Conceptus Uptake of the $^{106}$RuNO-nitro Complex in Relation to Gestational Stages

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(Received September 1, 1989)
(Revised version, accepted November 20, 1989)

$^{106}$RuNO-nitro complex was administered intravenously to pregnant rats to study uptake of the radioruthenium complex by the conceptus in relation to gestational stages. Each conceptus was sampled periodically with respect to its placenta, fetal membrane, fetal fluid and fetus. Perceptible radioactivity in the fetus was detected only in the later stage of gestation and its relative concentration, defined as the ratio radioactivity per unit weight in the body tissue at sacrifice to that in the whole-body at dosing, was very low compared with other tissues. The average number of fetuses in one litter was 13 and the transfer rate of nitro complex into the fetuses 24 hr after injection to rats on the 20th day of gestation was about 1% of initial maternal dose. The relative concentration in the placenta and fetal membrane was much higher than in the fetus and decreased with time after injection. These results indicate that the placenta and fetal membrane play significant roles as barriers to the transfer of $^{106}$RuNO-nitro complex into the fetus.

INTRODUCTION

Radioruthenium is a fission product radionuclide present in nuclear fuel cycles. It is a potential hazard to humans, because a significant fraction of fission product activity attributed to ruthenium isotopes and such isotopes can appear in the biosphere. The most hazardous radionuclide of ruthenium is $^{106}$Ru, with its high energy beta emissions from its daughter $^{106}$Rh and relatively long half-life of 368 days.

Ruthenium has one of the widest ranges of oxidation states, from +8 in the tetroxide to
zero in carbonyls and related compounds. Because of its elusive and variable chemical characteristics, the chemistry is complicated and many of the earlier investigations probably misinterpreted the ruthenium data and many contradictory reports have appeared in the literature.

When uranium fuel elements are removed from a reactor and dissolved in nitric acid for chemical processing, derivatives of nitrosyl ruthenium are formed. Therefore, among the most important ruthenium complexes are nitrogen complexes. There is some information available about the biokinetics of ruthenium in humans and animals. Bruce et al. reported the fraction of the gastrointestinal absorption for $^{106}$RuCl$_3$ to be about 3% in rabbits and 2% in rats; Burykina et al. about 5% in guinea pigs. Furchner et al. reported that the long-term biological half-life has been observed to be 220–300 days in the mouse, rat, monkey and dog after a parenteral administration. Yamagata et al. showed a larger absorption of nitrosyl complexes than chloro complexes and this may be related to different rates of conversion to a non-absorbable state that occurs on mixing with the contents of the GI tract. Enomoto et al. found that the RuNO-nitro complex had the highest whole-body retention when compared with RuNO-nitrato and Ru-chloro complexes after oral administration, but the smallest retention after intravenous injection. The cumulative urinary excretion of three days for the RuNO-nitro and RuNO-nitrato complexes was higher than that for the Ru-chloro complex. These results suggest that RuNO-nitro and RuNO-nitrato complexes in the body are excreted faster than Ru-chloride. Other than this, very few data are available on the effect of the chemical species including food-chain involvement of radioruthenium.

Only limited information on the absorption of ruthenium in newborn animals has been reported. Matsusaka et al. showed that newborn mice absorbed about 7% of orally administered Ru-chloride but that 21 day old and adult mice absorbed less than 1%. A similar result was obtained by Inaba et al. in rats. There are no data on age related changes in the retention and tissue distribution of ruthenium in experimental animals.

Recently, the radiation dose to the fetus as compared to the adult has become of particular interest in the unintentional intake of radionuclides, e.g. by emission from nuclear power plants, as well as in the medical use of radiopharmaceuticals. However, little information has been found in references in the literature dealing with the dosimetric model and biokinetic data for radiation doses to the conceptus. It is known that placental transfer and fetal uptake depend largely upon gestational stage. This suggests that it is important to know the gestational stage at the time of administration of the radionuclide in studying the internal dosimetry of the embryo and fetus. The present study, therefore, deals with the transfer of radioactive nitrosylruthenium-nitro complex to the fetus as well as its accumulation in the conceptus with special reference to gestational stage of the animal at the time of administration of radioruthenium.

**MATERIALS AND METHODS**

Wistar strain rats bred and supplied by the animal and plant section of the National Institute of Radiological Sciences were used in this experiment. In a cage, a pair of about 80 day old female
and male rats were housed for mating. When the presence of a vaginal plug was observed after mating, the gestation age was designated as day 0, whereupon the male rat was removed. All rats were kept in a room with controlled temperature and lighting, and were fed commercial rat diet (Funabashi Farm Co., Funabashi-shi, Japan) and tap water ad libitum. Radioactive ruthenium chloride (\(^{106}\text{RuCL}_3\)) was obtained from Radiochemical Centre, Amersham, England and nitrosylruthenium-nitro complex of the nuclide (\(^{106}\text{RuNO-nitro}\)) was prepared by the method of Iwashima and Watar10. \(^{106}\text{RuNO-nitro}\) complex was diluted to 37.5 kBq per ml with physiological saline solution and 0.5 ml of this solution was administered through the tail veins of pregnant rats in various gestation stages. Immediately after administration, the pregnant rat was placed in a polystyrene rat container and the initial activity was determined by in vivo counting using a small animal counter (Armac Model-446, Packard). Thereafter the rats were periodically sacrificed by carotid artery puncture under ether anesthesia. Maternal blood, liver, kidneys and whole product of conceptus with respect to its placenta, fetal membrane, fetal fluid and fetus were sampled from each pregnant rat group. The experimental design is summarized in Table 1.

### Table 1. Outline of experimental design and number of rats used

| Days of gestation at the time of sacrifice | Days of gestation at injection |
|------------------------------------------|------------------------------|
| 7 | 14 | 16 | 18 | 20 |
| 14 | 3 | 3 | 3 | 3 | 3 |
| 16 | 3 | 3 | 3 | 3 | 3 |
| 18 | 3 | 3 | 3 | 3 | 3 |
| 20 | 3 | 3 | 3 | 3 | 3 |
| 21 | 3 | 3 | 3 | 3 | 3 |
| Total number of rats | 15 | 12 | 9 | 6 | 3 |

RESULTS

Almost all the results are expressed in relative concentration (RC) which is defined as follows.

\[
\text{RC} = \frac{\text{Radioactivity in tissue}}{\text{Weight of tissue at sacrifice}} \times \frac{\text{Radioactivity administered to pregnant rat}}{\text{Weight of rat at administration}}
\]

The relative concentration in maternal blood, liver and kidneys after single intravenous administration to pregnant rats are shown in Table 2, 3 and 4, respectively. Each value is the mean and standard deviation for 3 animals.
Table 2. Relative concentration of $^{106}$RuNO-nitro complex in maternal blood after intravenous administration

| Days of gestation at sacrifice | Days of gestation when radioruthenium complex administered |
|-------------------------------|----------------------------------------------------------|
|                               | 7  | 14 | 16 | 18 | 20 |
| 14                            | 0.19 ± 0.02 |
| 16                            | 0.08 ± 0.03 0.84 ± 0.08 |
| 18                            | 0.06 ± 0.01 0.18 ± 0.01 0.79 ± 0.07 |
| 20                            | 0.04 ± 0.01 0.11 ± 0.04 0.19 ± 0.02 0.44 ± 0.04 |
| 21                            | 0.05 ± 0.01 0.07 ± 0.02 0.15 ± 0.03 0.31 ± 0.02 1.18 ± 0.12 |

Each value is mean of 3 maternal blood ± SD.

Table 3. Relative concentration of $^{106}$RuNO-nitro complex in maternal liver after intravenous administration

| Days of gestation at sacrifice | Days of gestation when radioruthenium complex administered |
|-------------------------------|----------------------------------------------------------|
|                               | 7  | 14 | 16 | 18 | 20 |
| 14                            | 0.51 ± 0.07 |
| 16                            | 0.34 ± 0.12 1.52 ± 0.10 |
| 18                            | 0.56 ± 0.07 0.66 ± 0.02 1.22 ± 0.76 |
| 20                            | 0.29 ± 0.02 0.52 ± 0.11 0.87 ± 0.04 1.08 ± 0.12 |
| 21                            | 0.29 ± 0.05 0.50 ± 0.09 0.78 ± 0.19 0.95 ± 0.08 1.77 ± 0.03 |

Each value is mean of 3 maternal liver ± SD.

Table 4. Relative concentration of $^{106}$RuNO-nitro complex in maternal kidney after intravenous administration

| Days of gestation at sacrifice | Days of gestation when radioruthenium complex administered |
|-------------------------------|----------------------------------------------------------|
|                               | 7  | 14 | 16 | 18 | 20 |
| 14                            | 3.58 ± 0.29 |
| 16                            | 2.72 ± 0.80 5.41 ± 0.44 |
| 18                            | 2.89 ± 0.36 4.40 ± 0.45 5.40 ± 0.40 |
| 20                            | 2.46 ± 0.57 4.61 ± 0.19 4.85 ± 0.19 6.33 ± 0.14 |
| 21                            | 2.53 ± 0.39 4.25 ± 0.89 4.81 ± 0.48 6.52 ± 0.75 7.53 ± 0.50 |

Each value is mean of 3 maternal kidney ± SD.
Table 5. Relative concentration of $^{106}$RuNO-nitro complex in fetus after intravenous administration

| Days of gestation at sacrifice | Weight* (g) | Days of gestation when radioruthenium complex administered | 7 | 14 | 16 | 18 | 20 |
|-------------------------------|-------------|----------------------------------------------------------|---|----|----|----|----|
| 14                            | 0.1         |                                                          | 0 |    |    |    |    |
| 16                            | 0.4         |                                                          | 0 | 0.05 ± 0.01 |    |    |    |
| 18                            | 1.1         |                                                          | 0 | 0.02 ± 0.01 | 0.04 ± 0.01 |    |    |
| 20                            | 3.3         |                                                          | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.05 ± 0.01 |    |    |
| 21                            | 4.3         |                                                          | 0.004 ± 0.001 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.06 ± 0.03 | 0.07 ± 0.02 |

* Average weight of one fetus.
Each value is mean of 15 fetuses ± SD.

Table 6. Relative concentration of $^{106}$RuNO-nitro complex in placenta after intravenous administration

| Days of gestation at sacrifice | Weight* (g) | Days of gestation when radioruthenium complex administered | 7 | 14 | 10 | 18 | 20 |
|-------------------------------|-------------|----------------------------------------------------------|---|----|----|----|----|
| 14                            | 0.12        |                                                          | 0.28 ± 0.05 |    |    |    |    |
| 16                            | 0.21        |                                                          | 0.12 ± 0.01 | 1.29 ± 0.18 |    |    |    |
| 18                            | 0.30        |                                                          | 0.07 ± 0.01 | 0.57 ± 0.10 | 1.40 ± 0.23 |    |    |
| 20                            | 0.36        |                                                          | 0.10 ± 0.02 | 0.54 ± 0.16 | 0.82 ± 0.08 | 1.43 ± 0.21 |    |    |
| 21                            | 0.38        |                                                          | 0.08 ± 0.01 | 0.39 ± 0.06 | 0.91 ± 0.06 | 1.25 ± 0.10 | 2.00 ± 0.41 |    |    |

* Average weight of one placenta.
Each value is mean of 15 placentas ± SD.

Table 7. Relative concentration of $^{106}$RuNO-nitro complex in fetal membrane after intravenous administration

| Days of gestation at sacrifice | Weight* (g) | Days of gestation when radioruthenium complex administered | 7 | 14 | 16 | 18 | 20 |
|-------------------------------|-------------|----------------------------------------------------------|---|----|----|----|----|
| 14                            | 0.01        |                                                          | 2.1 ± 0.5 |    |    |    |    |
| 16                            | 0.03        |                                                          | 1.0 ± 0.3 | 15.3 ± 2.8 |    |    |    |
| 18                            | 0.06        |                                                          | 1.5 ± 0.1 | 10.8 ± 1.2 | 20.3 ± 1.8 |    |    |
| 20                            | 0.11        |                                                          | 1.1 ± 0.1 | 10.5 ± 2.3 | 18.0 ± 4.3 | 26.3 ± 1.3 |    |    |
| 21                            | 0.11        |                                                          | 1.1 ± 0.1 | 11.8 ± 2.0 | 18.6 ± 3.5 | 23.2 ± 2.9 | 25.2 ± 3.5 |    |    |

* Average weight of one fetal membrane.
Each value was mean of 15 fetal membranes ± SD.
The concentration of $^{106}$Ru in the blood was very low and after its introduction into blood it seems to be rapidly cleared within the first two or three days after administration, after which clearance is much slower. On the other hand, several percent of administered dose was distributed to liver and kidneys and the clearance from those organs were rather slow. The relative concentration in the kidneys was higher than that of the liver and decreased slowly with time after administration.

Perceptible radioactivity in the fetus was detected only in later stage of gestation and found to be relatively low compared with other tissues. The relative concentration of $^{106}$Ru in the fetus was 0–0.07 and was lower than that in maternal blood (Table 5). The fetal transfer rate of $^{106}$Ru 24 hr after injection into rats at day 20 of gestation was calculated to be about 0.08% of initial maternal dose per one fetus. The average number of fetuses in one litter was 13 and about 1% of maternal dose was transferred to the fetuses. Practically no activity was observed in the fetal fluid. Table 6 and 7 show that the relative concentration of $^{106}$Ru in the placenta and fetal membrane was higher than in the fetus, and was greatest immediately after administration, followed by a gradual decrease thereafter. The concentration in the fetal membrane was highest in the conceptus.

**DISCUSSION**

Some information on the absorption and distribution of ruthenium complexes have been reported in experimental animals and in humans. In general the concentration of RuNO-nitro complex is significantly higher than other ruthenium complexes. Few data are available on the absorption and distribution of ruthenium complexes in juvenile animals. The absorption of Ru-chloro complex in newborn and suckling mice is higher than in the adult. In the case of rats, the whole-body retention of ruthenium complexes in newborn and sucklings after oral administration was extremely high during the suckling period, but this high retention decreased sharply at weaning. A similar remarkable retention pattern has been observed for $^{141}$Ce, $^{110m}$Ag and $^{51}$Cr.

There are few published reports on the transfer of ruthenium complex to the fetus. Nelson et al. reported an autoradiographic study of $^{103}$Ru-chloro complex in pregnant mice. A marked and consistent affinity of $^{103}$Ru for connective tissue was observed. Its passage to the central nervous system and the fetus was blocked but a considerable accumulation in the fetal membrane was noted.

Stather et al. administered $^{106}$Ru-chloro complex to pregnant rats at varying stages of gestation and measured the relative concentration of $^{106}$Ru in maternal and fetal tissues at birth. The greatest transfer of $^{106}$Ru to the fetus occurred following intravenous administration to the dam towards the end of pregnancy. Thus relative concentrations at birth, following administration to the dam in the week before conception, or at 14 or 19 days of gestation, were 0.0037, 0.015 and 0.051, respectively.

When uranium fuel elements containing fission products are removed from a nuclear reactor and dissolved in nitric acid for chemical processing, derivatives of nitrosyl ruthenium are formed.
Fig. 1. Relative concentration of $^{106}\text{RuNO}$-nitro complex in maternal blood, placenta, fetal membrane and fetus on 21st day of gestation after intravenous administration. Bars represent means and standard deviation.
Therefore, though the important ruthenium complexes are nitrogen complexes, many early investigations used the Ru-chloro complex.

The present study revealed that the accumulation of $^{106}\text{RuNO-nitro}$ complex in placental and fetal membrane was much greater than that in the fetus. Fig. 1 shows the relative concentrations of $^{106}\text{RuNO-nitro}$ complex in the maternal blood, placenta, fetal membrane and fetus on the 21th day of gestation after administration of the complex. The ratio of the concentration among the three entities, fetus:placenta:fetal membrane was estimated to be about 1:20–30:300–600. Relative concentration in the fetus is approximately $10^{-2}$ and the greater portion of the conceptus burden was found in the fetal membrane and placenta. These results indicate that the placenta and fetal membrane seem to play roles as barriers to the transfer of the RuNO-nitro complex into the fetus.

Ullberg et al.\textsuperscript{15} studied autoradiography of plutonium in mice. They reported that plutonium accumulated predominantly in the hard tissues but uptake and retention was also observed certain soft tissues such as liver, ovarian follicles, fetal membrane and mammary glands. Sikov and Mahlum\textsuperscript{16} also reported colloidal $^{239}\text{Pu}$, $^{237}\text{Np}$ and $^{233}\text{U}$ concentrates in the placenta and fetal membrane. These results are somewhat similar to the case of the $^{106}\text{RuNO-nitro}$ complex. Ruthenium is a transition metal of the platinum group that occupies an unusual position in nuclear technology and shows many valence states, has a relatively large mass and becomes colloidal easily. These data indicate that ruthenium can be used as a model experiment for plutonium.

Radioactivity in the fetus was detected only in later stages of gestation and the relative concentration was very low compared with other tissues. Moreover, the concentration in the placenta and fetal membrane was much higher than in the fetus. These results suggest that internal exposure of the fetus from $^{106}\text{Ru}$ is of no particular important, but that external exposure from maternal kidneys and liver, placenta and fetal membrane may be quite marked.

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