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Short Research Article

Use of stable isotope-labelled thyroid hormone to monitor the thyroid metabolism†

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Introduction

Former thyroid clinical studies involving radioactive molecules (^{131}I) exhibit potential hazards for human health and induce ethical problems. The use of stable isotope-labelled molecules allows a safe and precise method for monitoring thyroid hormone metabolism *in vivo*. We summarize the current state of our research using this method. Our main objectives are the production of labelled thyroid hormones and the development of the analytical protocols for their quantification in biological fluid (serum).

A first implementation by *in vivo* tests of [$^{13}C_9$]T4-like metabolic tracer was carried out under veterinary control on cat and rabbit. The thyroxine follow-up was realized by GC–MS and was based on double isotopic dilution with two isotopomers of the same hormone labelled with carbon 13. The present work shows the results obtained and discusses the limitations and possibilities of this high-tech methodology. The data demonstrate that the use of stable isotopes provides a valuable tool for monitoring thyroid hormone metabolism.

Materials and methods (ID-GC–MS)

We have synthesized a thyroxine hormone with two different isotopic enrichments. The biological tracer was thyroxine substituted on the inner ring with six stable isotopes of carbon or on the inner ring and lateral chain with nine stable isotopes of carbon ([$^{13}C_6$]thyroxine or T4* and [$^{13}C_9$]thyroxine or T4IS) (Figure 1). The first stable isotope was the form used as the pharmacological agent; the second isotope was used as the internal standard for the measurements. Analytical detection was based on gas chromatography coupled with mass spectrometry (single ion detection).

Two animals (one New Zealand White rabbit and one female domestic short-haired cat) were administered T4* 100 μg, once at time 0, immediately following blood collection. Blood collection (6 ml in plain tubes) was repeated at various intervals (time 0, 4 and 8 h and 1, 2, 4, 8 and 16 days).

The data presented here show that the use of stable isotopes provides a valuable tool to monitor thyroid hormone metabolism. Within a few hours of administering the stable isotope, both animals became hyperthyroxinemic, with serum total thyroxine (i.e. T4 and T4*) concentration reaching values at least ten times greater than the initial serum T4 value (before administration of stable isotope).

Results and discussion

In these two animals, there was an abrupt decrease of endogenous serum T4 (Figures 2 and 3), as reflected by the decrease of its serum concentration. While it is not possible to determine whether this is due to a decrease of thyroid synthesis or an increase of thyroid hormone catabolism, hyperthyroid states are known to induce a marked increase of deiodinase activity in peripheral tissues, mainly in the liver. We propose, then, the following scheme compatible with damped oscillations of endogenous T4:

- The first peak is likely to be associated with a displacement of endogenous T4 from the extracel-

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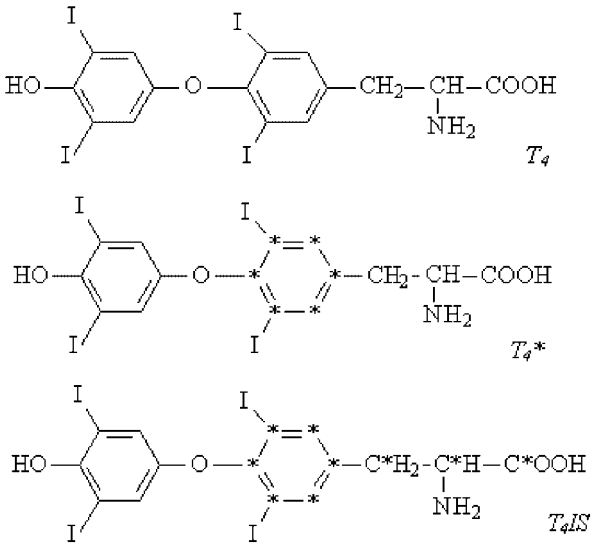

Figure 1 T4, T4* and T4IS structure (* denotes ^{13}C).

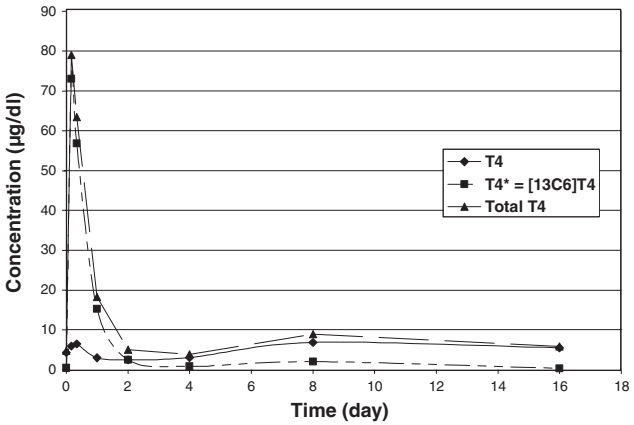

Figure 3 Endogenous and exogenous thyroxine follow-up on one New Zealand White rabbit after the administration of 100 µg T4* by GC–MS-SIM using [$^{13}C_9$]thyroxine as internal standard.[1]

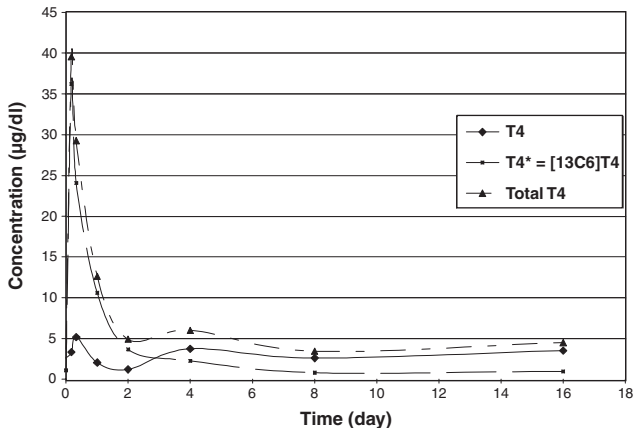

Figure 2 Endogenous and exogenous thyroxine follow-up on one female domestic short-haired cat after the administration of 100 µg T4* by GC–MS-SIM using [$^{13}C_9$]thyroxine as internal standard.[1]

lular compartment to the blood compartment, this displacement being involved by the large amount of exogenous T4 administered as stable isotope.

- After exogenous T4 administration, an increase in deiodinase activity, due to hyperthyroxinemia, involves, first, an increase of deiodinase 1 activity, and a drop of endogenous serum T4 when the animal is hyperthyroid.[2,3]
- In a second step, intratissular mechanisms exert a feedback mechanism on deiodinase activity, the activity of which decreases, resulting in increasing concentrations of endogenous serum T4. We have no hypothesis, at the moment, for this second-step feed-back mechanism.
- Points 2 and 3 repeated, until normal physiological serum T4 is attained.

Damped oscillations of serum thyroid hormone levels have been previously described in neonates.[4,5]

During the following days, as serum T4* is rapidly cleared and as T4 catabolism is increased, there is a transient state of hypothyroidism (low serum total thyroxine concentration), followed by a second wave of hyperthyroxinemia/hypothyroxinemia cycle.

This profile is typically the one described in ondulatory mechanics, and it reflects a quite efficient mechanism of homeostasis of serum T4 around a biological haemostat—the physiological serum T4 concentration. This aspect of wave response for dosing T4* was not analysable with radioactive tracers, as classical immunoassays do not distinguish between radioactive and cold hormones. The stable isotope methodology offers an interesting solution to this problem.

This first study is limited in the number of animal experiments and the possibilities of exploitation of these results in the field of the metabolic study. Our major aim was, in this work, to *demonstrate the potential of the technology based on the double isotopic dilution with two isotopomers for thyroid hormone metabolic study* for humans and animals. Some improvements in the analytical equipment and protocols will allow the T3 and T4 serum concentrations to be followed (for instance, the use of HPLC-MS/MS technique).

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