after administration of free nicotinic acid (–♦–) and the macromolecular pro-drug in rats (–■–)

The shape of the elimination curve indicates that, for free nicotinic acid (Group I), the maximum plasma concentration (C_{max}) is reached 1 h after administration (Fig. 2). Between 2 and 6 h after administration, there is a three-fold decrease in the plasma levels of nicotinic acid. This agrees well with previously published results showing that significant amounts of nicotinic acid do not remain in the blood 6 h after administration [15, 16]. Finally, after 24 h, only physiological levels of nicotinic acid are present.

In Group II, which received the macromolecular pro-drug, the plasma nicotinic acid levels reach a maximum 2 h after administration (Fig. 2). The value of the C_{max} is smaller than that obtained by administration of free nicotinic acid. The lower concentrations obtained with the macromolecular pro-drug can be explained by the slower release of the drug from the polymeric support. This may help prevent side-effects. After 3 h, the plasma concentrations of nicotinic acid slowly decreased, confirming its continuous release from the macromolecular pro-drug.

We next examined the plasma triglyceride levels. The plasma triglyceride level was inversely related to the plasma nicotinic acid level. In group I, 2 h after administration of free nicotinic acid, when the plasma level of nicotinic acid reaches the C_{max}, the plasma level of triglycerides was the lowest. Furthermore, as the plasma level of the drug decreases, the triglyceride level increases. Within 24 h, the triglyceride levels nearly returned to the original levels. In group II, the plasma triglyceride level was lower starting 6 h after administration, which corresponds to a satisfactory release of nicotinic acid from the macromolecular pro-drug. As in group I, the triglyceride levels nearly returned to the original levels within 24 h.

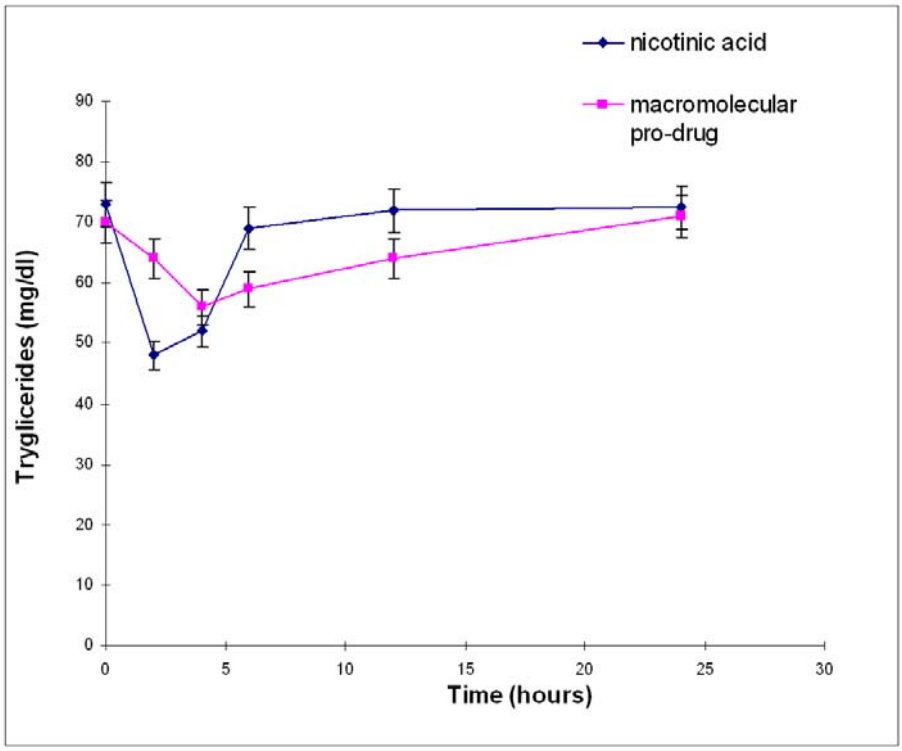


Fig. 3 The plasma level of triglyceride (mg/dl) after administration of free nicotinic acid (–◆–) and macromolecular pro-drug (–■–)

4 Discussion

The plasma decay curves show that in the animals receiving free drug (Group I), nicotinic acid reaches the C_{max} within 1 h. In addition, we found that the hypolipidemic activity was the highest and the plasma level of triglycerides the lowest at this point. Because of the fall of nicotinic acid concentrations, due to its rapid elimination from the body, the plasma level of triglycerides rise and the hypolipidemic activity decreases. After approximately 8 h, the triglyceride levels return to normal.

In rats receiving the macromolecular pro-drug (Group II), the plasma nicotinic acid level reaches the C_{max} 2 h after administration. The C_{max} value was smaller than that obtained for the rats receiving free nicotinic acid. As a result, there is a smaller decrease in the triglyceride level. In the following hours, the plasma level of nicotinic acid decreases very slowly, and 6 h after administration, it is higher than in animals receiving the free drug. In agreement with the prolonged presence of nicotinic acid in the blood, triglycerides levels remain at a reduced level, and hypolipidemic activity is maintained for almost 12 h. There was almost no hypolipidemic activity in either group 24 h after administration of the drugs.

Because this experiment examined only a short period of time, we were unable to study the effect of the macromolecular pro-drug on other atherogenic lipids, such as total LDL-cholesterol or lipoprotein a.

In conclusion, our results suggest that the macromolecular pro-drug provides pro-

longed delivery of nicotinic acid in rats. This reduces the maximum concentration in the blood and ensures prolonged hypolipidemic activity.

References

[1] L.A. Carlson: "Nicotinic acid and other therapies for raising high-density lipoprotein Nicotinic acid", *Curr. Opin. Cardiol.*, Vol. 21, (2006), pp. 336–344.

[2] Y. Lomnicky, M. Friedman, M.H. Luria, I. Raz and A. Hoffman: "The effect of the mode of administration on the hypolipidaemic activity of niacin: continuous gastrointestinal administration of low-dose niacin improves lipid-lowering efficacy in experimentally-induced hyperlipidaemic rats", *J. Pharm. Pharmacol.*, Vol. 50, (1988), pp. 1233–1239.

[3] *The Pharmaceutical Codex*, 11th ed., Pharmaceutical Press, London, 1979.

[4] N. Hengen, V. Seiberth and M. Hengen: "High-performance liquid-chromatoghaphic determination of free nicotinic acid and its metabolite, nicotinuric acid, in plasma and urine", *Clin. Chem.*, Vol. 24, (1978), pp. 1740–1743.

[5] J. Pan, J.T. Van, E. Chan, R.L. Kesala, M. Lin and M.A. Charles: "Extended-release niacin treatment of the atherogenic lipid profile and lipoprotein(a) in diabetes", *Metabolism*, Vol. 51, (2002), pp. 1120–1127.

[6] A. Vogt, U. Kassner, U. Hostalek, E. Steinhagen-Thiessen; NAUTILUS Study Group: "Evaluation of the safety and tolerability of prolonged-release nicotinic acid in a usual care setting: the NAUTILUS study", *Curr. Med. Res. Opin.*, Vol. 22, (2006), pp. 417–425.

[7] G. Mocanu, A. Airinei, and A. Carpov: "Macromolecular drug-conjugates. IV Nicotinic acid and nicotinamide/dextran derivatives", *STP Pharma. Sci.*, Vol. 4, (1994), pp. 287–291.

[8] A.M. Gotto Jr: "Triglyceride as a risk factor for coronary artery disease", *Am. J. Cardiol.*, Vol. 82, (1998), pp. 22Q–25Q.

[9] J. McKenney:" New perspectives on the use of niacin in the treatment of lipid disorders", *Arch. Intern. Med.*, Vol. 164, (2004), pp. 697–705.

[10] H.N. Ginsberg: "Identification and treatment of hypertriglyceridemia as a risk factor for coronary heart disease", *Curr. Cardiol. Rep.*, 1999, Vol. 1, (1999), pp. 233–237.

[11] I. Gouni-Berthold and W. Krone: "Hypertriglyceridemia-why, when and how should it be treated?", *Z. Kardiol.*, Vol. 94, (2005), pp. 731–739.

[12] K. Takikawa, K. Miyazaki and T. Arita: "High-performance liquid chromatographic determination of nicotinic acid and its metabolites, nicotinuric acid and nicotinamide, in plasma", *J. Chromatogr.*, Vol. 233, (1982), pp. 343–348.

[13] L. Saubetin, G. Galli, L. Puglisi, M.G. Ciapponi and R. Paoletti: "Analysis of free nicotinic acid released by a polymeric preparation using a mass fragmentographic technique", *Pharmacol. Res. Commun.*, Vol. 2, (1981), pp. 141–149.

[14] R. Gugler: "Clinical pharmacokinetics of hypolipidaemic drugs", *Clin. Pharmacokinet.*, Vol. 3, (1978), pp. 425–439.

[15] K. Shibata: "Simultaneous Measurement of nicotinic acid and its major metabolite in blood and urine by a reversed-phase high-performance liquid chromatography", *Agric. Biol. Chem.*, Vol. 52, (1988), pp. 2973–2976.

[16] M. Iwaki, T. Ogiso, H. Hayashi, E.T. Lin and L.Z. Benet: "Simultaneous measurement of nicotinic acid and its major metabolite, nicotinuric acid in urine using high-performance liquid chromatography; application of solid-liquid extraction", *J. Chromatogr.*, Vol. 661, (1994), pp. 154–158.