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Subcutaneous and Intraperitoneal Lipogranulomas Following Subcutaneous Injection of Olive Oil in Sprague-Dawley Rats

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Abstract

Olive oil is commonly employed as a solubilizing agent for lipophilic materials in preclinical studies in rodents. Here we report that following subcutaneous (SC) injection of olive oil to Sprague-Dawley (SD) rats, local SC lipogranulomas formed, which were associated with an unusual location of the same changes in the peritoneum. Macroscopically, multifocal white spots were found over the liver and mesentery. Histologically, lipid granulomas were seen in the SC injection site, as well as on the capsular or serosal surface of the abdominal organs. No abnormal clinical signs were noted except for swelling at the injection site. The olive oil may have reached the peritoneal cavity from the SC tissue passively via the lymphatic vessels or actively after engulfment by antigen-presenting cells via the lymphatic or blood vessels. These findings are of particular importance for drug safety assessments, as the occurrence of lipogranulomas in locations distant from the site of administration may lead to misinterpretation of histological results. We suggest that these aberrations may be induced by the administration of olive oil as a vehicle.

Keywords: lipogranuloma; olive oil; rats.

When performing preclinical assessments of potential new drugs, few or no effects should be attributable to the vehicle used to produce the formulation (Gad et al. 2006). Reporting any adverse clinical or histopathological effects caused by the vehicle compounds themselves therefore holds great importance. Vegetable oils are employed commercially in parenteral formulations owing to their ability to solubilize very lipophilic materials (Strickley 2004). Olive oil, specifically, is commonly used as a solubilizing vehicle in many preclinical studies in rodents (Sugiura et al. 2006). Although usually considered a safe and nontoxic compound, vegetable oil injected subcutaneously in humans and rats has caused cases of lipogranuloma (Marsden 1958), which is a foreign-body reaction around a deposit of injected material containing an oily substance (Mosby 2009). Formation of granulomas in distant peripheral organs following SC injection is, however, rare. We report the unusual formation of lipogranulomas in the peritoneal cavity of male and female SD rats following SC administration of olive oil as vehicle.

Five male and five female eight- to nine-week-old rats (280 $\pm$ 77.7 g) (Harlan Laboratories Ltd., Israel) were assigned to the vehicle group of a twenty-eight-day repeated SC toxicity study. They were maintained on standard chow (Harlan Teklad diet 2018S, Madison, WI, USA) and allowed free access to drinking water supplied to each cage via polyethylene bottles with stainless steel sipper tubes. The water was filtered (0.1 μm), chlorinated, and acidified. During the acclimation and throughout the entire study, animals were housed within a limited-access rodent facility and kept (a maximum of three rats/cage) in polypropylene cages (37.5 × 21 × 18 cm) fitted with solid bottoms and filled with wood shavings as bedding material. They were allowed at least a five-day acclimation period to facility conditions (20–24°C, 30–70% relative humidity, twelve-hour light/dark cycle) prior to inclusion in the study. All animal care and procedures were performed at a GLP-certified site (Harlan Biotech Israel Ltd., Rehovot, Israel) and in compliance with the OECD Principles of Good Laboratory Practice (revised 1997). The study was approved by the Committee for Ethical Conduct in the Care and Use of Laboratory Animals of the Hebrew University, Jerusalem, Israel.

Animals were injected on alternate days with 4 mL/kg olive oil (Sigma-Aldrich, O1514) and killed on day 28; they received a total of 14 injections, administered in a rotating fashion between the right and left flanks. All rats were observed for abnormal clinical signs once daily. At twenty-eight days following the first injection, animals were euthanized by CO_2 asphyxiation. Blood for hematology and biochemistry parameters was collected by retro-orbital sinus bleeding under light CO_2 anesthesia just prior to euthanasia. Complete necropsy and macroscopic examinations were performed on all animals. Samples from the following tissues and organs were collected and fixed in 10% neutral-buffered formalin: adrenals; aorta; brain; cecum; colon; duodenum; epididymides; femur; knee joint; heart; ileum; jejunum; kidneys; lacrimal gland; liver; gall bladder; lungs; mandibular, mesenteric, and inguinal lymph nodes; mammary gland; skin; esophagus; ovaries; pancreas;

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Abbreviations: APC, antigen-presenting cell; PAS, periodic acid-Schiff; SC, subcutaneous.

pituitary; prostate; rectum; salivary gland; sciatic nerve; seminal vesicles; skeletal muscle (left thigh); spinal cord; spleen; sternum with bone marrow; stomach; testes; thymus; thyroid and parathyroids; tongue; trachea; urinary bladder; uterus with cervix and vagina; and all injection sites. The eyes, optic nerve, and Harderian glands were fixed in Davidson's solution. Tissues were trimmed, embedded in paraffin, sectioned at approximately 5 μm thickness, and stained with hematoxylin and eosin (H&E). In addition, selected slides were stained with conventional Fontana-Masson and periodic acid–Schiff (PAS). Described histopathological changes were scored using a semiquantitative grading, based on Shackelford et al. (2002), taking into consideration the overall estimate of severity of the changes using a five-grade system, as follows: 0 = no lesion, 1 = minimal change, 2 = mild change, 3 = moderate change, 4 = marked change. The severity grades were determined by a relative estimate of the tissue involvement, as well as the magnitude of the specific granulomatous inflammatory cells present (e.g., composed of mixed collections of fat-storing histiocytes, mononuclear cells, and fibrotic septa).

No mortality occurred in any of the animals prior to termination of the scheduled study. Swelling at either the right or left injection sites was evident in most males and females following the first injection. Macroscopically, all animals exhibited increased thickness of the subcutis at injection sites, as well as multifocal white spots (approximately 1 mm in diameter) spread over the liver and mesentery (Figure 1A, B). Histologically, lipogranulomas were seen in different regions, such as the SC injection site, as well as the capsular or serosal surface of abdominal organs, such as the liver, spleen, pancreas, prostate, ovaries, stomach, duodenum, jejunum, ileum, and urinary bladder (Table 1, Figure 1C–F). The lipogranulomas appeared in the form of ovoid drops or irregular nodes and consisted of variably sized collections of unilocular large or multilocular fine fat-storing histiocytes, intermixed with histiocytes devoid of cytoplasmic fat droplets and sporadically present lymphocytes and plasma cells. Sometimes, focal fibrotic septa could be seen. The nodules were usually devoid of real capsular formation, but in some cases, mostly on the liver, a fibrotic capsule was present along a segment of the free (abdominal) surface or at the site of attachment to the liver surface.

No extension of inflammation or infiltration by histiocytes into the adjacent organs was noted. Frequently, multiple large extracellular empty vesicles were present, which apparently formed as a result of coalescence of fat vesicles from neighboring cells. These extracellular vesicles were particularly large at the SC injection site. Selected slides were stained with PAS and Fontana-Masson to detect lipofuscin pigment. Since all slides were negative for these stainings (data not shown), the possibility of lipogranuloma formation as a consequence of fat degeneration was discounted. There was no statistical difference between male and female rats. No histopathological changes were noted in lymphoid organs, such as the mandibular, mesenteric, and inguinal lymph nodes; spleen; and thymus.

Organ weights were compared to those in animals injected with SC saline from a previous, different experiment performed under the same conditions and using rats of a similar strain and age. A significant increase in mean liver and prostate weights ($p < .01$; Student t test) was detected in male rats from our study compared to the male rats from the previous study.

Lipogranuloma develops from a foreign-body reaction to administered mineral or vegetable oil, involving macrophages that react to the foreign material by surrounding it and forming giant multinucleated cells (Di Benedetto et al. 2002). This kind of granuloma is commonly seen in humans as a result of self-injection, either intramuscularly or subcutaneously, in order to augment or improve bodily contours (Darsow et al. 2000; Di Benedetto et al. 2002; Georgieva et al. 2003). Lipogranulomas probably occur more commonly after SC than intramuscular injection (Darsow et al. 2000). Lipogranulomas have also been reported in the peritoneal cavity of rodents after intraperitoneal injections of oil (Nacionales et al. 2006; Potter et al. 1994; Shaheen et al. 1999).

The means by which the olive oil traveled from the SC tissue to the peritoneal cavity in our case are not completely understood. The lymphatic vessels may constitute one possible channel. That lymphatic vessels react by sending sprouts that grow toward injected olive oil has been known for many years (Clark and Clark 1917). Supporting that finding is the discovery of silicone in regional lymph nodes after SC administration in mice (Ben-Hur et al. 1967). This trafficking through the lymphatic system may be mediated by antigen presenting cells (APCs), such as Langerhans cells residing in the dermis. The reaction of cells of the immune system to engulf olive oil in the skin was documented long ago (Clark and Clark 1917), and their migratory path was followed after injection of AK-5 tumor cells, which were engulfed by APCs, delivered to regional lymph nodes, and moved from there to the peritoneum (Mitra et al. 2004), a process that took a total of three days. Following these results, researchers suggested that the peritoneum functions as an organ with lymphoid characteristics, where the APCs perform their antigen presentation function and elicit an immune reaction (Mitra et al. 2004). The trafficking of foreign materials through the lymphatic system to the peritoneal cavity was also suggested after SC injection of asbestos fibers (Roe et al. 1967). However, since macrophages containing poly(lactide-co-glycolide) microspheres were detected in a peritoneal flush in mice as soon as ten minutes after SC injection (Peyre et al. 2004), movement may occur by a quicker route, presumably the circulatory system. A summary of the proposed trafficking methods of the subcutaneously injected olive oil is presented in Figure 2.

Despite developing lipogranulomas intraperitoneally, animals did not show any related adverse clinical effects. White blood cell counts were comparable to those observed in rats in a similar study performed with subcutaneously injected saline. These results suggest that despite being a prominent histological finding, intraperitoneal lipogranulomas probably do not have significant clinical implications.

We have described the occurrence of lipogranulomas in SC tissue and the peritoneal cavity following SC injection of olive oil to SD rats. The unusual peritoneal location is highly

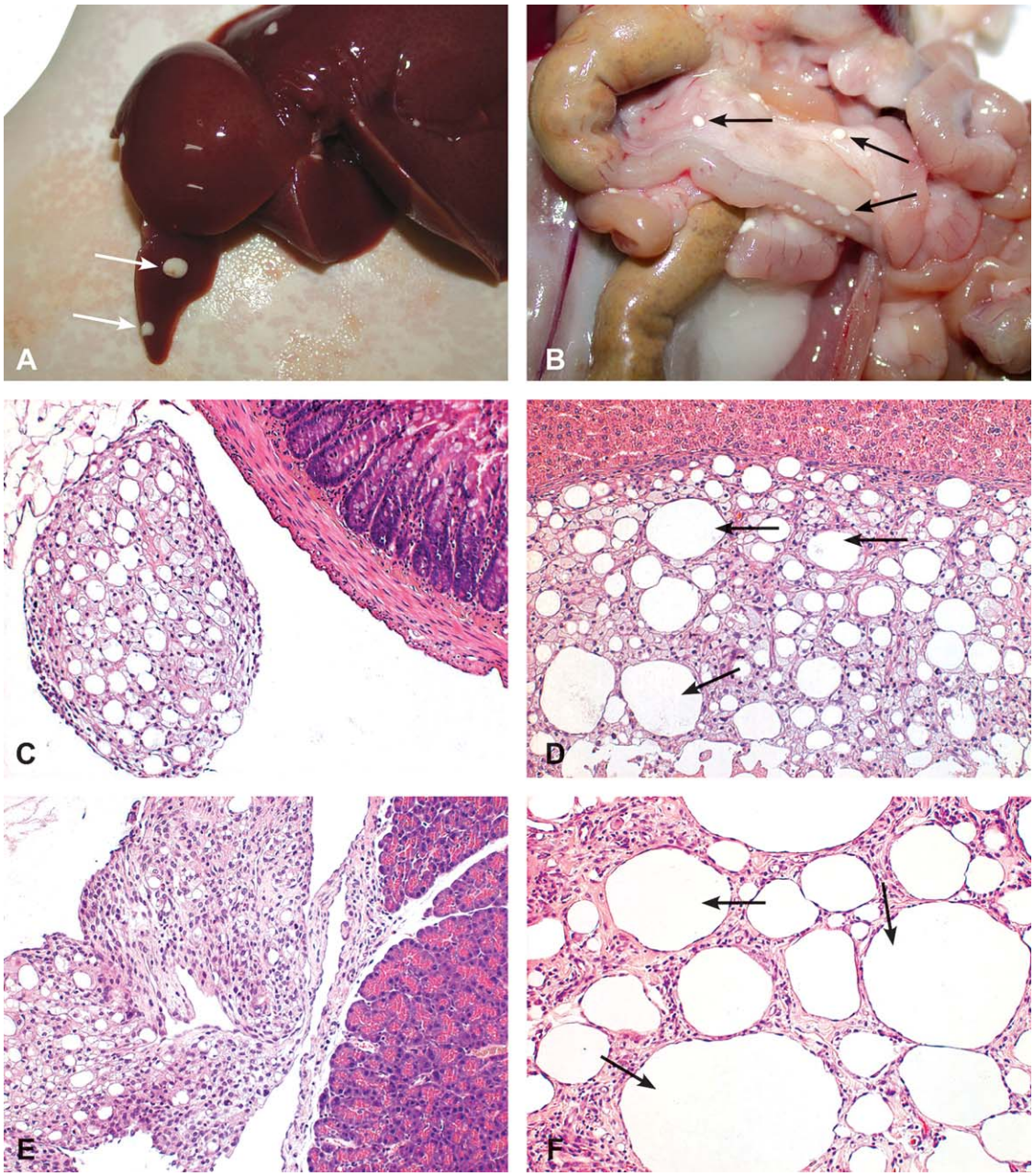

FIGURE 1.—(A, B) Macroscopic aspect of multifocal white spots (diameter approximately 1 mm, arrows) spread over the liver (A) and mesentery (B). (C) Histological section of ovoid lipogranulomas attached to the serosal surface close to the jejunum. Note the collection of unilocular large or multilocular fine fat-storing histiocytes, intermixed with histiocytes devoid of cytoplasmic fat droplets, and sporadically present lymphocytes and plasma cells. Multiple large extracellular empty vesicles (owing to washout of lipid during histological processing) can also be seen. H&E, 10×. (D) Histological section of ovoid lipogranuloma attached to the liver capsule with multiple large extracellular empty vesicles (arrows). H&E, 10×. (E) Histological section of irregular nodular lipogranuloma attached to the serosal surface close to the pancreas. H&E, 10×. (F) Histological lipogranuloma located in the subcutis. Note multiple large extracellular empty vesicles (arrows) surrounded by relatively abundant fibrotic septa. H&E, 10×.

important for drug safety assessments, as olive oil is a common vehicle for drug administration. We suggest that olive oil used as a vehicle may, in distant peripheral tissues, cause lipogranulomas, which should not be attributed to the tested drug.

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TABLE 1.—Mean severity and incidence of histopathological findings observed in male and female rats.

	Lesion grading (number affected / total number of animals)	
Tissue / Organ	Males	Females
Duodenum	0.0 (5/5)	0.0 (4/5)
Serosa: lipid granuloma, multifocal		0.2 (1/5)
Ileum	0.0 (4/5)	0.0 (5/5)
Serosa: lipid granuloma, multifocal	0.2 (1/5)	
Injection site: right and left flank (skin)		
Subcutis: lipid granuloma, multifocal	1.8 (5/5)	2.0 (5/5)
Jejunum	0/0 (5/5)	0.0 (4/5)
Serosa: lipid granuloma, multifocal		0.2 (1/5)
Liver	0.0 (1/5)	0.0 (2/5)
Capsule: lipid granuloma, multifocal	1.4 (4/5)	1.2 (3/5)
Ovary	–	0.0 (3/5)
Serosa: lipid granuloma, multifocal		0.6 (2/5)
Pancreas	0.0 (2/5)	0.0 (4/5)
Serosa: lipid granuloma, multifocal	1.2 (3/5)	0.2 (1/5)
Prostate	0.0 (4/5)	–
Serosa: lipid granuloma, multifocal	0.4 (1/5)	
Spleen	0.0 (1/5)	0.0 (3/5)
Capsule: lipid granuloma, multifocal	1.0 (4/5)	0.4 (2/5)
Stomach	0.0 (2/5)	0.0 (2/5)
Serosa: lipid granuloma, multifocal	0.8 (3/5)	1.0 (3/5)

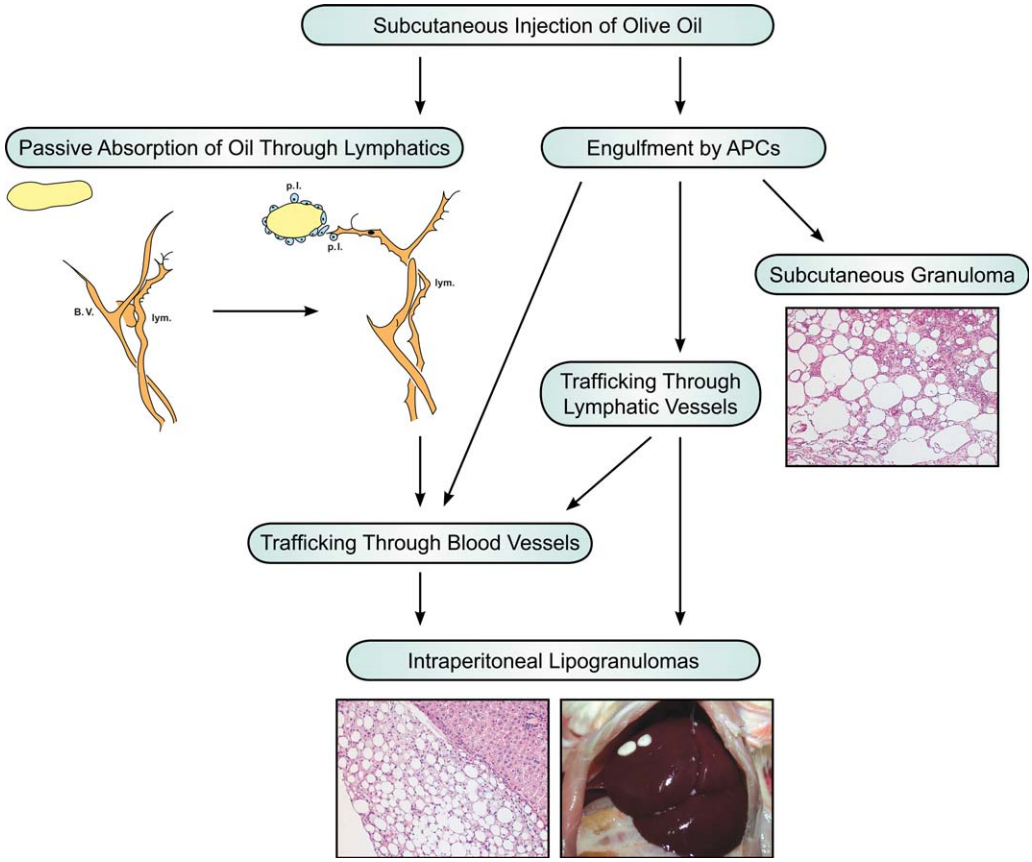

FIGURE 2.—Schematic describing possible trafficking routes by which olive oil may induce granuloma formation in the peritoneum.

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