The Pharmacokinetics of Nicotinamide in Humans and Rodents

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Nicotinamide, a derivative of the B vitamin niacin, is currently under trial for the prevention of insulin-dependent diabetes mellitus after success in the NOD mouse. However, the dose, route of administration, and formulation of nicotinamide given to humans is quite different from those used successfully in animals, and the aim of this study was to investigate the plasma pharmacokinetics of oral nicotinamide in humans in two doses and in two different formulations (standard and the long-acting Enduramide). There were no significant differences in the kinetics of the low dose of standard nicotinamide (2.5 mg/kg) and low-dose Enduramide (6.7 mg/kg) in young adult men. Nonlinear kinetics were found with both formulations at higher doses, e.g., a 10-fold increase in the dose of the standard nicotinamide produced a 62-fold increase in the area under the plasma concentration-time curve (AUC). The high dose of standard nicotinamide (25 mg/kg body wt) produced a mean peak plasma concentration 75% higher than that achieved with the sustained release nicotinamide preparation given in a dose similar to that currently used in prevention trials (2 g ≡ 26.6 mg/kg body wt for a 75-kg subject). The AUC was also significantly greater with the standard formulation, indicating a higher bioavailability. Long-term plasma levels for high doses of both formulations were modeled from the single-dose kinetics by computer program. The AUC for standard nicotinamide was 1.7 times higher than that for Enduramide. We conclude that standard nicotinamide offers greater bioavailability than the long-acting formulation tested and that the metabolic clearance pathways of nicotinamide are saturated at the doses currently used in human trials. *Diabetes* 44:152–155, 1995

Insulin-dependent diabetes mellitus (IDDM) is preceded by an asymptomatic prediabetic phase, and advantage has been taken of this opportunity to introduce medications aimed at halting the autoimmune destruction of β-cells. Nicotinamide is a derivative of the B vitamin, niacin. It not only is a weak free-radical scavenger, but also inhibits interleukin-1β–induced nitric oxide synthase (1) and poly(ADP-ribose) synthetase (2) in islets. Poly(ADP-ribose) synthetase is an intracellular enzyme that repairs DNA strand-breaks, utilizing the energy source NAD at the expense of other cell functions. Thioureas such as alloxan cause DNA strand-breaks and deplete NAD stores, and their diabetogenic action is prevented by nicotinamide (3). Nicotinamide at 500 mg/kg body wt also prevents the onset of IDDM in the spontaneously diabetic NOD mouse (4), although the effect is possibly only short-term. Observations in the mouse have led to human studies with nicotinamide, first in the secondary prevention (5,6) and more recently in the primary prevention of IDDM (7,8). Studies in patients with newly onset diabetes have shown only marginal and inconsistent improvements in glucose tolerance (5,6).

Nicotinamide is believed to be essentially nontoxic, but its safety at high doses in humans has been assumed rather than demonstrated, and its pharmacological use in the past has been largely restricted to adults. Nicotinamide is methylated in both humans and rodents to *N'*-methylnicotinamide (9), and the proportion methylated rises with the dose (9). Methyl groups are required in the utilization of protein for growth, but the methylation of nicotinamide takes precedence, and the doses needed to prevent IDDM in the young rat cause growth retardation (11).

In diabetes prevention studies in animals, nicotinamide has been administered to mice as a solution in a daily dose of 500 mg/kg body wt (4). In contrast, it is currently given to humans as a long-acting formulation rather than a solution and in a dose of 1–3 g/day equivalent to 15–45 mg/kg, some 10–33 times less than the dose observed to prevent IDDM in rodents (7).

Little is known of the kinetics of high-dose nicotinamide in humans. The aim of this study was to investigate the plasma pharmacokinetics of nicotinamide in humans in two doses and in two different formulations.

RESEARCH DESIGN AND METHODS

The pharmacokinetics of nicotinamide were studied in eight adult men with an average age of 20 years (range 18–22 years) and an average body weight of 75 kg (range 66–80 kg). All subjects gave written informed consent to the protocol, which had been approved by the local Ethics Committee.

Two different preparations of nicotinamide were studied: pure nicotinamide as a powder (supplied by Ferrosan, Copenhagen), and a sustained release preparation, Enduramide (supplied by Innovite, NJ). Enduramide was in tablet form with each tablet containing 500 mg nicotinamide. The pharmacokinetics of each preparation were studied at both a low dose and a high dose: *1*) low-dose standard nicotinamide (2.5 mg/kg body wt); *2*) high-dose standard nicotinamide (25 mg/kg body wt); *3*) low-dose Enduramide (500 mg ≡ 6.7 mg/kg body wt for a 75-kg subject); and *4*) high-dose Enduramide (2 g ≡ 26.6 mg/kg body wt for a 75-kg subject).

The different doses and different preparations of nicotinamide were studied in each volunteer on four separate occasions, each dose being separated by at least 1 week. The dose of low-dose Enduramide used was determined by the manufacturer's tablet size.

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IDDM, insulin-dependent diabetes mellitus; AUC, area under the plasma concentration-time curve.

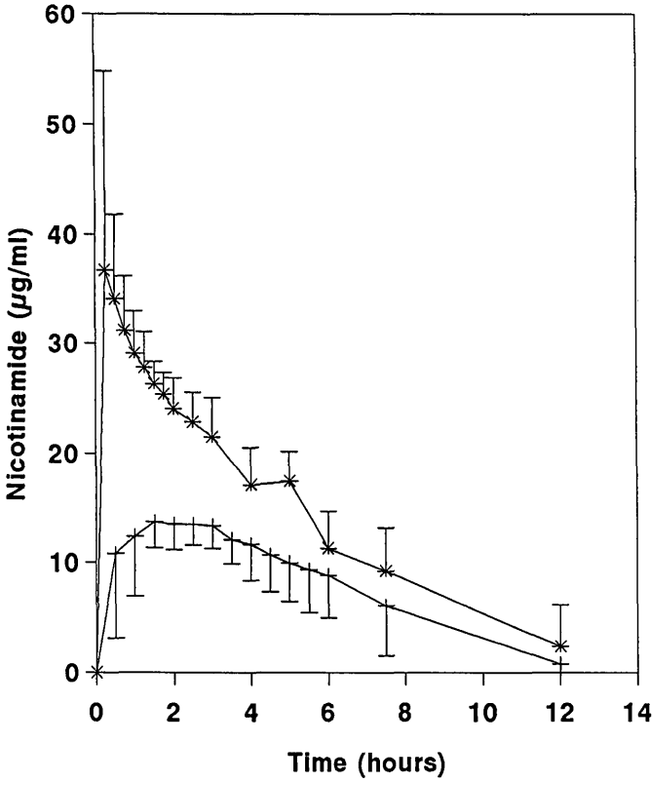

FIG. 1. The pharmacokinetics of standard nicotinamide (25 mg/kg body wt) and a sustained release formulation (2 g Enduramide, equivalent to 26.6 mg/kg body wt) after oral administration to eight young adult men of 75 kg mean body weight (*, standard nicotinamide; +, Enduramide).

After an overnight fast, each subject was given a single oral dose of nicotinamide, either dissolved in 30 ml water for the standard nicotinamide or swallowed with water for the sustained release preparation. Subjects remained semirecumbent and continued to fast for a period of 4 h after which time a light lunch was eaten. Blood samples (8 ml) were withdrawn from a venous cannula into heparinized tubes before the dose of nicotinamide and subsequently every 15 min for 2 h, then at 2.5, 3, 4, 6, 7.5, and 12 h for the standard nicotinamide and every half hour until 6 h, then at 7.5 and 12 h for the Enduramide formulation. The samples were centrifuged, and the plasma was stored at −20°C until the assay.

The plasma samples were analyzed for nicotinamide using the reverse-phase high-performance liquid chromatography method of De Vries et al. (12). The method involved the addition of the internal standard (5 g isonicotinamide) to blank plasma, to plasma standards (containing 0–10 μg nicotinamide), and to samples. The samples and standards (0.2, 0.5, or 1.0 ml depending on dose given) were diluted to 2 ml with water and passed through an activated Sep-Pak C cartridge (Waters). After washing with water (0.5 ml), the components were eluted from the cartridge with 1.5 ml chromatographic mobile phase. After centrifugation for 2 min, an aliquot (150-μl) was injected onto the column. Peaks of nicotinamide and internal standard were detected at 254 nm and their areas were integrated. The ratio of nicotinamide to isonicotinamide was linear over the range 0–10 g/sample. Samples were analyzed in duplicate, and the values for each sample were routinely within ±3% of the calculated mean.

The maximum plasma concentration of nicotinamide (C_{max}) and the time to C_{max} (t_{max}) were the observed values. The terminal half-life of the different nicotinamide formulations and doses was derived by least-squares regression analysis applied to the post-peak, log-linear part of the plasma concentration-time curve. The area under the plasma concentration-time curve (AUC) was determined by the linear trapezoidal method, with extrapolation to infinity by dividing the last measurable plasma concentration by the terminal slope. Long-term plasma levels for the high-dose regimen of both formulations were modeled from their single-dose kinetics by computer program (SIPHAR).

Statistical analysis. Analysis of all data was by Student's t test for paired or unpaired data as appropriate.

RESULTS

The standard nicotinamide was absorbed rapidly after doses of 2.5 and 25 mg/kg (Figs. 1, 2 and Table 1). At both doses, the plasma concentration-time curves were fitted by a simple monoexponential decrease, but the 10-fold increase in dose resulted in a significant increase in plasma terminal half-life ($P < 0.001$). The 10-fold increase in dose gave a 13-fold increase in mean peak plasma level, from 3.3 to 42.1 mg/l ($P < 0.001$), but a 62-fold increase in the AUC ($P < 0.001$), which indicates considerable nonlinearity in the pharmacokinetics.

Enduramide was absorbed more slowly than the standard nicotinamide (Figs. 1 and 2 and Table 1). A 4-fold increase in dose of Enduramide did not significantly change the half-life, but resulted in an 8-fold increase in C_{max} and a 24-fold increase in AUC ($P < 0.001$). Thus, the AUC values for high doses of both Enduramide and standard nicotinamide were sixfold higher than predicted from linear extrapolation of the corresponding low dose.

The two preparations of high-dose nicotinamide were administered in similar doses so that it was possible to directly compare their absorption (Fig. 1). The high-dose standard nicotinamide gave a 75% higher mean peak plasma concentration ($P < 0.05$), was absorbed more rapidly ($P < 0.05$), and resulted in a higher AUC ($P < 0.01$) than high-dose Enduramide (Table 1). However, there was no significant difference in their plasma terminal half-lives. Figure 2 shows corresponding concentration-time curves of the low-dose

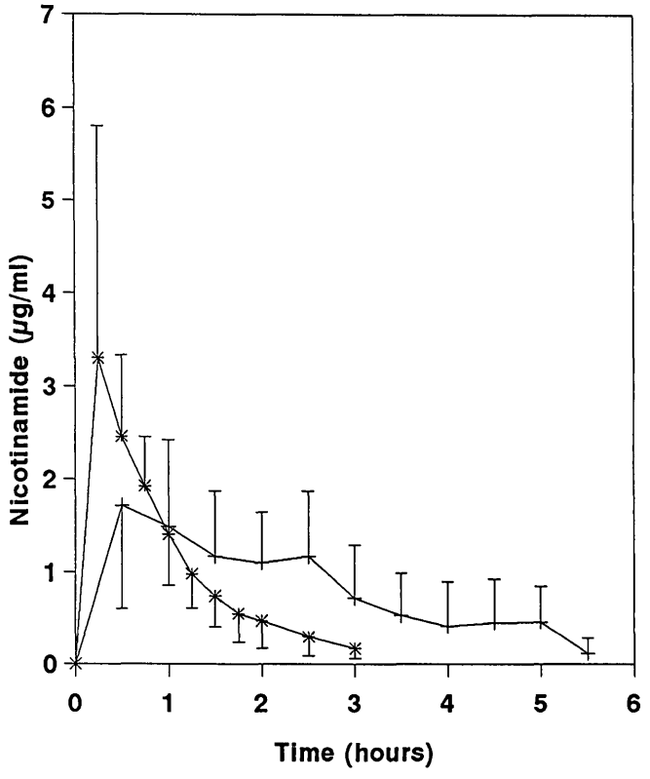

FIG. 2. The pharmacokinetics of standard nicotinamide (2.5 mg/kg body wt) and a sustained release formulation (500 g Enduramide, equivalent to 6.7 mg/kg body wt) after oral administration to eight young male adults of 75 kg mean body weight (*, standard nicotinamide; +, Enduramide).

TABLE 1
Summary of the mean C_{max}, t_{max}, the AUC, and plasma half-life observed in humans given two different preparations of nicotinamide orally in two different doses

Dose	C_{max} (g/ml)	t_{max} (hs)	AUC ($g \cdot ml^{-1} \cdot h^{-1}$)	Plasma half-life (hs)
Low standard	3.3 ± 2.6	0.3 ± 0.1	3.0 ± 1.4	0.6 ± 0.2
Low Enduramide	2.1 ± 0.7	1.0 ± 0.8	4.5 ± 2.2	1.0 ± 0.5
High standard	42.1 ± 15.2	0.5 ± 0.3	187.1 ± 51.8	3.5 ± 1.0
High Enduramide	16.2 ± 3.5*	1.9 ± 1.2*	107.0 ± 41.1†	2.7 ± 1.6

Data are means ± SD for eight volunteers. * $P < 0.05$, ‡ $P < 0.01$ compared with standard nicotinamide.

preparations of nicotinamide. Although the sustained release preparation was at a higher dose than standard nicotinamide, there was no difference in AUC or half-time between the low-dose Enduramide (6.7 mg/kg) and standard nicotinamide (2.5 mg/kg).

Computer-modeled data on long-term plasma levels using the high dose of both formulations of nicotinamide are shown in Fig. 3. The peak levels obtained from the standard formulation were 2.2 times higher than those attributable to the Enduramide, and the AUC was 1.7 times higher.

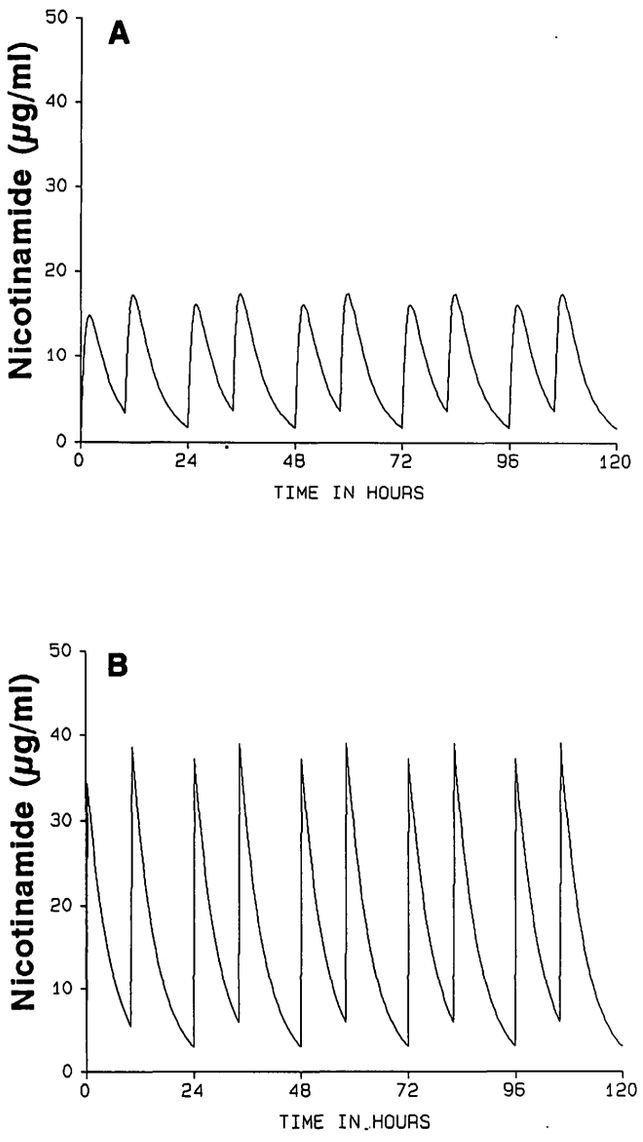

FIG. 3. *A*: high-dose Enduramide and *B*: high-dose nicotinamide. Predicted plasma concentrations of nicotinamide modeled on single-dose data assuming twice-daily administration at 10- and 14-h intervals.

DISCUSSION

The pharmacokinetics of sustained release preparations of nicotinamide have not previously been compared with those of standard nicotinamide.

Our data show very different kinetics between high- and low-dose nicotinamide and between standard and long-acting preparations. As anticipated, the peak plasma concentrations and AUC observed with the high dose were greater than those that resulted from the low dose of each formulation. After oral administration, the AUC for a drug depends on dose, bioavailability, and clearance. For first-order (linear) kinetics, both bioavailability and clearance are constant, and therefore the AUC is directly proportional to the dose. In the case of nicotinamide, both high doses gave disproportionately (six times) higher AUC compared with those predicted by the corresponding low dose, which indicates either increased bioavailability or decreased clearance at the higher dose. The plasma half-life for standard nicotinamide was much greater at the high dose (3.5 h compared with 0.6 h), which is consistent with saturation of elimination (for example, due to cofactor depletion) and a dose-dependent decrease in clearance. The same was true for Enduramide, although the variation was greater.

The plasma terminal half-lives of the two preparations at the high dose were similar, but the peak plasma concentration and the AUC attributable to the long-acting preparation were lower. These kinetic data indicate a lower bioavailability of the long-acting nicotinamide, probably due to poor absorption compared with the standard drug. There seems little rationale, given these data for high doses of nicotinamide, to use the more expensive long-acting formulation in clinical trials. The larger tablets, which are more difficult to swallow by children, pose an additional problem with Enduramide.

One potential concern with the use of nicotinamide to prevent IDDM is toxicity. The doses shown to prevent IDDM in the NOD mouse cause growth inhibition in the rat (10,11). At these doses, the excretion of nicotinamide by methylation to *N*-methylnicotinamide is achieved at the expense of other crucial methyl-requiring pathways, which include protein anabolism (13). The clearance pathways for nicotinamide in humans are the same as those for the rodent (9), and if the nonlinear kinetics of nicotinamide found in humans at doses of 25 mg/kg arise from depletion of *S*-adenosylmethionine (the methyl donor), as seems likely, the effects on protein anabolism described earlier may be expected at this dose.

Nicotinamide was introduced into human trials because it

was believed to be both safe and effective at high dose. Little, however, was known of its pharmacokinetics at high dose in humans (14). Comparison of the dose used in humans and in the NOD mouse, in which 500 mg/kg body wt of nicotinamide are needed to prevent IDDM, questions whether the plasma concentrations and AUC achieved in humans are sufficient to prevent IDDM. Conversely, if the exposure to nicotinamide that prevents IDDM in the NOD mouse were achieved in humans, it seems likely to prove toxic. Of course, serum levels of nicotinamide may not reflect concentrations inside the β-cell. Evidence from animal studies indicates active transport of nicotinamide into islets (15), so that it remains uncertain what connection exists between plasma and tissue (β-cell) levels.

Extrapolation from animal models to humans is, however, the cornerstone of drug development and safety evaluation. If nicotinamide is to be widely used for trials in humans, the data from humans presented here need to be supplemented with more detailed investigation of its metabolism at high dose. There certainly seems to be no justification on the basis of present knowledge to routinely prescribe high doses of nicotinamide for prevention of IDDM. It is particularly important to learn more about the metabolic basis of the saturation kinetics of the drug, which profoundly affect the clearance of nicotinamide at the doses currently used in clinical trials. It is also important to compare the growth of children recruited into these trials with carefully matched control subjects, rather than historical growth standards, which are largely out of date.

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