Transport of Orally Administered 9, 10-Dimethyl-1, 2-benzanthracene in the Sprague-Dawley Rat

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SUMMARY

Chylomicrons were separated from lymph collected from thoracic duct-cannulated rats receiving 20 mg 9, 10-dimethyl-1, 2-benzanthracene (DMBA) and 25 μCi DMBA-9-14C intragastrically. In order to ascertain the moiety of the chylomicron with which DMBA is associated, the chylomicrons were disrupted by freeze-thawing and subjected to chromatography and autoradiography. These procedures showed that the lipophilic DMBA molecule remains dissolved in the lipid fraction of the chylomicron. In another study, animals were divided into three groups and each rat was given hourly i.v. injections of either 0.9% NaCl solution, 1.0 mg protamine sulfate, or 25 USP units of heparin in a volume of 0.25 ml. Injections were begun 1 hr prior to and continued for 5 hr following the administration of 20 mg DMBA and 25 μCi DMBA-9-14C. DMBA uptake by mammary parenchymal cells of rats receiving either protamine or heparin was similar. However, in both groups, the parenchymal cell uptake of DMBA was significantly less than that of rats given injections of 0.9% NaCl solution. Although heparin was effective in promoting chylomicron lysis, it is apparent that this compound has an additional action. This effect of heparin upon DMBA uptake by mammary gland parenchymal cells may be occurring at the membrane of either the arterial endothelial cell and/or mammary parenchymal cell. These data do indicate that the majority of the DMBA taken up by the mammary gland parenchymal cells is mediated through transport in chylomicrons.

INTRODUCTION

Numerous investigators have observed the development of mammary cancer after the intragastric administration of 3-methylcholanthrene and DMBA² and the response of such chemically induced tumors to a hormonally altered environment (11–13, 19, 24). Following absorption from the gastrointestinal tract, the carcinogens are transported primarily through the lymphatic network to the blood in association with the chylomicrons (2, 5, 15). However, it is not known whether binding of DMBA to chylomicrons occurs or, if it does, what the moiety of the chylomicron (protein, triglycerides, cholesterol, etc.) is with which this compound associates. Hamilton and Jacobson (10), using zone electrophoresis, have shown that at 24 hr postfeeding, approximately 35% of DMBA-3H is bound to albumin, 20% remains with neutral fats and chylomicrons, and the remainder is distributed evenly among the other protein fractions.

Although approximately 40% of p.o. DMBA is absorbed from the gastrointestinal tract (2, 15), Janss and Moon (14) have found that only a small fraction of the carcinogen is concentrated by the mammary parenchymal cells. Therefore, the possibility exists that the carcinogen concentrated by these cells may have arisen from the intravascular lysis of the chylomicrons through activation of the serum heparin: lipoprotein: lipase system. Since this system may be either stimulated or inhibited by the administration of heparin or protamine, respectively, it appeared important to determine whether or not these compounds would modify the uptake of DMBA-9-14C by the mammary parenchymal cells.

MATERIALS AND METHODS

Chylomicron Fractionation. Thoracic duct cannulation was performed on 50-day-old female Sprague-Dawley rats. Each animal was allowed 24 hr to recover before receiving 20 mg nonradioactive DMBA and 25 μCi DMBA-9-14C (Amersham/Searle Corp., Arlington Heights, Ill.; specific activity, 6.53 mCi/mmole) in 1.0 ml of sesame oil via a stomach fistula. Rats were allowed free access to Purina laboratory chow except for the 12-hr period preceding DMBA administration. Lymph was collected from the unanesthetized, restrained animal for 24 hr and the chylomicrons were separated and fractionated by the following modification of the procedure of Zilversmit (25). Effective isolation of chylomicrons from lymph was obtained by centrifugation at 10° in a Sorvall angle-head centrifuge at 30,000 x g for 4 hr. The chylomicrons, suspended in 5 ml of water, were alternately frozen in a mixture of Dry Ice and acetone and thawed to room temperature 10 to 15 times. This process liberated a surface layer of oil, which fluoresced intensely under UV. Ten ml of water were added and the oil phase was separated by centrifugation (700 × g). Subsequent washings of the oil phase with 10-ml aliquots of water (3 to 4 times) removed those chylomicrons that had not been disrupted. To precipitate any remaining protein, we extracted the oil phase with 10 ml of ethanol:ethyl ether.

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2The abbreviations used are: DMBA, 9, 10-dimethyl-1, 2-benzanthracene; DFFT, dry, fat-free tissue.

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The precipitated protein fraction was placed in a scintillation vial, digested with 0.5 M NaOH, and counted for radioactivity. Samples of the evaporated ethanol:ethyl ether extract (chylomicron lipids), corn oil, DMBA-9-14C diluted with sesame oil, sesame oil, and DMBA-9-14C were dissolved in benzene and applied to thin-layer Silica Gel G plates (20 x 20 cm, 20 μm thick). The plates were developed in tanks containing a solvent system of Skellysolve B:benzene (50:50, v/v). Lipid spots were visualized by spraying the plates with glacial acetic acid : water : sulfuric acid (97:2:1, v/v/v) and charring at 200°C for 4 hr. Autoradiography was accomplished by exposing a sheet of Kodak No-Screen X-ray film to the chromatogram for 48 hr prior to photographic development.

**Heparin and Protamine Administration.** In another experiment, the right external jugular vein of 48-day-old female rats was cannulated under ether anesthesia by the method of Popovic and Popovic (22). The distal end of the cannula was passed beneath the skin and exteriorized through a small skin incision located between the scapulae. The cannula was flushed with 0.9% NaCl solution and then sealed by inserting the pointed tip of a straight pin (Dorcas).

Four days following the cannulation, the rats were divided into 3 groups (5 rats/group) and each rat was given hourly i.v. injections of either 0.9% NaCl solution, 1.0 mg protamine sulfate, or 25 USP units heparin in a volume of 0.25 ml. Injections were begun 1 hr before and continued for 5 hr after the gastric intubation of DMBA-9-14C.

One hr after the last injection, the animals were sacrificed and the various tissues were analyzed for either total lipid content or radioactivity. Total lipid of the plasma, perirenal fat, mammary fat pad, and parenchymal cell intracellular lipid was determined by the method of Folch et al. (9). Details for the removal of the tissue fractions, the separation of mammary parenchymal cells from adipose tissue, and the measurement of radioactivity have been reported previously (14, 20). The Student t test was performed for determining the difference between means and all reported differences at a level of confidence of 95% or greater were considered to be statistically significant.

**RESULTS**

Samples of a benzene solution of corn oil, chylomicron lipids from animals fed DMBA-9-14C, DMBA-9-14C dissolved in sesame oil, sesame oil, and DMBA-9-14C were subjected to thin-layer chromatography (Chart 1). The solvent system used allowed only minimal migration of the various lipid fractions (Rf values from 0.0 to 0.2), while the DMBA molecule exhibited an Rf value of 0.6. Corn oil was chromatographed to illustrate the migration of triglycerides. Both chromatography and autoradiography (Chart 2) suggest that DMBA-9-14C remains as an intact molecule in the lymphatic system and that DMBA-9-14C does not associate with any particular lipid fraction. In addition, the autoradiograph revealed an unidentified contaminant near the origin of the DMBA-9-14C sample which was not visible in samples of either the chylomicron lipid or DMBA-9-14C dissolved in sesame oil. Radioactivity was not detectable in the protein fraction of the chylomicron.

The total lipid content of plasma, mammary fat pad, and perirenal fat of rats receiving either 0.9% NaCl solution, protamine, or heparin is shown in Table 1. Although the administration of heparin or protamine did not alter the lipid content of the mammary fat pad or the perirenal fat, the lipid content of the fat pad and perirenal fat between the 3 groups was significantly different. However, the plasma lipid content of the rats receiving protamine increased 36% while that of the heparinized animals decreased 28% when compared to 0.9% NaCl solution controls.

The specific activity of the various mammary gland fractions and other tissues at 6 hr after feeding of DMBA-9-14C is shown in Table 2. The specific activity of the perirenal fat from the protamine group was 40% less than that of the 0.9% NaCl solution group, while the mammary gland fat cell fraction showed a decrease of 54%. Furthermore, the specific activity of the parenchymal cell lipid fraction of rats receiving protamine was 49% less than that of the 0.9% NaCl solution group. The specific activity of the fat pad, vascular area, and parenchymal cells (tissue relatively low in lipid content) showed a decrease of 17, 23, and 26%, respectively, when compared to the corresponding tissues of animals given 0.9% NaCl solution. The mammary parenchymal DFT from animals receiving protamine exhibited a 58% reduction in specific activity from those given 0.9% NaCl solution.

The gastrointestinal tract and contents of animals receiving protamine contained 12% less radioactivity than that of rats given 0.9% NaCl solution (Table 2). This table also shows that the specific activity of the lipid extracted from the plasma of animals receiving protamine increased 54%. Although the plasma level of carcinogen increased in rats given protamine, liver content decreased 25% when compared to the 0.9% NaCl solution controls.

The specific activity of the various mammary gland fractions of the heparin-treated rats was similar to that exhibited by those fractions obtained from protamine-treated animals (Table 2). In addition, the specific activity of the liver and the gastrointestinal tract and contents between the protamine and heparin groups did not differ. However, the percentage of total lipid of the plasma of the heparinized rats was 28 and 47% less than that of the plasma of the 0.9% NaCl solution and protamine groups, respectively (Table 1).

**DISCUSSION**

Earlier studies (6, 8) have suggested that hydrocarbon-induced neoplastic alteration of mammary parenchymal cells is subsequent to uptake and release of the carcinogen by mammary fat cells. However, Janss and Moon (14, 16) have demonstrated that an increase in the concentration of DMBA in the mammary gland parenchymal cells does not occur while the concentration of this carcinogen in mammary fat cells is decreasing. Rather, the binding of DMBA...
Transport of p.o. DMBA

Chart I. Thin-layer chromatogram of benzene extracts of corn oil, chylomicron (chylo.) lipids obtained from a rat fed 20 mg DMBA plus 25 μCi DMBA-9-14C in 1 ml sesame (ses.) oil by gastric intubation, DMBA-9-14C dissolved in sesame oil, sesame oil, and DMBA-9-14C. Chromatogram was developed in a tank containing Skellysolve B: benzene (50:50, v/v). F, front; O, origin.

To parenchymal cell DNA and cytoplasmic proteins appears to be dependent upon the uptake of carcinogen by the parenchymal cell intracellular lipid. Janss and Moon (16) have also shown that the level and time of binding of DMBA can be altered by either ovariectomy or hypophysectomy. Furthermore, Janss et al. (17) found that DMBA binding to mammary parenchymal cell protein was undetectable 14 days after feeding, whereas binding to DNA was still evident at 42 days after intragastric administration of the hydrocarbon.

It is generally accepted that lipids are hydrolyzed in the gastrointestinal tract and absorbed into the mucosa cells primarily as monoglycerides and fatty acids. Before entering the intestinal lymphatics, these substances are resynthesized into triglycerides and encapsulated with a protein coating to form the chylomicron. Apparently, within this chain of events, approximately 40% of the p.o. dose of DMBA becomes associated with the chylomicrons (2, 15). After entrance into the blood, the chylomicrons are lysed by the heparin:lipoprotein:lipase system with release of its various components. An alteration of this system, resulting from the administration of exogenous heparin or protamine, could result in either an increase or decrease, respectively, of chylomicron breakdown and DMBA release.

In the present study, rats receiving protamine showed a significant increase in plasma lipid content while heparinized animals exhibited decreased quantities of plasma lipids when compared to 0.9% NaCl solution-treated rats. These data agree with that of Bragdon and Havel (4) and demonstrate the effect of these 2 compounds on the lysis of chylomicrons. The greater specific activity of the plasma lipid and lower activity of the other tissues studied after protamine administration suggest that DMBA was still associated with the chylomicrons. Although the mammary parenchymal cells were exposed to high plasma levels of DMBA, the compound was apparently “trapped” within the chylomicron and thus was not available for transport through the cell membrane. The decreased binding of DMBA to the parenchymal cell DFFT of animals receiving protamine probably resulted from the low level of the carcinogen found in the intracellular lipid fraction.

The data obtained from the plasma lipid determination indicate that heparin was effective in promoting lysis of the plasma chylomicrons by activation of the heparin:lipoprotein:lipase system. However, the increased lysis of chylomicrons did not result in an increased uptake of DMBA by the mammary parenchymal cells or by the other tissue samples. In fact, the opposite was true. The specific activity of the parenchymal lipid and parenchymal DFFT would appear to indicate that binding of DMBA to cellular components is probably not impaired but that the decrease

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<th>Lipid content of plasma, perirenal fat, and mammary fat pad of rats receiving 0.9% NaCl solution, protamine, or heparin</th>
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* Mean ± S.E.
in parenchymal DFFT is a reflection of the decrease in parenchymal lipid. This suggested a failure in the transport of the carcinogen into the parenchymal cell and indicated that heparin exerts an effect on DMBA uptake in a manner other than that of increasing serum lipoprotein:lipase activity.

Utilizing ascites tumor cells, it has been demonstrated that the intact plasma membrane acts as a barrier to heparin penetration and that, unless cell injury occurs, heparin must exert its effect at this level (23). Norman and Norrby (21) have observed that heparin, injected i.v. or s.c. in small amounts to rule out osmotic effects, can alter the cell volume of tumor cells and blood cells. Ambrose (1) has also shown that the highly negatively charged heparin molecule will cause expansion of the isolated nuclear membrane; however, in the presence of calcium or magnesium ions, contraction of the membrane will occur. A similar effect is thought to occur at the cell membrane and, because of the change in surface area, a change in permeability apparently occurs. Lazzarini-Robertson (18) has shown that heparin is extremely effective in inhibiting the incorporation of labeled serum-bound lipids by arterial endothelial cells in vitro. Since DMBA is transported in conjunction with a serum lipid or lipoprotein fraction, the effect of heparin upon DMBA uptake by parenchymal cells may be occurring at the level of either the endothelial cell membrane and/or the mammary parenchymal cell membrane.

Since the level and time of peak uptake of DMBA by the mammary gland has been shown to be important for cancer induction (7), the interference with the mechanism(s) responsible for transport of this carcinogen by either heparin or protamine could alter subsequent tumor development.

ACKNOWLEDGMENTS

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REFERENCES


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