**Distribution of Technetium-99m Sulfur Colloid in Mice Bearing Melanomas or Mammary Carcinomas**

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**SUMMARY**

Three groups of 24 C57BL/6J black mice were studied. One group was implanted with B16 malignant melanoma, another was implanted with mammary adenocarcinoma, and the third was not given tumor implants. After 14 to 17 days, the mice were given injections i.v. of technetium-99m sulfur colloid and killed 30 min later. Organs were weighed, and radioactivity was counted. The ratios of specific radioactivities of the spleens to those of the liver were higher from the circulation by the reticuloendothelial cells of these organs. This finding suggests that the “hot spleen” phenomenon observed in humans with malignant melanomas may be due to increased specific activity rather than increased splenic volume.

**INTRODUCTION**

Sulfur colloid labeled with $^{99m}$Tc$^3$ is widely used in scanning of the liver and spleen because it is quickly extracted from the circulation by the reticuloendothelial cells of these organs. Under normal conditions about 80 to 85% of the injected dose is localized in the liver, 2 to 3% in the spleen, and most of the remainder in the bone marrow (7, 8). In addition to technical factors and artifacts, such as the presence of the $^{99m}$Tc as pertechnetate or variations in the particle sizes of the colloid (10, 12), a variety of disease states, such as cirrhosis, hepatitis, and anemia (2, 13), are known to increase the relative uptake of the $^{99m}$Tc sulfur colloid in the spleen as compared with the liver. This has been termed the “hot spleen” phenomenon or reversed liver:spleen ratio. Recently, several reports (5, 9, 13) have appeared in the literature which suggest that the relative distribution of $^{99m}$Tc sulfur colloid in the liver and spleen may also be modified in a similar fashion in patients harboring certain neoplasms.

Goldman et al. (5) reported reversed liver:spleen ratios to be much more common in patients with malignant melanoma (34%) compared with patients with other types of cancers (2%). These authors excluded from their series patients who had clinical, laboratory, or scan evidence of hepatic disease or hepatic metastases. On the other hand, Wilson and Keyes (13) found that about 50% of patients with all types of cancers had spleen densities higher than those of the liver. However, in their series a large number of patients had liver involvement, as judged by the clinical, laboratory, or scan findings. A more recent study by Harvey et al. (9) tends to confirm the findings of Goldman et al. (5). Harvey et al. (9) found a 3% incidence of relative increased splenic density in patients without evidence of liver involvement with a variety of neoplasms (13 of 403 patients). These authors do not give the breakdown of the scanned patients according to the types of cancers, except to state that melanoma is an uncommon cancer at their institution; yet approximately 50% of all of the patients having increased splenic density were melanoma patients.

In the above reports, the criterion used to judge the relative distribution of $^{99m}$Tc sulfur colloid was visually increased density of splenic scans compared with hepatic scans in an anterior or posterior projection. This is a qualitative and highly subjective judgment that does not fully represent the total or the per g amount of radioactivity accumulated in the liver or spleen. In our study, direct measurements of radioactivity of hepatic, splenic, and control tissues were made in a murine model harboring malignant tumors in order to better understand the hot spleen phenomenon and to determine whether it crossed species lines.

**MATERIALS AND METHODS**

**Animals.** Female C57BL/6J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, in 2 batches of 36 mice. When they were 6 to 7 weeks old, they were placed into 1 of 3 groups. Group 1 consisted of 24 mice that received B16 malignant melanoma implants into the right axillae. The method of implantation is described elsewhere (1). Group 2 consisted of 24 mice that received BW-10232 mammary adenocarcinoma implants into the right axillae at the same times that the mice in Group 1 received melanoma implants. Group 3 consisted of 24 mice that were not implanted with tumor and thus served as controls. To facilitate the experiments, 6 mice from each group (18 mice) were selected for implants on each of 4 occasions.

**Distribution Studies.** $^{99m}$Tc sulfur colloid was prepared from a commercial kit (Tesuloid; E. R. Squibb and Sons, Inc., Princeton, N. J.) according to the instructions provided. The amount of free pertechnetate in the labeled sulfur colloid was checked for each preparation, with as-
cending paper (Whatman No. 1) chromatography with 85% methanol as solvent. Any preparation that showed more than 5% free pertechnetate was discarded. For the distribution studies, 5 to 15 μCi of the labeled material in about a 0.1-ml volume was injected into a tail vein of each mouse 14 to 17 days after tumor implantation. A standard of known amount of radioactivity was prepared at this time. The mice were killed 30 min after the injections. The liver, spleen, kidneys, lungs, tumor, tail, and samples of blood and marrow-containing bone were put into preweighed test tubes. The tubes were reweighed to determine the net weights of the samples. The test tubes containing samples and the test tube containing the standard were counted in a Picker well scintillation Gamma counter to determine the radioactivity of each sample. Data from mice in which the amount of radioactivity in the tail exceeded more than 10% of the injected dose were excluded, since this was interpreted as evidence of infiltration of the injection sites. From the data, ratios of the radioactivity in whole spleens to those in the whole livers, as well as ratios of the radioactivities per g in the spleens to those in livers, were calculated.

The above procedure was repeated 3 times/week (Monday, Tuesday, and Thursday) for 4 weeks, with 2 animals from each group used each day.

Statistical Analysis. Since the experimental data resulted in non-Gaussian distribution, nonparametric statistical procedures were used to detect significant differences in the mean value of the various ratios among the control, melanoma, and breast cancer groups. Overall differences among the groups were tested at a p of 0.01 by the Kruskal-Wallis procedure, and, when significant, comparisons were made with the 2-sample Wilcoxon test (6) at a p of 0.01 again.

RESULTS

The distribution of ratios of liver weights to mouse body weights in the 3 groups is shown in Chart 1. The mean values of the liver weights per g of mouse weight in the melanoma, breast carcinoma, and control group are 57.7 ± 4.6 (S.D.), 59.3 ± 7.5, and 53.7 ± 5.5 mg/g, respectively. The distribution of ratios of spleen weights to mouse body weights in the 3 groups is shown in Chart 2. The mean values of the spleen weights per g mouse weight for the melanoma, breast carcinoma and control groups are 7.0 ± 1.2, 7.9 ± 2.6, and 5.1 ± 1.2 mg/g, respectively. The average weights of livers and the average weights of spleens in both the melanoma and breast cancer groups were greater than the average weights of the livers and spleens of mice in the control group. These differences were statistically significant (p < 0.01). There was no statistical difference in the average weights of livers and spleens from mice in the melanoma group compared with those in the mammary carcinoma group (p = 0.49 and 0.36, respectively). In order to see whether there was a difference in the splenic weights relative to the liver weights in the 3 groups, the ratios of spleen weights to liver weights in the 3 groups were calculated. These are shown in Chart 3. Statistical analysis showed that the weights of the spleens relative to the liver weights in the melanoma and breast carcinoma groups were significantly higher than those in the control group (p < 0.01). No difference (p = 0.82) was found between melanoma and breast cancer groups in this respect.

The ratio, R, of the radioactivity in the whole spleen to that in the whole liver in each mouse was calculated. The distributions of these ratios in each of the 3 groups are shown in Chart 4. The mean values of the ratio R, are 0.087
Sulfur Colloid Uptake by Spleen in Mice with Melanomas

The specific radioactivity (cpm/g) was calculated for each spleen and each liver. The ratio, $R_2$, of specific radioactivity of the spleen to that of the liver was obtained for each mouse. The distributions of these ratios in each of the 3 groups are shown in Chart 5. The mean values for $R_2$ are 0.72 ± 0.27, 0.48 ± 0.31, and 0.41 ± 0.20, for the melanoma, breast carcinoma, and control groups, respectively. The melanoma group showed a statistically significant increase ($p < 0.01$) in the specific spleen: liver radioactivity, $R_2$, compared with the other 2 groups, between which there is no significant difference ($p = 0.68$).

These results, along with those illustrated in Chart 3, clearly demonstrate that the increase in the total splenic radioactivity in the breast carcinoma group is due to the increase in the spleen weights compared with liver weights in this group. In the melanoma group, however, there is a significant increase in the splenic radioactivity even when the relative increase in the spleen weights has been taken into account (Chart 5); i.e., there has been an increase in the specific radioactivities of the spleens compared with those in the liver of mice bearing melanomas.

The amount of the radioactivity in tissues other than spleen, liver, and bone marrow was always less than 2% of the injected dose, which indicates that there was little, if any, in vitro or in vivo degradation or breakdown of the 99mTc sulfur colloid, since a large amount of free pertechnetate would have increased the radioactivities in these other tissues markedly.

To make certain that the relative increase in the splenic uptake of 99mTc sulfur colloid was not a result of the hepatic cell blockade or suppression, the ratio of the specific radioactivities of the lung to that of the liver was calculated for

\[ \frac{\text{CPM}_{\text{spleen}}}{\text{CPM}_{\text{liver}}} \times 10^3 \]

Chart 4. Distribution of the ratios of cpm in the spleens to cpm in the livers, $R_1$, after an injection of sulfur colloid labeled with 99mTc.

\[ \pm 0.03, 0.058 \pm 0.025, \text{ and } 0.036 \pm 0.011, \text{ respectively, for the melanoma, breast carcinoma, and control groups. Statistical analysis of the data showed significant (}$p < 0.01$\text{)} relative increase in the radioactivity of the whole spleen over that of the whole liver, $R_1$, in the melanoma and breast cancer groups compared with the control groups. There is also a statistically significant increase ($p < 0.01$) in the

radioactivity of the whole spleen over that of the whole liver, $R_1$, in the melanoma group compared with the breast carcinoma group.

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Each mouse. No statistical differences were found in these ratios among the 3 groups. The mean values of these ratios are 0.036 ± 0.013, 0.041 ± 0.017, and 0.042 ± 0.022 for the melanoma, breast carcinoma, and control groups, respectively. That is, the lung: liver ratio was not increased in the melanoma group.

An attempt was made to correlate the ratios R₁ and R₂ with the tumor mass in the melanoma and breast carcinoma groups. No correlation of R₁ and R₂ with the tumor mass was found in either group.

No visceral metastases were found in mice bearing melanomas. In 1 mouse with mammary carcinoma, visceral metastases were found; this mouse was excluded from the study.

DISCUSSION

Reversed liver:spleen ratios or hot spleens with ⁹⁹ᵐTc sulfur colloid scanning have been reported in cirrhosis of the liver, diabetes mellitus, hepatitis (13), and anemia (2). These findings have usually been attributed to changes in blood flow in these conditions. Several investigators also report hot spleens in association with malignant neoplasms in the absence of detectable liver disease. Goldman et al. (5) report this finding specifically in patients with malignant melanoma. Harvey et al. (9) tend to support this. Our experiments quantitatively confirm this observation in an animal model, indicating that the cause of this phenomenon is probably independent of species and is specific for melanoma. Under the controlled conditions of our experiment, the relative increase in the uptake of ⁹⁹ᵐTc sulfur colloid in the spleen in the melanoma group over that in the control or breast carcinoma group can be explained as follows. In the melanoma group (a) there is an increased blood flow to the spleen, and/or (b) the reticuloendothelial cells of the spleen have been selectively stimulated so that their trapping efficiency for ⁹⁹ᵐTc sulfur colloid is increased. Whether only one or both of these mechanisms are responsible for the hot spleen phenomenon cannot be decided on the basis of the information now available.

Since malignant melanoma is thought to have unique antigenic properties, as evidenced by its capacity to evoke humoral as well as cellular immunity, its propensity for late recurrence, and its remission or regression following immunotherapy, it is possible that some tumor antigen or a substance produced as a result of host defense against melanoma may be stimulating the splenic reticuloendothelial cells (3, 4, 11). This stimulation may be either a part of the defense mechanism or simply a by-product of it.

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REFERENCES

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