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Rates of intestinal absorption of molybdenum in humans

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Abstract

The intestinal absorption of molybdenum in healthy human volunteers has been measured by simultaneous oral and intravenous administration of the stable isotopes ^{95}Mo and ^{96}Mo, and the results were analysed using the convolution integral technique. The results showed that molybdenum ingested in liquid form was rapidly and totally absorbed into the circulation under ordinary intake regimes. The rates and extent of absorption were lower for composite meals, and also for increasing levels of administration. This information can be helpful in the application of the new ICRP model of the human alimentary tract.

Keywords: Molybdenum; Humans; Stable isotopes; Human alimentary tract model; Intestinal absorption

1. Introduction

The knowledge of the rates and extent to which radioactive materials are absorbed through the human gut wall into the systemic circulation is crucial for the reliable evaluation of the internal dose after ingestion (Roth et al., 1998). For many elements of radiological importance, no specific and detailed information on this process are available, and therefore, generic parameters are used in the recommended models, which might lead to incorrect dose estimates.

Techniques enabling the use of stable isotopes as tracers represent a valid and ethically justifiable tool for conducting repeated biokinetic investigations in healthy human volunteers. In contrast to radioactive tracers, they are free from radiation risk, however, both careful experimental design and thorough data analysis are required. Measurements of stable isotopes are indeed limited to assays of biological fluids and excreta, and the techniques required are usually more complex and less sensitive than those used with radiotracers. In recent years, stable isotope techniques have been developed and applied to the study of the biokinetics of molybdenum (Cantone et al., 1995, 1997a,b; Giussani et al., 1995, 1998a; Werner et al., 1998, 2000). Molybdenum biokinetics is of interest for radiological protection, mainly due to the use of ^{99}Mo as ^{99m}Tc generator in medical applications, and also for nutrition, since it is an essential oligo-element (Sardesai, 1993). Molybdenum is primarily accumulated in the human body due to ingestion. The reported intake values range in average around 100–200 μg Mo d^{-1}, with significant regional variations between different countries, and intakes as high as 10–15 mg Mo d^{-1} have been also observed (Hamilton and Minski, 1972/73; Huisingh and Matrone, 1976; Tsongas et al., 1980; Chatt et al., 1988; Becker and Kumpulainen, 1991; Holzinger et al., 1998).

Initial studies on three healthy volunteers (Cantone et al., 1995, 1997a,b; Giussani et al., 1995), combined with the results of similar investigations by Turnlund et al. (1995a,b, 1999), led to the proposal of possible revisions to the biokinetic model for molybdenum recommended by ICRP (ICRP, 1993; Giussani et al., 1998b, 2000). These preliminary analyses also identified factors that required better characterization, particularly with regard to the mechanisms that regulate the absorption and excretion and total body content of molybdenum in the case of increased

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intake of the element. The proposed revised model, therefore, served as a starting point for the design of a second series of investigations, involving additional volunteers, with the aim of assessing how the intestinal absorption, systemic kinetics and urinary excretion are influenced by the amount and form of administration. Preliminary results from these new studies have already been published (Giussani et al., 1998a; Werner et al., 1998, 2000).

This paper presents an analysis on the intestinal absorption of molybdenum and on its rate, based on that subset of investigations for which measurements of tracer concentrations in blood plasma are available. The analysis was performed applying the convolution integral technique to the experimental data (Sparacino et al., 2001). The reason for using this kind of analysis is that no a priori assumptions concerning the kinetics governing the passage, or on the structure of the model are needed, and artificial biases in the obtained results are thus avoided.

2. Materials and methods

2.1. Tracer kinetic investigations and determination of tracers' concentrations in blood plasma

Tracer kinetic investigations were conducted at GSF, National Research Center for Environment and Health, using stable isotopes by a double tracer technique involving the simultaneous intravenous injection of one isotopic tracer and the oral administration of another isotope. Isotopic solutions were prepared using metal powders enriched in ^{95}Mo and ^{96}Mo, respectively, purchased from Chemotrade, Düsseldorf, Germany (isotopic enrichment approx. 95%). The solutions used for intravenous injections were appropriately filtered and sterilized. The concentrations of the solutions were checked by combined ICPMS and activation measurements.

The investigations were started in the morning, with each subject having fasted overnight. Informed consent was given by the volunteers before each investigation. Injections were performed, immediately before the oral administration, into a vein in the arm opposite to that used for blood sampling. After 2 h, the subjects were allowed to consume a standard continental breakfast, consisting of black coffee and 2 bread rolls with butter and jam. After administration, blood and urine samples were collected following appropriate time schedules over a period of at least 6 h. A time interval of at least 2 months was observed between different investigations conducted on the same person.

This work concentrates on a subset of 23 investigations on 7 volunteers (3 males, 4 females, ages ranging from 28 to 59, mean 46 ± 10 years), for which the tracer concentrations in blood plasma samples were determined using proton activation analysis. The activation and other analytical procedures have been discussed in detail elsewhere (Molho et al., 1993; Cantone et al., 1995).

As indicated in the introduction, the new series of studies was focussed on the modifications of the biokinetics of molybdenum at increased intake levels. In the subset of investigations considered here, the oral tracer was administered in liquid form, dissolved in 100 ml water or in 100 ml black tea, and also as a marker of composite meals (vegetables or an homogenised infant formula) in amounts varying from 0.5 to 5 mg, comparable or greater to the average daily intake. In most of the studies, the oral load was accompanied by the injection of about 0.35 mg Mo taken from the sterile solutions.

2.2. Data analysis

Fig. 1 shows a typical example of the results obtained. The plasma clearance of the injected tracer (0.35 mg total Mo) may be described by a multi-exponential function, whereas the concentration of the oral tracer (0.53 mg total Mo) shows a fast rise and then a slower descent, asymptotically assuming the behaviour of the curve of the injected tracer.

The convolution integral technique was used for the analysis of the curves that describe the behaviour of the tracers in blood plasma. The concentration per unit intake of the oral tracer at time t, expressed by $G(t)$, is linked to the plasma clearance per unit intake of the injected tracer $F(t)$ by the following convolution integral:

$$G(t) = B(t) \otimes F(t) = \int_0^t B(\tau) \cdot F(t-\tau)\,\mathrm{d}\tau, \qquad (1)$$

where $B(t)$ is the rate of entry of the oral tracer into blood plasma at time t, and τ is the variable of integration. The symbols $\cdot$ and $\otimes$ indicate the common product and the convolution product, respectively.

For each study, proper analytical expressions of $F(t)$ and $G(t)$ were looked for. Since the convolution integral

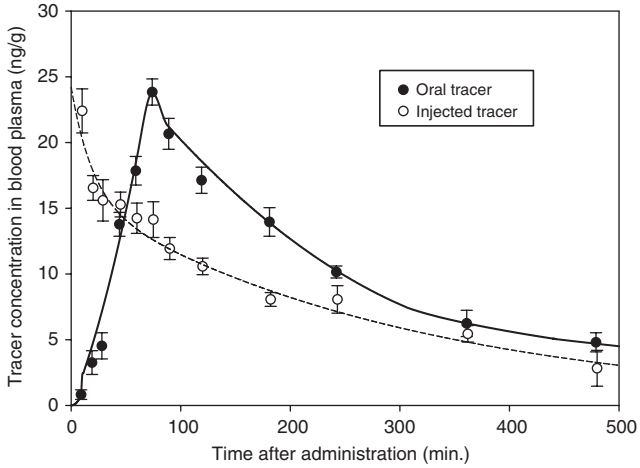

Fig. 1. Concentration in blood plasma of oral tracer ^{96}Mo and injected tracer ^{95}Mo for volunteer #7 (female) following intravenous injection of 0.35 mg molybdenum and oral administration of 0.53 mg molybdenum in 100 ml water. Error bars represent the experimental uncertainties. The lines represent the fit to the experimental data, as explained in the text.

technique does not require a priori model or kinetic assumptions, those functions were chosen that could be better fitted to the experimental data. A bi-exponential function was generally used for $F(t)$. In contrast, no simple analytical expression was found, that would describe satisfactorily the patterns of the oral tracer over the whole duration of the investigation. Data for each study were therefore divided into overlapping subsets each consisting in general of 4–7 data points, and a different polynomial function was fitted to the data in each subset. The polynomial functions relating to one investigation were then combined by applying an appropriate smoothing procedure in order to avoid discontinuities in the form of $G(t)$. For a few investigations only the oral tracer was administered, and the pattern for the injected tracer was derived from the results of other investigations in the same subject.

The bi-exponential and the polynomial functions were fitted to the respective data points using the fitting algorithm of the program SigmaPlot (SSI, Richmond, CA, USA). The combination of the polynomial functions in order to obtain $G(t)$ was performed using a dedicated code developed by the authors. The lines in Fig. 1 represent the functions $F(t)$ and $G(t)$ obtained with the procedure described above.

Deconvolution of Eq. (1), by applying the Laplace transform operator, yields $B(t)$:

$$\mathbf{L}(G(t)) = \mathbf{L}(B(t) \otimes F(t)) = \mathbf{L}(B(t)) \cdot \mathbf{L}(F(t)). \tag{2}$$

Thus

$$\mathbf{L}(B(t)) = \frac{\mathbf{L}(G(t))}{\mathbf{L}(F(t))}. \tag{3}$$

The inverse Laplace transform of Eq. (3) would provide the expression of $B(t)$. In the present analysis, however, no simple analytical expression of $G(t)$ was available. Therefore, a numerical solution of Eq. (1) was preferred. First of all, finite time intervals Δt were considered, and the integral expression in Eq. (1) substituted with a sum

$$G(k \cdot \Delta t) = \sum_{i=0}^{k} B(i \cdot \Delta t) \cdot F((k-i) \cdot \Delta t) \cdot \Delta t,$$
$$k = 0, 1, 2, \ldots, \tag{4}$$

where F and G were calculated at discrete time points. By considering a time interval of $\Delta t = 1$ min, it was possible to obtain the value of $B(0)$ from the expression of Eq. (4) for $k = 0$

$$G(0) = B(0) \cdot F(0) \Rightarrow B(0) = \frac{G(0)}{F(0)}. \tag{5}$$

For $k = 1$,

$$G(1) = B(0) \cdot F(1) + B(1) \cdot F(0) \Rightarrow$$
$$B(1) = \frac{G(1) - B(0) \cdot F(1)}{F(0)}. \tag{6}$$

By iteration of this procedure it was thus possible to build the whole vector $B(i)$, i.e. to obtain a numerical expression for $B(t)$. The iterations were made using a self-developed code, and they were conducted only up to times corresponding to the duration of the studies, since the expressions of the functions $F(t)$ and $G(t)$ were based only on the experimental data, and extrapolation at later times is not justifiable.

If $B(t)$ is the function describing the rate of intestinal absorption of the oral tracer, the fraction absorbed up to time T (duration of the investigation) is given by

$$f_T = \int_0^T B(t)\mathrm{d}t \tag{7}$$

or alternatively by

$$f_T = \sum_{j=1}^{n} B(j \cdot \Delta t) \cdot \Delta t \tag{8}$$

with $n = T/\Delta t$.

3. Results and discussion

Fig. 2 shows the absorption rates ($B(t)$ functions) obtained in 6 investigations after administration of 0.5 mg molybdenum dissolved in 100 ml water. The irregular shapes of the functions are a consequence of the numerical approach and of the methodology chosen for the definition of $G(t)$. The absorption into the systemic circulation was relatively rapid. The transfer rate functions peaked between 10 and 70 min, and for all volunteers but #3 (female) the greatest part of the absorption took place in the first 80 min. The differences observed are mainly ascribable to physiological inter-individual variations and, to a lesser extent, also to the sampling schedule. With such a rapid process, the timing of sample withdrawal may indeed play a significant role in the characterization of the transfer rate functions, in particular during the rapidly

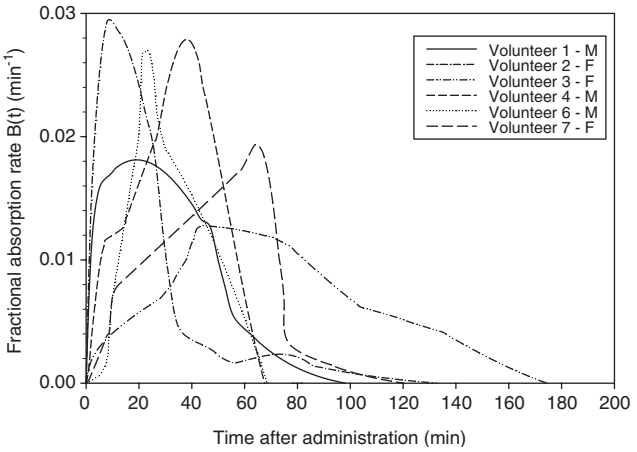

Fig. 2. Fractional rate of absorption $B(t)$ for six investigations in 6 subjects, 3 females (designated with F in the legend) and 3 males (designated with M in the legend), after administration of approx. 0.5 mg molybdenum in 100 ml water.

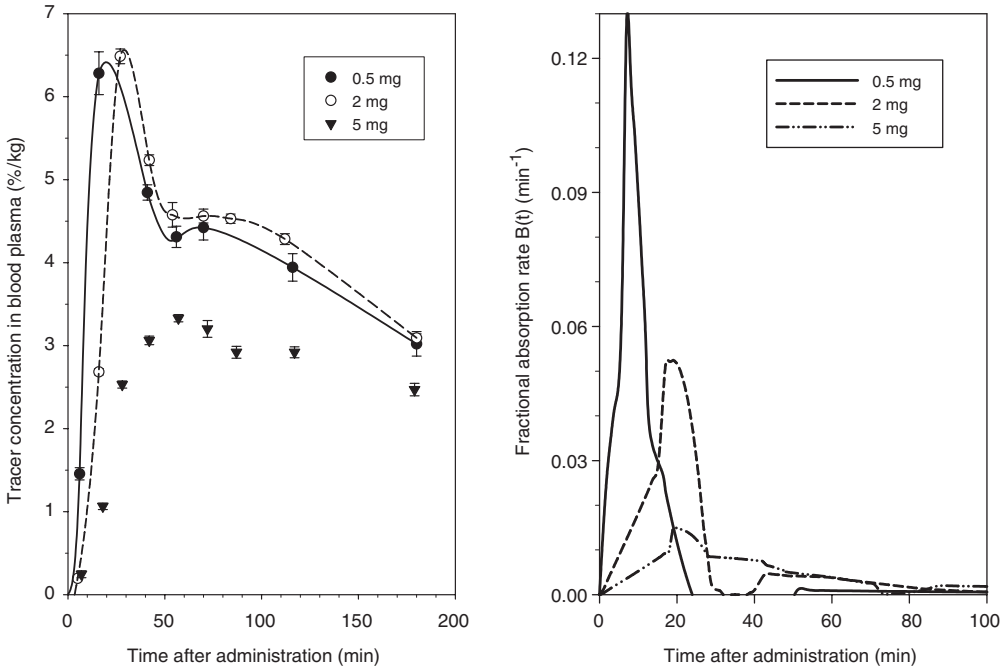

Fig. 3. The left panel shows the concentrations in blood plasma of the oral tracer following administration in various forms to volunteer #5 (female). For the sake of comparison, data are expressed as percentage of the intake per unit mass of blood plasma, and lines have been drawn to indicate the different rising patterns for the two investigations with lower levels of administration. The right panel shows the corresponding fractional rates of absorption $B(t)$.

rising phase. This can be better explained observing Fig. 3. The left panel shows the tracer concentration in blood plasma as measured in 3 different investigations conducted on volunteer #5 (female) by administration of different amounts of molybdenum dissolved in 100 ml water. The right panel shows the corresponding $B(t)$ curves. It is interesting to note that the tracer data for the two studies with lower levels of administration (0.5 and 2 mg molybdenum, respectively) show a similar pattern, however the slight difference in the rising part of the curve determines a significant difference in the forms of the $B(t)$ functions. A precise and detailed definition of the initial part of the $B(t)$ curve would therefore require frequent measurements at very short times after ingestion, but this is limited by ethical considerations related to the conduct of volunteer studies and practical considerations regarding the number and modality of sampling.

In spite of the differences in the form of the transfer rate functions presented in Fig. 2, important common features could be deduced. Firstly, the values of the absorbed fraction, calculated from Eq. (8) were very similar for all studies: the f_T estimates were distributed around the central value 0.98 with a standard deviation of 0.11. These estimates can be considered as a very good approximation of the total absorbed fraction (corresponding to $T \to \infty$), since the studies were conducted for a period T that was much longer than the duration of the absorption process.

Fig. 3 shows that at increasing level of administration the absorption pattern was protracted over a longer time, and also the total absorbed fraction was lower. Similar results were found also for the other subjects, and are summarized in Fig. 4. The absorbed fraction for all investigations is given as a function of the oral load (expressed per unit of body weight) and of the form in which the element was administered.

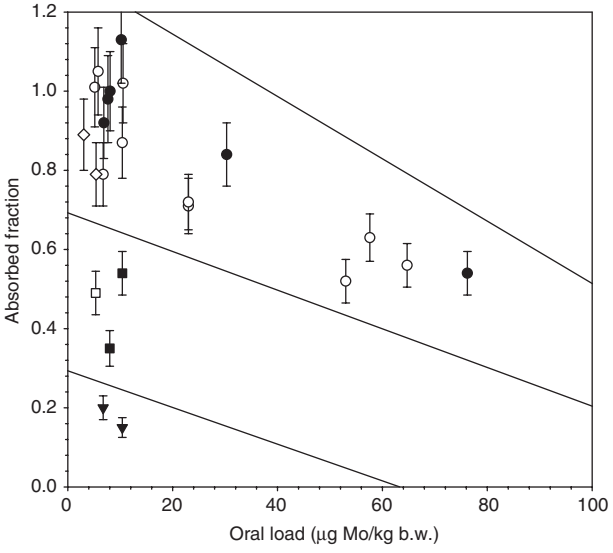

Fig. 4. The variation of the absorbed fraction with form and amount of the administered molybdenum. Circles: molybdenum dissolved in 100 ml water; squares: together with a composite meal; diamonds: intrinsic tracer of cress; triangles: dissolved in 100 ml black tea. Full symbols designate female volunteers, open symbols designate male volunteers (b.w. = body weight).

For a better interpretation of the results, the graph area has been divided into three regions, representing

administration of molybdenum in liquid form, with composite meals and with black tea, respectively. No differences were observed between the results from males (empty symbols) and females (full symbols). For intakes in liquid form lower than 20 μg molybdenum per kg body weight the data are in agreement with the value of $f_1 = 1$ assumed in ICRP Publication 67 for the fractional absorption of molybdenum (ICRP, 1993). For higher intake regimes or for administration with solid foods the absorbed fraction is significantly reduced, and also delayed. However, in all investigations the uptake was completed well within the duration of the study. Interesting is that the administration of black tea dramatically affected the absorption, reducing it at least of a factor 5. The influence of the form of administration on the uptake is further illustrated by the data in Figs. 5 and 6 which compare the absorption rate curves as obtained in the investigations with aqueous solutions and solid meals conducted on volunteers #1 (male) and #3 (female), respectively.

The reduction and the delay of the entry rate of molybdenum for increasing levels of administration in liquid form might be explained by considering that absorption through the gut walls is a saturable process; alternatively, a regulatory mechanism may exist which tends to control the body burden of molybdenum by affecting its intestinal absorption when the level of systemic molybdenum is too high. The slower absorption from composite meals than from liquids may be explained considering the differences in the characteristic times of gastric emptying and intestinal transit (Malagelada et al., 1984; ICRP, 2002). However absorption of molybdenum from other sites than the upper part of the alimentary tract seems to be negligible. The reduced absorption for administration with meals is ascribable to a lower bioavailability of molybdenum due to interactions with components of the foods; the dramatic effect of black tea is probably due to the presence of phenolic compounds (tannins), which are known to be powerful inhibitors of gut uptake (Disler et al., 1975; Manach et al., 2004).

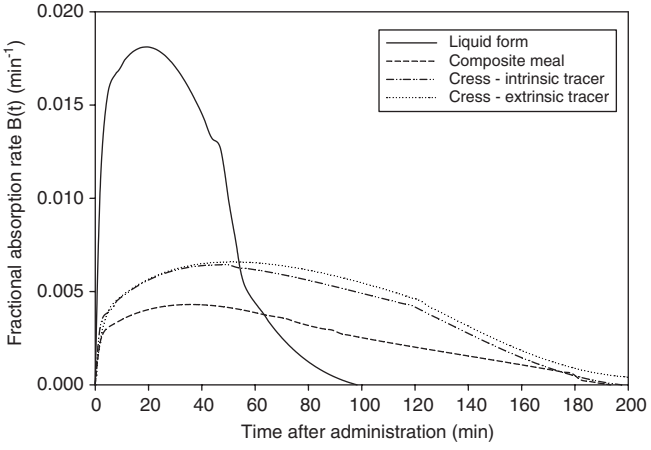

Fig. 5. The variation of the fractional rate of absorption $B(t)$ following administration in various forms to volunteer #1 (male).

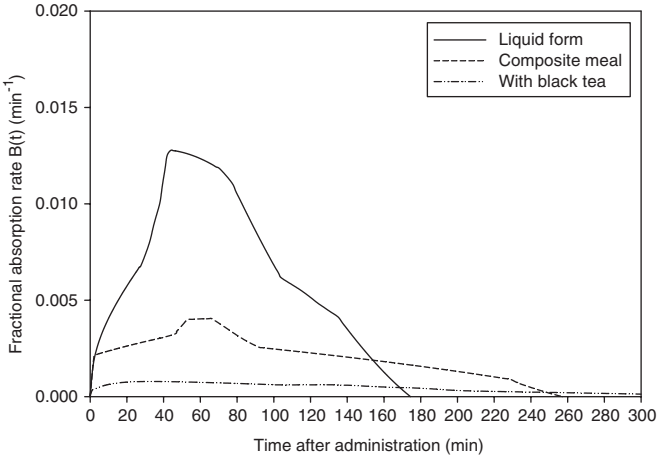

Fig. 6. The variation of the fractional rate of absorption $B(t)$ following administration in various forms to volunteer #3 (female).

The results thus confirm that, depending on the form of intake, separate sets of parameters are required for describing the biokinetics of ingested materials, as is actually foreseen in the new ICRP Model of the Human Alimentary Tract (Metivier, 2003; Harrison et al., 2005) which is going to replace the ICRP Publication 30 model (ICRP, 1979). They also indicate that uptake of molybdenum cannot be easily described as a first-order kinetics process, and that probably a more complicated structure would be required, as the model should also account for non-linearities in the equations governing entry into the systemic circulation. It should be observed that also other authors (Thompson et al., 1996) suggested the need to use intake-dependent parameter values to describe the biokinetics of molybdenum. These details, of great interest in the fields of physiology and nutrition, may be however too specific and will introduce undesirable complexities in the model application to radiation protection purposes, where intakes with very high concentrations and masses of molybdenum would be a very unlikely scenario.

4. Conclusions

In this work, interesting characteristics of the passage of molybdenum isotopes through the gut walls were investigated by using stable tracers and the integral convolution technique. The results showed that molybdenum is rapidly and efficiently absorbed into the systemic circulation, when it is administered in liquid form. The rate of absorption is lower and delayed for composite meals, and also for increasing levels of administration in liquid form. The information obtained may prove useful for the application of the new ICRP HAT Model to radionuclides of molybdenum.

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