Quantitative Investigations on the Diaplacental Transfer of Thallium by Field Desorption Mass Spectrometry *

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Z. Naturforsch. **35 c**, 902–906 (1980); received August 8, 1980

Thallium, Placental Transfer, Ultratrace Determination, Mass Spectrometry, Field Desorption

The placental transfer of thallium cations in pregnant mice was investigated by determining the thallium concentrations in fetal and maternal tissue 0.5 to 24 h after application of thallium. The maternal dose of thallium was 8 mg/kg body weight throughout. Uterus and fetus were found not to differ from other organs like heart and liver in time course and magnitude of thallium uptake with an initial surge during the first few hours of exposure to thallium and a rapid decrease to steady 12 and 24 h values somewhat lower than those found in the kidney. Diaplacental transfer is therefore assumed comparatively rapid and a specific placental barrier for thallium does not seem to exist. For the determination of thallium concentrations Field Desorption Mass Spectrometry was utilized as a reliable, fast, and sensitive method for the analysis of metal cations in biological material. This method does not require extensive pretreatment of the tissue and total sample amounts in the range of milligrams and less are sufficient for quantitative analysis.

Introduction

It has recently been shown that Field Desorption (FD) Mass Spectrometry (MS) can be used for ultratrace determination of metal cations directly from very small amounts of biological samples [1–5]. The use of this technique for quantitative analysis of metal cations from tissue samples is favored by two reasons: First, ultratrace determinations down to a range of ppb and ppt [6] are possible from smallest samples on the emitter (≤ 1 µg). Secondly, tissue samples require no pretreatment other than homogenizing the cell fractions such that it is possible to load the sample onto the FD – emitter by a syringe technique. Moreover, the determination of elements with more than one stable isotope is feasible within an acceptable error range for medical problems using the stable isotope dilution technique [5, 7]. Determinations of cesium and thallium directly from animal tissue were the first applications of this method for trace metal analysis in biological sciences [1, 2, 8]. We were thus able to study the pharmacokinetics of thallium in the first hours after administration of thallium to test animals [5].

After applying single doses of thallium it was established that a first wave of thallium is swept into the organs very rapidly (1 to 2 h after poisoning). This does not apply to the brain, however, which showed a dose dependent barrier for thallium [5]. In the other organs a rapid wash-out then takes place within the first four hours depending on the function of the kidneys as the first organ of thallium excretion. This first wave of thallium in the initial period after poisoning is possibly determining for later lethal effects. It is well known that thallium enters the cells in exchange for potassium ions [9] due to their similar ionic radii and same electric charge. One can conclude that the first few h of acute poisoning are decisive for the mechanism of the toxic effects observed.

In studies on the cardiovascular apparatus Ku and coworkers [10, 11] reported a transient positive inotropic effect of thallium which coincides with our own observations (unpublished). This they supposed to be due to an inhibition of the sodium – potassium exchange pump (similar to the action of cardioglycosides) and to an accumulation of thallium inside the cell. Enhanced intracellular thallium levels by a factor of 45 in comparison to the external medium was also reported by Lameijer and van Zwieten [12] who, however, observed only a rapid onset of hypotensive and negative chronotropic effects as the result of acute cardiovascular toxicity of thallous ions.

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* Quantitative Field Desorption Mass Spectrometry: Part XXI; for Part XX see ref. [21].

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0341-0382/80/1100-0902 $ 01.00/0

During the methodological development of FD-MS as an analytical tool for metal trace analysis, preliminary studies [4] have shown that thallium is likely to cause fetal deformities. From this work and the considerable environmental problems involved with thallium(I)-salts the question therefore arose whether thallium ions actually enter fetal organs when maternal animals were contaminated with thallium due to diaplacental transfer. Investigations on a thallium poisoned cat showed that thallium had entered the fetus [13]. The authors reported that the thallium intoxication led to abortion in the pregnant cat. On determining thallium concentrations in different organs of the cat and in one fetus using polarography similar thallium concentrations in maternal and fetal tissue were found. Fetal lung and heart levels were comparatively enhanced. While earlier investigations of the placental transfer of thallium using ^{201}Tl in rats by Gibson and Becker [14] showed a 30fold higher thallium content in maternal blood compared to fetal blood, the study of Fitzek and Henning [13] gave evidence that thallium passes through the placenta easily. Earlier investigations in animals [15] also showed a marked placental transfer of thallium, when thallium was given on day 18 to 19 of pregnancy. Thallium application on day 20 of gestation of rats however showed thallium concentrations much lower in the fetus than in the maternal animals during the initial 32 min period [14]. Single doses of 20 mg/kg thallium sulphate at the 13th day given to rats were thought to be mostly retained by the placenta, when fetal liver and brain thallium contents were measured by atomic absorption spectrometry 12 and 48 h after application (about 10 ppm were found in fetal liver [16]). From these results Sabbioni and Manzo [16] suggested that the passage of thallium across the rat placenta would be a rather slow process.

Methods

Pregnant laboratory mice from the international standard strain SWS were used. The mice were fed with 8 mg/kg thallium at the 9th day after conception. In all experiments the mice were fed by a stomach tube with a thallium solution containing 8 mg/kg weight of the mouse. This Tl^+ solution was prepared from Tl_2SO_4 (Merck, Darmstadt). The volume was varied according to the weights of the mice. The mice were killed 30 min to 24 h after thallium feeding. Nauseated animals were disregarded. Immediately after death brain, kidney, and uterus with all fetal parts were carefully removed. The fetal part of uterus preparations was found to be about 90% of the preparation. The organs were rinsed in 0.9% NaCl solution, weighed after removal of superficial fluid, and deep-frozen at −18 °C. Before the determination of thallium by FD-MS, the thawed organs were homogenized manually in glass homogenators after addition of 1 ml isopropylalcohol and 0.25 to 1 ml 10^{-4} M isotopically enriched Tl^+ solution (Rohstoff Einfuhr GmbH, Düsseldorf) [2]. Deep freezing and isopropylalcohol were used to ensure that the cells and protein structures were highly distorted during homogenizing. The concentration of the enriched thallium solution was in the order of the thallium concentration expected in the sample. The homogenate was centrifuged for 3 min at 5000 rpm. Several microliters of the more or less clear fluid were then loaded onto the emitter by microsyringe. The mass spectrometric measurements were performed on a simple, home-built single focussing spectrometer with a mono FD ion source. For control all quantitative experiments were repeated by a different operator on a double focussing, commercially available Varian MAT 731 instrument. Electric detection was performed operating the secondary electron multiplier at −2 kV. The ion source potentials were +8 kV for the field anode and −3 kV for the slotted counter electrode. For integrated accumulation of FD ions a multichannel analyzer of type Tracor Northern NS-570A was used, which was triggered by the cyclic magnetic scan of the mass spectrometer. This procedure and experimental details in order to improve the signal/noise ratio and to avoid the detrimental influence of fluctuations in the ion currents produced have been reported previously [17, 18]. For all measurements the standard, high temperature activated carbon FD emitters with a 10 µm tungsten core were employed. Direct heating of the emitter wire was achieved by raising the emitter heating current linearly by a programmable heating unit up to 70 mA. If the corresponding temperature is not sufficient for desorption of other metal cations such as Cu^+, Cr^+, and Ba^+ indirect heating with an argon ion laser, Spectra Physics model 166 can be performed in addition [19, 20].

Kidney and brain samples of the maternal animals were used as a reference for the uptake of thallium by fetal tissue. The idea was to compare the thallium concentrations in fetal tissue to an organ with a high thallium uptake such as the maternal kidney on the one hand and to an organ with low thallium content such as the brain on the other.

Results and Discussion

Fig. 1 shows two characteristic FD recordings of the thallium isotopes 203 and 205 obtained from kidney and uterus samples treated according to the methods described above. The ratios of the peaks heights determine the thallium concentration and are employed to calculate the pharmacokinetic data.

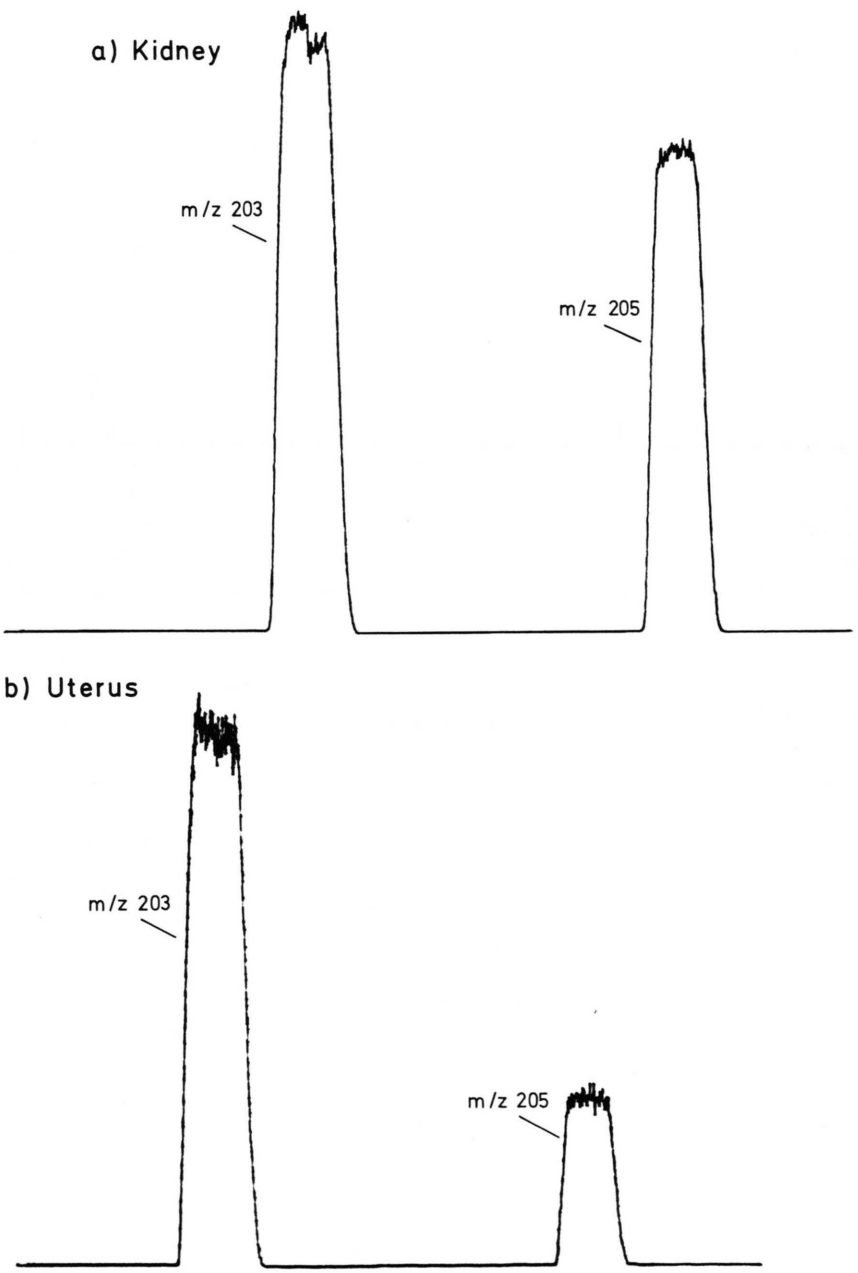

Fig. 1. a) Thallium determination in kidney tissue of a pregnant mouse. The original plot shows the results obtained by using FD-MS and the stable isotope dilution technique for internal calibration. Accumulated were 62 repetitive magnetic scans in the emitter heating current programm between 40 and 50 mA. The integrated ion currents of the Tl^+ cations corresponded to a recorded number of counts in the 62K range of the multichannel analyzer. The concentration of thallium was found to be $1.26 \cdot 10^{-4}$ mol/l (see Fig. 2). b) Thallium determination of an uterus including the fetus of a test animal which was sacrificed three h after the dosage of the heavy metal sulphate. Altogether 30 repetitive magnetic scans were recorded between 30 and 45 mA emitter heating current (range 32 K). The concentration of thallium was found to be $2.9 \cdot 10^{-5}$ mol/l. From the observed signal to noise ratio in this plot it can clearly be derived that the detection limit of the FD method in this complex matrix is a few orders of magnitude lower. The amount of thallium actually consumed in one FD measurement lies in the nanogram range.

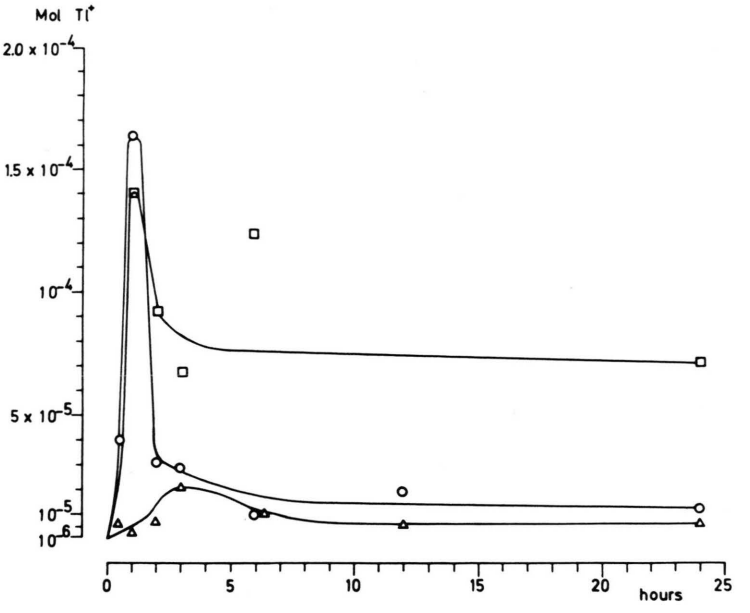

Fig. 2. Time course of thallium levels in maternal kidney (□), maternal brain (△), and fetal tissue (○) after acute poisoning with 8 mg/kg body weight thallium.

The time course of thallium levels are shown in Fig. 2. There is an initial surge in thallium content in kidney followed by a short wash-out period. Kidney thallium concentrations level off to reach a steady 24 h value. The thallium uptake of the brain remains lower, wash out is prolonged and long-term values are consistantly low. These findings are identical to the time courses determined in our previous study [5] for the kidney and the brain, thus verifying our hypothesis that there is a high initial thallium uptake in abdominal organs whereas, apparently, there is a barrier for thallium in the brain. Actual organ thallium concentrations are, of course, somewhat lower in this study compared to those determined previously [5] due to the lower thallium doses employed here.

The uterus thallium content mirrors that of the kidney in time course and, initially, in concentration. Long-term thallium values, however, are lower. Thallium uptake by the uterus and the fetus therefore does not differ from that of other organs like heart and liver where 12 and 24 h thallium values also were distinctly lower than those determined in the kidneys. These findings suggest that, if the uterus displays a behaviour similar to that of abdominal organs, there is no specific placental barrier for thallium. Beyond this, diaplacental transfer should be considered quite rapid and in equilibrium with the overall uptake and elimination processes governing thallium metabolism.

Since the dosage of thallium in this study is rather low a secondary thallium uptake in the kidney does not take place nor do the long-term uterus values for thallium change. Wash-out naturally proceeds from the uterus to the kidneys which could explain why kidney thallium is enhanced in the long run. Therefore, one can imagine that the first surge of thallium should enable a rather large fraction of thallium ions to cross the cell membranes and to enter the cells. These high thallium concentrations expected at intracellular sites presumably cause a number of acute toxic effects in the fetus quite similar to those described above. Short-term exposure of pregnant animals to toxic doses of thallium could therefore lead to delayed malfunctions in the fetus on the grounds of the mechanism shown in this study.

Long-term exposure to low but chronic doses of thallium should possibly have a mechanism distinct from an acute poisoning, since fetal thallium concentrations can be expected to be so low as not to produce the symptoms found at high concentrations.

Acknowledgements

This work was supported financially by the Deutsche Forschungsgemeinschaft, the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen, and the Umweltbundesamt.

We are particularly indepted to F. Köhler, Institute of Human Genetics, University of Bonn, for his expert assistance in the animal preparations.

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