Radiation Doses to the Tissues of Rat from Tritiated Thymidine Administered by Three Different Routes

HIROSHI TAKEDA, TETSUO IWAKURA and YASUO MABUCHI

Division of Environmental Health, National Institute of Radiological Sciences
9-1, Anagawa-4-chome, Chiba-shi 260, Japan
and
Department of Pharmaceutical Science, Toho University
2-1, Miyama-2-chome, Funabashi-shi 274, Japan
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Biological behaviour of tritiated thymidine were investigated in rat over 120 days after oral, intraperitoneal or intravenous administration and the absorbed doses to different tissues were estimated.

The result of present study revealed that the absorbed dose from tritiated thymidine varied with the route of administration. Among the three routes of administration, intraperitoneal injection gave the highest dose to all of the tissues examined. A significant difference due to the route of administration was found in spleen and small intestine, where the doses were, respectively, 3.3 and 4.5 times higher after intraperitoneal injection than after oral ingestion. The difference was substantially dependent on the dose value from non-volatile tritium which would be incorporated into DNA.

Present observation suggests that the radiation hazards of tritiated thymidine differ depending on the route of entry into the body.

INTRODUCTION

Tritiated thymidine is used widely in research laboratories to study the cell population kinetics because of its preferential incorporation into deoxyribonucleic acid (DNA) of the cell. But this property also makes tritiated thymidine a potential hazard to the laboratory workers dealing with it. Biological effects of tritiated thymidine have been extensively investigated at many endpoints. In most of these studies, tritiated thymidine was administered by intraperitoneal or intravenous injection. While, the main route of entry into workers in research laboratory is more likely to be that of ingestion. Several investigators indicated that the route of administration have a measurable effects
on the in vivo uptake of the compound by animal tissues\textsuperscript{12-14}. However, these reports deal with only short-term uptake within 24 hours after administration of tritiated thymidine. For appropriate evaluation of radiobiological hazards of tritiated thymidine, it is necessary to obtain the information on the long-term retention of the radioactive material in the body as well as the uptake data.

In the present study, the metabolic fate of tritium after oral, intraperitoneal or intravenous administration of tritiated thymidine was investigated over 100 days, and the radiological hazards due to tritiated thymidine from different routes of administration were compared on the basis of the average absorbed doses.

**MATERIALS AND METHODS**

Wistar strain male rats (3 to 4 month-old), ranging from 370 to 410 g in body weight, were used for this study. Throughout the experiment, rats were fed standard cubed diet and water *ad libitum* and were maintained in the room with controlled air and temperature.

Tritiated thymidine (6-\textsuperscript{3}H) with a specific activity of 23.0 Ci/mmol was obtained from the New England Nuclear. The labelled compound was diluted to an appropriate concentration with distilled water and was administered to animals orally, intraperitoneally or intravenously with a radioactivity of 0.2 \textmu Ci (7.4 kBq) per gram body weight in a volume of 0.5 ml saline solution. The radioisotopes were always administered between 9 and 10 AM to reduce variations in gastric absorption and fluctuations that might be caused by diurnal rhythm in cellular proliferative activity.

The animals were sacrificed at various time intervals between 1 day and 120 days after the administration and were dissected to obtain the following tissues: liver, kidney, testis, spleen, small intestine, brain, muscle and lung. A part of each tissue was weighed immediately and total tritium activity in wet tissue was determined. Another part of each tissue was lyophilized with liquid nitrogen for analysis of non-volatile tritium activity in dry tissue. Lyophilization was repeated twice by adding distilled water to remove water-form tritium. Total tritium and non-volatile tritium activity were determined by a liquid scintillation spectrometer after the combustion by an oxidizer (Model 306 Tri-Carb Packard Instrument Co.).

Volatile tritium activity was calculated by subtracting non-volatile tritium activity from total tritium activity of the same sample. This volatile tritium, therefore, refers to tritium in tissue water, namely tritiated water. In this study, all data were expressed as relative concentration which had been calculated as follow:
relative concentration = \frac{\text{activity per g sample weight}}{\text{activity administered per g body weight}} \times 100

Relative concentration of volatile tritium for each tissue was calculated using the water content of each tissue which had been reported in our previous publication\(^{15}\).

RESULTS

Table 1 shows the relative concentration of volatile and non-volatile tritium in various tissues of rat at 24 hours after the administration of tritiated thymidine by three different routes. Volatile tritium was almost uniformly distributed among the tissues in all three cases. The concentration levels of volatile tritium for oral ingestion and intraperitoneal injection were very similar, but were different from the lower levels observed in the case of intravenous injection. At 24 hours after the administration, total amount of volatile tritium present in the body was calculated using the average concentration of volatile tritium for all of the tissues examined and the water content of the animal which was assumed to be 65%. The result of this calculation showed that the amount of volatile tritium in the whole body was 63%, 67% and 30% of total tritium administered to the animal for oral ingestion, intraperitoneal injection and intravenous injection, respectively.

As can be seen in Table 1, distribution of non-volatile tritium was not uniform among the tissues and relatively high concentrations were found in spleen and small intestine in all cases. The concentration level of non-volatile tritium in the case of oral ingestion were lower than those observed in the other two cases. The concentration level for intraperitoneal and intravenous injection was almost the same in most tissues, but exceptionally higher concentrations were observed in

Table 1. Distribution of volatile and non-volatile tritium in various tissues of rat at 24 hours after oral, intraperitoneal or intravenous administration of tritiated thymidine.

| Tissue     | Oral ingestion | I. P. injection | I. V. injection |
|------------|----------------|-----------------|----------------|
|             | Non-volatile $^3$H | Non-volatile $^3$H | Non-volatile $^3$H |
| Liver      | 102            | 11              | 101            | 22             | 42             | 25             |
| Kidney     | 99             | 7.4             | 115            | 47             | 47             | 52             |
| Testis     | 96             | 10              | 104            | 66             | 43             | 67             |
| Spleen     | 91             | 72              | 118            | 1104           | 59             | 252            |
| Brain      | 93             | 5.2             | 117            | 8.8            | 46             | 9.2            |
| Muscle     | 95             | 3.2             | 99             | 11             | 38             | 12             |
| S. Intestine | 101          | 87              | 106            | 2336           | 68             | 743            |
| Lung       | 95             | 11              | 96             | 101            | 46             | 83             |

Each value represents the average of determinations on three rats.
spleen and small intestine after intraperitoneal injection. These findings were more clearly represented in Table 2 in term of the ratio of non-volatile tritium concentration for intravenous or intraperitoneal injection to the concentration for oral ingestion at 24 hours after the administration. It also became clear from the table that the distribution pattern among tissues of non-volatile tritium after oral ingestion was somewhat different from those observed in the other two cases.

| Tissue     | Ratio of non-volatile tritium concentration | Ingestion | I.P. injection | I.V. injection |
|------------|---------------------------------------------|-----------|----------------|----------------|
| Liver      | 1                                           | 2.9       | 2.3            |
| Kidney     | 1                                           | 6.4       | 7.0            |
| Testis     | 1                                           | 6.5       | 6.7            |
| Spleen     | 1                                           | 15.3      | 3.6            |
| Brain      | 1                                           | 1.7       | 1.8            |
| Muscle     | 1                                           | 3.4       | 3.8            |
| S. intestine | 1                            | 26.9      | 8.5            |
| Lung       | 1                                           | 9.2       | 7.5            |

Long-term retention of volatile and non-volatile tritium was investigated in various tissues after oral, intraperitoneal or intravenous administration. The retention curves for volatile tritium showed a very similar pattern in all tissues examined irrespective of the route of administration (results not shown here). On the other hand, as shown in Figs. 1—4, the retention pattern of non-volatile tritium was different from tissue to tissue. However, a significant difference of the retention pattern due to the route of administration was not found in any tissue.

On the basis of these experimental data, the radiation dose to each tissue from volatile and non-volatile tritium was calculated. For this calculation, the concentrations of tritium in volatile and non-volatile fraction of tissue were recalculated to give concentrations in living tissues using the water content for different tissues. In the calculation, it was also assumed that the tritium activity in each tissue remained constant during the first 24 hours after administration and the activity decreased as an exponential function of time from the level measured at a given time to the level measured at the succeeding time. The result was shown in Table 3.

In the case of ingestion, majority of the dose was delivered from volatile tritium and the contribution from non-volatile tritium to total dose ranged from
5.6% in testis to 12.7% in spleen. On the contrary, the dose contribution from non-volatile tritium after intravenous and intraperitoneal injection was relatively higher than those after oral ingestion. In the case of intraperitoneal injection, the dose values from non-volatile tritium were almost the same as those after intravenous injection in most of the tissues, but in spleen and small intestine the values after intraperitoneal injection were about 1.7 times as high as those after intravenous injection. Also, the doses from non-volatile tritium in these two tissues after intraperitoneal injection were 20–30 times as high as those after oral ingestion.

The total doses from both volatile and non-volatile tritium were highest when administered by intraperitoneal injection in all the tissues examined. In spleen and small intestine the doses after intraperitoneal injection were 3.3 and 4.5 times higher than those after oral ingestion, respectively.
Fig. 3. Retention curves of non-volatile tritium in liver and kidney after oral, intraperitoneal and intravenous administration of tritiated thymidine.

Fig. 4. Retention curves of non-volatile tritium in brain and muscle after oral, intraperitoneal and intravenous administration of tritiated thymidine.

Table 3. Doses accumulated during 100 days after ingestion, intraperitoneal or intravenous injection of tritiated thymidine.

| Tissue  | Ingestion | I.P. injection | I.V. injection |
|---------|-----------|----------------|----------------|
|         | from Volatile $^3$H | from Non-volatile $^3$H | from Volatile $^3$H | from Non-volatile $^3$H | from Volatile $^3$H | from Non-volatile $^3$H |
| Liver   | 13         | 1.6            | 15             | 7.5            | 9              | 6.5              |
| Kidney  | 15         | 1.3            | 16             | 8.0            | 11             | 8.5              |
| Testis  | 15         | 0.9            | 17             | 6.8            | 12             | 7.9              |
| Spleen  | 16         | 2.3            | 15             | 44.7           | 11             | 26.3             |
| Brain   | 14         | 1.3            | 15             | 2.2            | 9              | 2.1              |
| Muscle  | 14         | 1.2            | 15             | 2.9            | 9              | 2.5              |
| S. intestine | 14       | 1.8            | 19             | 62.5           | 12             | 31.2             |
| Lung    | 13         | 1.2            | 15             | 13.8           | 10             | 13.3             |

DISCUSSION

In the present study, the tritium activity in the tissues measured after the administration of tritiated thymidine by three different routes was estimated in the two fractions of volatile and non-volatile fraction. The non-volatile tritium activity in the tissues was considered to exist mainly in DNA molecules, as shown by some investigators using chemical analysis and autoradiography\(^{16-20}\). In contrast, tritium in volatile fraction should be in tritiated water which was
produced by catabolism of tritiated thymidine. Present study should that the amount of volatile tritium in the whole body determined at early time after intravenous injection was less than that after intraperitoneal injection or oral ingestion. In the case of intravenous injection, thymidine is transported directly to heart through venous return system from the tail vein of injection site, and then distributed to various tissues. On the other hand, when thymidine is given by oral ingestion or intraperitoneal injection, the compound is absorbed from the small intestine and transfers to liver which is the main site of thymidine catabolism. This could account for the larger amount of volatile tritium in the tissues of rat after oral ingestion or intraperitoneal injection.

Present study also showed that the concentration levels of non-volatile tritium in various tissues after oral ingestion were lower than those after intraperitoneal or intravenous injection. This result suggests that catabolism of tritiated thymidine to tritiated water also occurs in the gastrointestinal tract. Lambert et al. indicated that tritiated thymidine administered orally was not only catabolized to tritiated water, but also metabolized to some different tritiated compounds other than tritiated thymidine on the passway of gastrointestinal absorption. This could relate to the present result which showed that the distribution pattern of non-volatile tritium after oral ingestion was different from that for the other two routes.

The concentrations of non-volatile tritium after intraperitoneal injection and intravenous injection were substantially similar in most of the tissues observed, but in spleen and small intestine extremely high concentrations were observed for intraperitoneal injection. Similar results have been reported by Petersen et al. and Skougaard et al., although they used mice as experimental animal. Such a result is probably because of the direct absorption by these tissues from the site of injection.

As shown in Figs. 1-4, the retention patterns of non-volatile tritium were different from tissue to tissue, but the pattern for each tissue showed a similar tendency irrespective of route of administration. Since the non-volatile tritium should be bound to DNA in the cell nucleus, its pattern depends primarily on the rate of cell turnover. Therefore, the result of present study will give us some fundamental information about cell turnover of each tissue. However, theoretical analysis about biological significance of the retention patterns have not been performed in this report because of the complexity of the analysis.

From a viewpoint of radiation protection which was the original purpose in this study, the absorbed doses to the tissues from tritiated thymidine administered by three different routes were estimated. The result revealed that the doses varied considerably with the route of administration. Among the three routes of administration, intraperitoneal injection gave the highest dose to all of the tissues examined. A significant difference due to the route of
administration was found in proliferative tissues such as spleen and small intestine where the dose contribution from tritium incorporated into DNA (non-volatile tritium) was relatively high. In these two tissues, the average absorbed doses were 3–5 times higher after intraperitoneal injection than after oral ingestion. These findings will lead to the conclusion that the possible radiation hazards of tritiated thymidine differ depending on the route of entry into the body.

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