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PHARMACOKINETICS AND TOLERANCE OF NICOTINAMIDE COMBINED WITH RADIATION THERAPY IN PATIENTS WITH GLIOBLASTOMA MULTIFORME

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The pharmacokinetic properties of nicotinamide and its tolerance were studied in seven patients affected by glioblastoma multiforme and treated with two fractions per day of radiation therapy. Nicotinamide was given orally at two daily doses of 4 g and then 2 g separated by a 6-h-interval. The treatment was well tolerated in almost all patients and had no effect on blood pressure, cardiac rhythm or body temperature. Pharmacokinetic analysis showed peak plasma levels (C_{max}) above 100 mg/l 45 minutes after the administration of both doses. This was followed by a biexponential decay of plasma concentrations with a thermal half life of 9.4 h. Tumours were irradiated 1 hour after each drug dose to match with drug C_{max} in plasma, and although it is too early to evaluate the tumour response, the drug levels achieved should be sufficient to improve radiation therapy.

The positive results obtained recently with radiation sensitizers (Dahanca trials) (1, 2) provide additional evidence of the importance of hypoxia as a cause of failure in the treatment of cancer by radiotherapy. In animal models there are some arguments showing that hypoxia is a combination of acute (3) and chronic hypoxia (4, 5) and while there are many different treatments that can specifically reduce the number of chronic hypoxic cells during irradiation, like hyperthermia (6, 7), perfluorochemical emulsions (8, 9) and carbogen or oxygen breathing (10–12), the only agent that appears to influence acute hypoxia is nicotinamide which prevents the development of acute hypoxia by reducing the transient fluctuations in blood flow (13–15).

Nicotinamide has also been widely used in the past for treatment of various diseases including pellagra, diabetes mellitus, granuloma annulare, psoriasis, schizophrenia and daily doses of up to 12 g have been given orally for prolonged periods of time with low toxicity (16–20).

The aim of our present was to evaluate the pharmacokinetics and tolerance of nicotinamide in patients with glioblastoma multiforme, so as to optimize the timing of drug administration and radiation treatment in cancer patients given the combined treatment.

Material and Method

Design of the study. Between January and September 1992, 9 patients who gave informed consent (the protocol was accepted by a local ethical committee), age (24–70 years), with glioblastoma multiforme, after previous debulking surgery, with haemathological and biochemical analysis in the normal range (complete blood counts, blood chemistry profile, urinalysis, liver function studies), and a Karnofsky index of 60 to 100, received radiation therapy with curative intent. The following accelerated fractionation schedule was chosen: 1.5 Gy per fraction, two times/day Monday through Friday up to a total dose of 60 Gy. Nicotinamide was used in combination with radiation therapy and its pharmacokinetic properties were investigated. The drug was supplied as a powder (Roche

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Prodotti Farmaceutici S.p.A., Milano, Italia) and prepared, as 1 g capsules. It was administered orally with 200–300 ml of water after at least 3 h of fasting in the morning and in the afternoon of the days of irradiation and continued for the entire course of therapy. The first dose of 4 g was given 1 h before the first radiation fraction (1.5 Gy) and the second dose 2 g was given 1 h before the second radiation fraction (1.5 Gy). The daily radiation dose was 3 Gy and the total amount of drug was 6 g. The study of the pharmacokinetic properties was based on venous blood samples collected up to 24 h after the drug ingestion, and drug plasma levels were measured using the high pressure liquid chromatography (HPLC) method reported by Horsman et al. (21). On day 1, blood samples were taken at 15, 30, 45, 60 min, 3 and 6 h after the first administration of nicotinamide (4 g) and repeated with the same timing after the second drug administration (2 g). The last sample was taken 24 h after the first dose and before the second day of therapy.

Additional samples were collected from 4 patients immediatley before the first morning dose, and 1 h after the morning and afternoon doses for 5 consecutive days and each Monday of the 2nd, 3rd, 4th week of treatment before the morning dose. Haematological and biochemical analysis were performed at regular intervals (every week), and cardiac rhythm, blood pressure and temperature were registered in each subject before and up to 3 h after ingestion of the drug.

HPLC analysis of nicotinamide plasma concentration. An indwelling intravenous catheter was inserted to facilitate blood sampling. Plasma was immediately separated with centrifugation and stored at −20°C until analysis. The high-pressure liquid chromatographic analysis described by Horsman et al. (21) was followed, with minor modifications. The column was changed to Spherisorb S5 ODS1 (150 × 4.6 mm e.d.; Phase Separations Ltd, Deeside, England) and the chromatographic system was a Gilson (Villiers le Bel, France) equipped with model 116 variable wavelength absorbance detector set at 254 mm.

Pharmcokinetic calculations. The peak concentration in plasma (C_{max}) and time to peak concentration (t_{max}) were obtained by inspection of the plasma concentration-time curves. The elimination rat constant (k_{el}) was calculated by use of linear regression of the terminal linear portion of the log concentration-time curve. Terminal elimination half life ($t_{1/2\beta}$) was calculated as $0.693/k_{el}$, while $t_{1/2\alpha}$ was obtained as $0.693/\alpha$ were α is the slope of the initial (distribution) phase. The area under the plasma concentration-time curve (AUC) for nicotinamide was calculated by use of linear trapezoidal rule from time zero to 24 h. Other pharmacokinetic parameters, included clearance, MRT (mean residence time: the mean time during which the drug resides in the body after the administration), volume of distribution (Vd) and absorption rate-constant (K_{abs}), were calculated as reported by Gibaldi & Perrier (22).

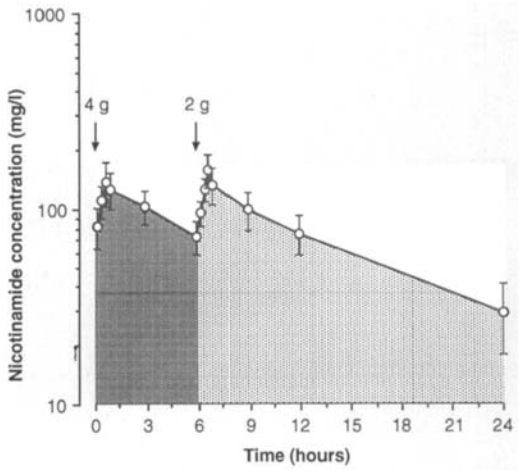

Fig. 1. Semilogarithmic plot of plasma concentration vs. time of nicotinamide after two oral doses (arrows) at 10:00 (4 g) and 16:00 h (2 g) to 7 patients. Vertical bars indicate standard error of the mean. Shaded areas indicate the AUC after the first and second drug dose.

Results

The plasma concentration vs. time profile of nicotinamide obtained during the first 24 h after two administrations is shown in Fig. 1. Peak plasma concentration was reached 45 min after the first ingestion of 4 g and following the second dose of 2 g.

The pharmacokinetic profile indicates a rapid absorption phase, after the first dose with a C_{max} of 138.4 ($\pm$ 16.2) mg/l. After the second ingestion the C_{max} was achieved rapidly and its mean value ($\pm$ S.E.) was 157 $\pm$ 19.1 mg/l (Fig. 1 and Table). Twenty-four hours after the first ingestion of nicotinamide, the mean drug plasma concentration was 30 mg/l (Fig. 1). The pharma-

Table

*Summary of mean plasma pharmacokinetic parameters of nicotinamide**

	Dose A	Dose B
C_{max} (mg/l)	138.4	157.0
t_{max} (h)	0.75	6.75
$t_{1/2\alpha}$ (h)	6.33	0.44
$t_{1/2\beta}$ (h)		9.40
k_{el} (1/h)	0.11	0.18
$AUC_{0\to6}$ (h · mg/l)	560.10	
$AUC_{6\to24}$ (h · mg/l)		1502.00
$AUC_{0\to00}$ (h · mg/l)	1233.00	1572.00
Clearance (l/h)	3.24	1.27
Vd (l)	29.65	17.29
MRT (h)	9.39	19.24
k_{abs} (1/h)	4.06	1.57
$t_{1/2abs}$ (h)	0.17	0.44

* S.E. was less than 15% of the mean value.
Dose A: nicotinamide 4 g p.o., h 10:00.
Dose B: nicotinamide 2 g p.o., h 16:00.

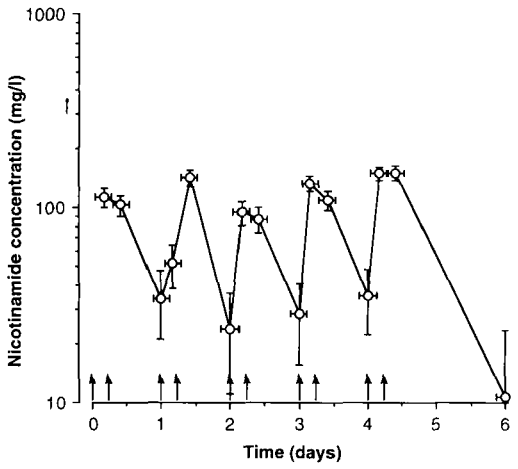

Fig. 2. Semilogarithmic plot of plasma concentration vs. time of nicotinamide after muliple oral doses (4 and 2 g, arrows) at 10:00 and 16:00 h of each day up to day 4 to 4 patients. Horizontal and vertical bars indicate the standard error of the mean.

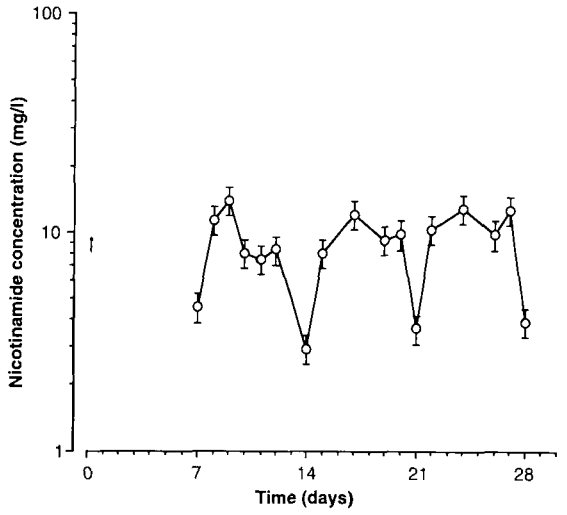

Fig. 3. Semilogarithmic plot of plasma concentration vs. time of nicotinamide after multiple oral doses (4 and 2 g) at 10:00 and 16:00 h of each day to 6 patients. Plasma was drawn before the morning dose of nicotinamide. Note the low plasma concentrations on each Monday (day 7, 14, 21 and 28) 56 h after the last drug administration (Friday). Vertical bars indicate the standard error of the mean.

cokinetic parameters are summarised in the Table. The examination of the pharmacokinetic data shows that after the second drug dose there is a rapid drop in plasma concentration, followed by a second slower elimination phase. It is therefore possible to calculate two different half-lives after the second daily drug administration: the $t_{1/2\alpha}$, was 0.44 h, while the $t_{1/2\beta}$, was 9.4 h (Table). The Table also shows that the drug absorption is rapid: the k_{el} absorption rate-constant has a value of 4.06 h^{-1} after the first dose and is lower (1.57 h^{-1}) after the second.

In the 4 patients monitored daily for 5 consecutive days (Fig. 2), the measurement of drug concentrations did not show nicotinamide accumulation in plasma. With the exception of one time point, the peak drug levels, reached each day over a 5-day period, showed a reduced variation.

The tolerance to nicotinamide was usually good, with minor side-effects and the patients showed only slight or medium transient symptoms, such as headache, nausea and temporary, reversible, elevation of transaminases: the AST-GOT from 45 to 60 U/L (normal range under 45 U/L) and the AST-GPT between 50 and 80 (normal range under 45) and the GAMMA-GT around 90 U/L (normal range under 50 U/L). Difficulties in swallowing the capsules, was sometimes found simply because of their large size. Only one patient developed repeated episodes of severe nausea and vomiting and the nicotinamide treatment was interrupted, while radiation therapy continued without major related symptoms. In another case the patient refused to take the drug (the view of the capsules after a few ingestions caused nausea) and the treatment was discontinued.

In these last two cases, the pharmacokinetic study was interrupted and the patients were evaluable for drug-induced adverse reactions.

Discussion

In the current study we administered a total of 6 g of nicotinamide to patients with glioblastoma multiforme. This dose was chosen because previous studies did not show significant toxicity following its administration to healthy volunteers (21, 23). Furthermore, the daily treatment of nicotinamide was divided in two different doses of 4 and 2 g separated by a time interval of 6 h, and combined with an accelerated fractionation schedule of 2 fractions of 1.5 Gy/day, five times a week up to a total dose of 60 Gy.

After the first oral ingestion, the absorption of nicotinamide was rapid and the peak plasma concentration was achieved after 45 min (Fig. 1). Following the second ingestion of the drug (2 g) the peak plasma concentration was achieved after 45 min and reached a mean level of 157 mg/l comparable to that seen after the first dose (138.4 mg/l). The drug concentration before the morning dose demonstrated that there was no significant accumulation of nicotinamide after repeated treatments during the following days and at the beginning of each week of therapy. After the second daily dose of the drug a rapid drop of the plasma level concentration occurred, followed by a second slower phase of elimination; the curve thus assumes the features of a biexponential decay as in two-compartment model for distribution and elimination even if the plasma concentration vs. time points was only five.

On the basis of the volume of distribution it can be concluded that the drug is widely distributed in the body fluids and tissue. In addition to this the AUC values indicate a large exposition of the tissues to the drug.

In the past there have been few pharmacokinetic studies on nicotinamide in man. De Vries et al. (24) reported peak plasma concentrations of 5 ng/ml within 30 min after oral ingestion of 0.2 g of drug and an elimination half-life of 1 h. More recently Stratford et al. (23) and Horsman et al. (21) published pharmacokinetic data in healthy human volunteers following oral administration of escalating single doses of nicotinamide up to 6 g. Both studies found that peak plasma concentration and terminal half-life were increased with dose taken, and at 6 g the peak plasma concentration was above 100 mg/l and $t_{1/2\alpha}$ of approximately 9 h. Horsman et al. also found that the t_{max} was within 45 min, while in the study of Stratford et al. (23) it occurred unusually later (48–180 min), probably because in the former study the subjects fasted prior to taking the drug, but in the latter study did not. Our results are therefore entirely consistent with those of Horsman et al. (21). Horsman et al. have also shown that for maximal radiosensitisation by nicotinamide in mouse tumours, peak plasma levels above 100 mg/l are required. These levels were easily reached in our studies with both the 4 g dose and subsequent 2 g supplement. Thus, although it is too early to evaluate the tumour response it can be concluded that the drug plasma levels at the time of irradiation were in the optimum range.

As already described there was an acceptable toxicity: only one patient presented severe nausea and vomiting after taking the drug, which could be ascribed to nicotinamide itself. While another patient showed psychological distress at taking nicotinamide; the cause of that could be referred to the number and size of the capsules rather than the drug itself and a previous better selection could have avoided this problem in this specific case. In the other patients, the mild nausea and sometimes the transient episodes of vomiting, were easily managed with the usual antiemetics and did not represent a major side-effect.

In the patients who showed a slight elevation of serum transaminases without other symptoms, it was not possible to ascribe that phenomenon to nicotinamide because they were also taking, simultaneously, drugs like fenobarbital, which was required to prevent the epilectic crisis due to the disease and related treatments; the liver function test (as previously reported) increased after 15–20 days of therapy and fenobarbital, as a precautionary measure, was interrupted and replaced by another medicine while nicotinamide was continued. The transaminases slowly decreased in a few weeks after the end of the treatment.

The results of the present study show that nicotinamide can be safely used as a potential radiation sensitizer in patients with cancer. Furthermore, the total dose chosen appears to be reasonable and the schedule used allowed a combination with unconventional fractionation. We do not know yet if the dose investigated (6 g divided into two doses) is sufficient for clinical use and if the dose can be further increased safely. Looking at previously available data (14, 15, 25) which show the effectiveness of nicotinamide in affecting acute tumor hypoxia, the drug seems to be a useful radiosensitizing agent as suggested by Denekamp et al. (26).

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