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LUNG UPTAKE OF ^{99m}Tc-SULFUR COLLOID SECONDARY TO INTRAPERITONEAL ENDOTOXIN

William C. Klingensmith III and Vincent J. Lovett, Jr.

USAF Medical Center, Scott AFB, Illinois

The intraperitoneal injection of endotoxin in rats 6 hr prior to the intravenous injection of ^{99m}Tc-sulfur colloid (TcSC) results in a marked increase in lung uptake of TcSC that is easily appreciated on scintigrams. Previous animal studies suggest that the most likely mechanism of increased lung uptake is an increased number of macrophages in the pulmonary capillary bed, which are derived from the circulation, and that the liver and spleen are a possible source. This study indicates that, in the case of endotoxin, the liver and spleen are not the source of the postulated increased number of macrophages in the pulmonary capillary bed.

Recently a number of studies have reported increased lung uptake of ^{99m}Tc-sulfur colloid (TcSC) during liver–spleen studies, which could not be attributed to technical factors (*1–4*). In addition, three studies have presented evidence that the mechanism responsible for this phenomenon is increased phagocytic activity in the pulmonary capillary bed rather than in vivo macroaggregation of the TcSC (*1,2,5*). In one of these studies, Quinones reported a marked increase in lung uptake of TcSC 6 hr after the intraperitoneal injection of endotoxin in rats (*5*). As there have been two reports in the literature of the rapid migration of large numbers of macrophages from the liver and spleen to the lungs (*6,7*), we undertook to confirm Quinones' finding and to determine if intraperitoneal endotoxin causes the rapid migration of liver and spleen macrophages to the lungs.

MATERIALS AND METHODS

Female rats (250 gm) of Wistar origin (National Laboratory Animal Co., St. Louis, Mo.) were divided into two control and two experimental groups. The first control group contained five animals and each one was given 100 μCi TcSC intravenously and sacrificed 20 min later. The corresponding experimental group contained seven animals and each one was given 20 mg of lipopolysaccharide B E. coli endotoxin (Difco Laboratories, Detroit, Mich.) reconstituted in 4 cc of isotonic saline intraperitoneally. Six hours later each animal was given 100 μCi TcSC intravenously and sacrificed 20 min later. These two groups were designed to repeat Quinones' study and to confirm that intraperitoneal endotoxin 6 hr prior to TcSC results in a marked increase in lung uptake.

The second control group contained five animals and each one was given 100 μCi TcSC intravenously. However, they were not sacrificed until 6 hr later. The corresponding experimental group contained seven animals. Each animal was given 100 μCi TcSC intravenously and 20 mg of lipopolysaccharide B E. coli endotoxin intraperitoneally. Six hours later they were sacrificed. These two groups were designed to determine whether the increased lung uptake 6 hr after intraperitoneal endotoxin was secondary to a redistribution of macrophages which were initially in the intravascular space and specifically whether there was a migration of macrophages from the liver and spleen to the lungs.

All TcSC injections were through a femoral vein cut down under local anesthesia using 1% lidocaine. Those animals that were to be left for 6 hr before sacrifice had their wounds closed with interrupted skin sutures.

After sacrifice anterior scintigrams were made using a Nuclear-Chicago Pho/Gamma HP camera with a pinhole collimator. Then both lungs, tissue

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For reprints contact: William C. Klingensmith III, Div. of Nuclear Medicine, The Johns Hopkins Medical Institutions, 615 N. Wolfe St., Baltimore, Md. 21205.

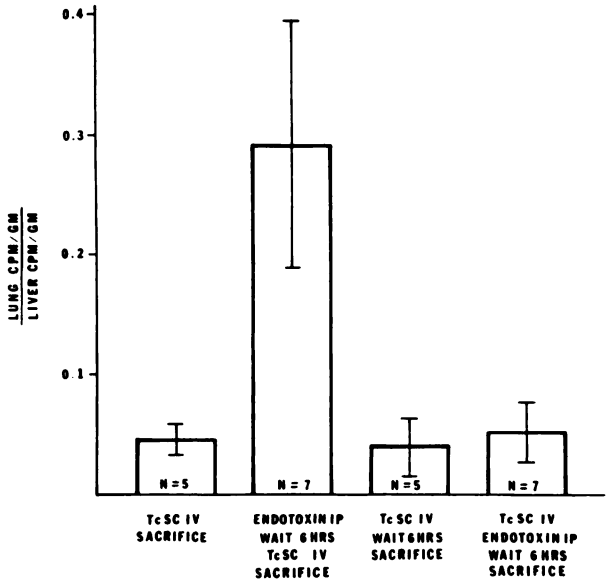

FIG. 1. Mean ± s.d. of lung uptake of ^{99m}Tc-sulfur colloid as compared with liver uptake is shown for each of four control and experimental groups of rats. Treatment protocol for each group is summarized below corresponding bar.

from two lobes of the liver, and the spleen of each animal were weighed on a Mettler balance and counted in a Picker Spectroscaler IIIA well counter. Lung uptake was quantitated by comparing the counts per minute per gram of lung to the counts per minute per gram of liver for each animal (*5*). Spleen uptake as compared with liver uptake was also quantitated for each animal.

The TcSC was prepared using ^{99m}Tc pertechnetate from a 100-mCi UltraTechneKow FM generator and a TechneColl kit (Mallinckrodt/Nuclear, St. Louis, Mo.).

RESULTS

The degree of lung uptake of TcSC for each animal group is shown in Fig. 1. The first control group showed a lung-to-liver ratio of 0.046 ± 0.024 (mean ± s.d.) and the corresponding experimental group in which endotoxin was injected 6 hr prior to injection of TcSC showed a lung-to-liver ratio of 0.291 ± 0.103. This represents a sixfold increase in lung uptake of TcSC and is significant at $p < 0.001$. Figure 2 shows that the increased lung uptake in the experimental group was sufficient to be easily appreciated by imaging.

The second control group with the 6-hr delay before sacrifice showed a lung-to-liver ratio of 0.040 ± 0.024. This result was not significantly different from the first control group although others using a different preparation of TcSC have reported a significant decrease in lung activity by 6 hr (*8*). The corresponding experimental group in which TcSC was injected initially before the endotoxin showed a lung-to-liver ratio of 0.051 ± 0.024, which was not significantly different from its control group.

The spleen-to-liver uptake ratios showed no significant difference between any two of the four groups.

DISCUSSION

Recent studies have shown that increased lung uptake of TcSC is associated with a number of serious diseases: malignant lymphomas (*2,3*), metastatic carcinomas (*1,3,4*), intra-abdominal abscesses (*3*), cirrhosis (*3*), and liver transplants (*9*). In addition, three different types of studies have all indicated that the mechanism of the increased lung uptake is increased phagocytic activity in the pulmonary capillary bed rather than in vivo macroaggregation (*1,2,5*). Our results confirm Quinones' finding that intraperitoneal endotoxin 6 hr prior to injection of TcSC results in a marked increase in lung uptake (*5*). This finding provides a partial explanation of the mechanism by which intra-abdominal abscesses in man result in increased lung uptake. In a related study, Saba (*10*) showed that laparotomy alone in rats under clean conditions resulted in increased lung uptake of ^{131}I-lipid emulsion as early as 15 min after operation. A number of previous animal experiments seem pertinent to these findings.

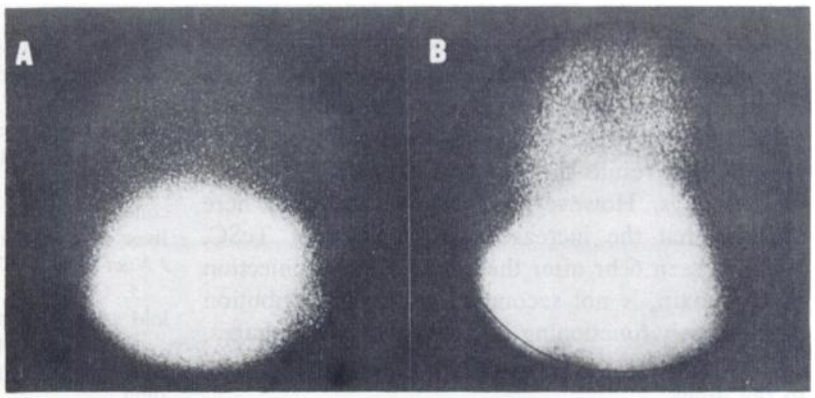

FIG. 2. Anterior scintigrams of normal control (A) and experimental animal (B) showing marked increase in lung uptake of ^{99m}Tc-sulfur colloid after treatment with intraperitoneal endotoxin.

If increased lung uptake of TcSC is secondary to increased phagocytic activity, the increased phagocytic activity may be due either to increased activity of the small number of macrophages normally present in the pulmonary capillary bed or to an increased number of macrophages. Studies have shown that the phagocytic activity of an animal varies with the weight of its liver and spleen and that stimulation of the phagocytic activity results in a corresponding increase in liver and spleen weights (*11,12*). In addition, it has been shown that the increased liver weight after stimulation of the macrophage system is secondary to proliferation of Kupffer cells and not hepatocytes (*13*). Although variation in phagocytic activity among macrophages is likely, the large increase in phagocytic activity of one organ in 6 hr as reported by Quinones (*5*) and confirmed here is more likely to be secondary to an increased number of macrophages.

If the increased lung uptake of TcSC is secondary to an increased number of macrophages in the pulmonary capillary bed, the most likely source is from the circulation. Several animal studies have shown that macrophages released into the blood stream are temporarily trapped in the pulmonary capillary bed (*6,14,15*). In addition, when organ weights are used as a measure of response to macrophage system stimulants, the lung weight peaks before the liver and spleen weights (*11*). This finding would be consistent with the migration of macrophages from the bone marrow to the liver and spleen with a temporary stop in the pulmonary capillary bed, in which case the amount of lung uptake of TcSC would be an index of the level of circulating macrophages (blood monocytes). Such an index might be useful as circulating macrophages are difficult to distinguish from lymphocytes histologically and a functional criterion is needed (*16,17*).

Other studies would support three possible mechanisms for an increased number of circulating macrophages (blood monocytes) and thus an increased number of macrophages in the pulmonary capillary bed. Simpson (*6*) showed that repeated intravenous injections of colloids into rabbits resulted in showers of macrophages from the liver and spleen to the lungs, and Schneeberger-Keeley and Burger (*7*) have presented evidence that open-chest ventilation of cats for 1 hr results in the migration of Kupffer cells to the lungs. However, the results presented here indicate that the increased lung uptake of TcSC, which is seen 6 hr after the intraperitoneal injection of endotoxin, is not secondary to the redistribution of previously functioning intravascular macrophages, i.e, macrophage migration from the liver and spleen to the lungs.

A second possible mechanism is an increased rate of release of macrophages from the bone marrow. Under most conditions macrophages originate in the bone marrow from promonocytes, circulate in the blood as monocytes with a half-time of 22 hr, and then become fixed in the liver, spleen, and lungs (alveolar macrophages) or enter the serous cavities such as the peritoneum (*18*). In fact, van Furth and Cohn (*19*) have shown that the injection of 1 ml of newborn calf serum into the peritoneum of mice results in a threefold increase in blood monocytes in 24 hr and that these monocytes can be traced to the bone marrow because of previous labeling with tritiated thymidine.

A third possible mechanism is raised by a recent report of elevated levels of chemotactic-factor inactivator in the serums of patients with Hodgkin's disease (*20*). Macrophages in these patients might be expected to leave the circulation at a reduced rate. If in addition the rate of release of macrophages from the bone marrow is not depressed, an elevated level of circulating macrophages will result and increased lung uptake of TcSC might be expected. However, this mechanism would not be expected to result in a sixfold increase in lung uptake in 6 hr if the half-time of circulating macrophages is normally 22 hr.

From the above considerations, it is suggested that the increased lung uptake of TcSC 6 hr after intraperitoneal endotoxin is most likely secondary to an increased rate of release of macrophages from the bone marrow. However, other mechanisms are possible and further study will be required to determine the correct explanation.

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