Response of Hepatocytes Transplanted into Syngeneic Hosts and Heterotransplanted into Athymic Nude Mice to Peroxisome Proliferators


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ABSTRACT

The development of a transplantation system by which rat hepatocytes can be implanted and remain viable in the dorsal fascia of two-thirds hepatectomized syngeneic hosts provides an opportunity to examine whether such transplanted hepatocytes retain the capacity to recognize and respond to the peroxisome proliferators 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropionic acid (ciprofibrate), a hypolipidemic drug, and di-(2-ethylhexyl)phthalate (DEHP), an industrial plasticizer. Male F344 rats with transplanted rat hepatocytes were fed a control diet or a diet containing either 0.05% ciprofibrate (w/w) or 2% DEHP (v/w). After 4 weeks, the animals were sacrificed, and transplanted hepatocytes as well as pieces of homotopic (host) rat liver were processed for electron microscopy and for the cytochemical localization of catalase. Morphometric analysis of transplanted hepatocytes revealed a significant increase in the numerical density of peroxisomes in both ciprofibrate- and DEHP-fed rats. The volume density of peroxisomes in transplanted hepatocytes increased 9.2- and 5.3-fold, respectively, in ciprofibrate- and DEHP-fed rats, whereas the volume density of mitochondria remained essentially unchanged. The magnitude of increase in peroxisome volume density in transplanted hepatocytes was comparable to increases in the volume density of these organelles in the liver parenchymal cells of syngeneic hosts. The present study also demonstrates that hepatocytes isolated from cat liver and heterotransplanted into partially hepatectomized athymic nude mice retain their biological integrity and respond to the peroxisome proliferative effect of ciprofibrate. This observation suggests the possibility that hepatocytes obtained from small segments of liver of humans, primates, and other species and heterotransplanted into nude mice might provide an opportunity to examine whether such transplanted hepatocytes retain the capacity to recognize and respond to other xenobiotic agents in a manner analogous to that of hepatocytes in the liver.

INTRODUCTION

The system of transplantation of hepatocytes into the interscapular fat pads of partially hepatectomized syngeneic hosts was developed as a potential tool to quantitate the clonogenic potential of hepatocytes exposed either in vivo or in vitro to physical and chemical agents (6). Although this system has been used to determine survival of liver cells exposed to ionizing radiation (8) and to assess the response of transplanted hepatocytes to the mitogenic stimulus following partial hepatectomy in the host (7), it is not certain whether these extrahepatic hepatocytes retain their biological integrity and respond to chemical carcinogens and other xenobiotic agents in a manner analogous to that of hepatocytes in the liver.

The objective of the work described here was to assess the response of rat liver cells transplanted into syngeneic hosts to peroxisome proliferators ciprofibrate,³ a hypolipidemic drug, and DEHP, a widely used industrial plasticizer (19, 22). Exposure of rodents and certain nonrodent species to peroxisome proliferators is predictably associated with a profound increase in peroxisomes in parenchymal cells of the liver (2, 20, 21, 24), hepatocyte-like cells in hamster pancreas (17), and to a lesser extent in the proximal tubular epithelium of kidney (10). The tissue specificity of this response suggests the possible role of a receptor(s) which can bind these peroxisome proliferators and mediate the subsequent enhanced expression of peroxisomal enzymes (9). A positive response of transplanted hepatocytes to these chemicals, therefore, would reflect their structural and functional integrity. In this study, we also examined the feasibility of using hepatocytes heterotransplanted into nude mice for assessing their ability to respond to a peroxisome proliferator.

MATERIALS AND METHODS

Animals. Male Fischer 344 rats (weighing approximately 100 g), obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA, were used as hepatocyte donors and syngeneic recipients. Male athymic nude mice (BALB/c strain) were also obtained from the above source and served as hepatocyte recipients for feline hepatocytes prepared from an adult male mongrel cat. The animals were housed in temperature- and humidity-controlled rooms with an automatic 12-hr light and dark cycle.

Hepatocyte Preparation and Transplantation. The hepatocytes from rat liver were dispersed by collagenase using the in situ 2-step collagenase perfusion technique which was described previously (8). The dispersed hepatocytes in L-15 medium (Grand Island Biological Co., Grand Island, NY) were mixed with an equal volume of 50% rat brain homogenate (1:1, w/v, in L-15 medium). A volume of 0.06 ml of this suspension containing approximately 1 x 10⁶ hepatocytes was injected into the interscapular fat pads of syngeneic male F344 rats, in which two-thirds...
partial heptectomy (3) was performed 1 to 2 hr before (8). As reported elsewhere (8), brain homogenate improves the clonogenicity or transplantability of hepatocytes.

The hepatocytes from a 2- to 3.5-cm piece of cat liver survived by a hepatic capsule on 3 sides were dispersed according to the procedure outlined for the isolation of human hepatocytes from surgically removed segments of liver (23). These cells were transplanted into two-thirds partially heptactomized athymic nude mice as described previously (8). These procedures were performed in the laboratories of R. L. Jirtle and G. Michalopoulos at Duke University Medical School, and the animals were shipped to the laboratory of J. K. Reddy at Northwestern University Medical School within 1 week following hepatocyte transplantation.

Administration of Peroxisome Proliferators. Groups of 6 to 8 rats with transplanted syngeneic hepatocytes were fed ciprofibrate (Sterling-Winthrop Research Institute, Rensselaer, NY) or DEHP (Eastman Kodak Co., Rochester, NY) at 0.05% (w/w) and 2% (v/v), respectively, in powdered chow for 4 weeks (11, 21). Three athymic nude mice with transplanted cat hepatocytes were fed ciprofibrate at 0.05% (w/w) for 14 days. Six control rats and 3 nude mice with transplanted hepatocytes were fed chow without added chemicals. After the treatment period, animals were killed by cervical dislocation under ether anesthesia.

Morphology and Morphometry. Brown patches (~1 to 2 mm in diameter) representing transplants of hepatocytes, were removed from the interscapular fat pads of rats and athymic nude mice. These transplants as well as small segments of homotypic (host) liver of these animals were minced into small fragments and fixed in 2.5% glutaraldehyde in 0.15 M sodium cacodylate, pH 7.4, and processed for electron microscopy (11, 20). For the cytochemical localization of peroxisomal catalase (15), tissues fixed in glutaraldehyde for 4 hr were processed as described previously (17, 20). One-μm-thick sections of Epon-embedded liver tissue were examined in a Zeiss Ultraphot III microscope unstained or after counterstaining lightly with toluidine blue. Thin sections were examined in a JEOL JEM-100 CX II electron microscope at an accelerating voltage of 60 kV.

Morphometric analysis of alterations in volume density of peroxisomes and mitochondria in transplanted hepatocytes and in the host liver of rats fed control diet and diet containing peroxisome proliferator was carried out according to the method outlined by Weibel (25) as described elsewhere (11, 13, 17). The numerical density of peroxisomes in transplanted rat hepatocytes was determined by counting the number of peroxisome profiles and is expressed as number of peroxisomes per sq μm of cytoplasm.

RESULTS

Rat Hepatocytes Transplanted into Rats. Histological examination of hematoxylin-eosin-stained sections of transplantation sites in rats fed normal chow revealed sheets or groups of hepatocytes as described previously (6). In rats fed either ciprofibrate or DEHP for 4 weeks, the clusters of transplanted hepatocytes were hypertrophic with abundant eosinophilic, granular cytoplasm and prominent nucleoli. The appearance of transplanted hepatocytes in semithin (1-μm-thick) Epon-embedded tissue sections is illustrated in Fig. 1. The individual hepatocytes appear normal and display trabecular or acinar configurations. These clusters are surrounded by connective tissue or adipocytes (Fig. 2).

Peroxisomes in these transplanted hepatocytes stained positively for the peroxidatic activity of catalase by the alkaline 3,3'-diaminobenzidine cytochemical method. They appear as black dots in the cytoplasm in black and white illustrations of semithin sections (Figs. 2 to 4). Numerous intensely stained peroxisomes were present in the cytoplasm of transplanted hepatocytes of rats fed ciprofibrate (Fig. 3) and DEHP (Fig. 4), when compared to untreated controls (Fig. 2). Electron microscopic features of transplanted hepatocytes of control and peroxisome proliferator-fed rats are illustrated in Figs. 5 to 7. Ultrastructurally, transplanted hepatocytes from animals that were fed control diet revealed morphological features characteristic of normal rat hepatocytes, with few peroxisomes (Fig. 5). These transplanted hepatocytes showed a characteristic peroxisome-proliferative response following ciprofibrate (Fig. 6) or DEHP feeding (Fig. 7) to recipient animals. The extent of peroxisome-proliferative response in hepatocytes located in the transplantation sites appeared similar to that noted in the hepatic parenchymal cells of the host liver.

Morphometric analysis (Table 1) demonstrated that ciprofibrate caused a 9.2-fold increase in the volume density of peroxisomes in transplanted rat hepatocytes, whereas DEHP administration resulted in a 5.3-fold increase in peroxisome volume density. The increase in the collective peroxisomal volume in transplanted hepatocytes of rats fed either ciprofibrate or DEHP was accompanied by an increase in the numerical density of peroxisomes. In recipients fed normal chow, the peroxisome number was 9.1 ± 1.3/100 sq μm of cytoplasm as compared to 72 ± 15.7/100 sq μm and 37 ± 9.6/100 sq μm, respectively, in recipients fed ciprofibrate and DEHP. The size distribution of peroxisomes in transplanted hepatocytes from recipients fed normal and peroxisome proliferator-containing diet is illustrated in Chart 1. The results show a small increase in the average peroxisome radius in animals treated with peroxisome proliferators, which also contributed to the change in the collective peroxisomal volume. Administration of these compounds caused no significant changes in the volume density of mitochondria. Table 1 also presents data on the changes in peroxisome and mitochondrial volume densities in hepatic parenchymal cells of liver of the rats bearing transplanted hepatocytes.

Qualitative evaluation of the distribution of peroxisomes (microperoxisomes) in fat cells (5, 16) surrounding the hepatocytes Table 1

Morphometric analysis of the effect of the hypolipidemic drug ciprofibrate, and plasticizer DEHP, on peroxisome proliferation

Male F344 rats with transplanted rat hepatocytes or athymic nude mice with transplanted cat hepatocytes were fed a diet containing either ciprofibrate (0.05%) or DEHP (2%) for 4 weeks. Approximately 14 electron micrographs of randomly selected areas of host liver cell cytoplasm or cytoplasm of hepatocytes transplanted in the interscapular fat pads from each group (4 animals/group) were subjected to morphometric measurement as described by Weibel (25).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mitochondria</th>
<th>Peroxisomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat liver Control</td>
<td>18.0 ± 3.88</td>
<td>1.8 ± 0.62</td>
</tr>
<tr>
<td>Ciprofibrate</td>
<td>21.4 ± 3.12</td>
<td>16.5 ± 2.19</td>
</tr>
<tr>
<td>DEHP</td>
<td>17.4 ± 1.56</td>
<td>10.4 ± 0.39</td>
</tr>
<tr>
<td>Transplanted rat hepatocytes Control</td>
<td>19.0 ± 4.15</td>
<td>1.8 ± 0.98</td>
</tr>
<tr>
<td>Ciprofibrate</td>
<td>22.7 ± 4.83</td>
<td>16.6 ± 6.00</td>
</tr>
<tr>
<td>DEHP</td>
<td>23.3 ± 2.57</td>
<td>9.5 ± 1.99</td>
</tr>
<tr>
<td>Transplanted cat hepatocytes Control</td>
<td>11.7 ± 1.92</td>
<td>2.4 ± 1.5</td>
</tr>
<tr>
<td>Ciprofibrate</td>
<td>12.8 ± 2.79</td>
<td>9.5 ± 2.6</td>
</tr>
</tbody>
</table>

a Points overlying cytoplasm, mitochondria, and peroxisomes were determined to obtain the volume density of mitochondria and peroxisomes. The values are expressed as the percentage of cytoplasmic volume.

b Mean ± S.D.

c Significantly different from controls, p < 0.001 (Student’s t test).
at transplantation sites showed no alteration in the number of these organelles in rats fed peroxisome proliferators (Fig. 8) when compared to controls.

Cat Hepatocytes Transplanted into Athymic Nude Mice. Hepatocytes isolated from cat liver, when injected into the interscapular fat pads of two-thirds hepatectomized athymic nude mice, formed discrete brown nodules. Fig. 9 illustrates the appearance of clusters of transplanted cat hepatocytes. These cells are rich in glycogen and contain few peroxisomes, some of which display a nucleoid and marginal plate (Fig. 10) characteristic of this species (4). A marked increase in the number of peroxisomes was observed in these transplanted cat hepatocytes when nude mice with these transplants were fed ciprofibrate at the 0.05% level in the diet for 14 days (Figs. 11 and 12). The results of morphometric analysis of changes in peroxisome and mitochondrial volume density in transplanted feline hepatocytes in normal and ciprofibrate-fed nude mouse hosts are presented in Table 1.

Electron microscopic examination of the homotopic liver of ciprofibrate-fed athymic nude mice with transplanted cat hepatocytes revealed peroxisome proliferation in hepatocytes similar to that reported previously in rats and mice (12, 19, 24).

DISCUSSION

The results of these studies demonstrate clearly the potential value of the transplanted hepatocytes as a system to investigate the effects of xenobiotics. This paper illustrates that hepatocytes transplanted at an extrahepatic site in syngeneic hosts or heterotransplanted in athymic nude mice retain the capability to respond to peroxisome-proliferative effect of a hypolipidemic drug or an industrial phthalate-ester plasticizer administered in the diet to the host. Hypolipidemic drugs and phthalate-ester plasticizers comprise 2 important chemical classes of known peroxisome proliferators (14, 18, 19). The magnitude of increase in the volume densities of peroxisomes in rat hepatocytes at the extrahepatic locations is comparable to the response observed in hepatocytes of syngeneic host liver (11, 13). This clearly suggests that blood-borne stimulus for peroxisome proliferation reaches the environs of transplanted hepatocytes in the interscapular fat pads. The same degree of peroxisome proliferation in transplanted hepatocytes and the host liver also suggests that the concentration of the stimulus at the intrahepatic and extrahepatic locations could be similar. Alternatively, the hepatocytes at these sites may be differentially sensitive and still give similar response. This stimulus most likely represents the chemical or its active metabolite(s) which is recognized by extrahepatic hepatocytes. In this regard, it is pertinent to note that hypolipidemic drugs nafenopin or Wy-14,643, administered to lactating rats result in transfer of the active moiety in milk which exerts peroxisome proliferation in neonatal livers (1). Emerging evidence indicates that rat hepatocytes contain a cytosolic receptor which binds to nafenopin, a potent peroxisome proliferator (9). In the present study, peroxisome proliferation was used as a marker for physiological function in the transplanted hepatocytes. The ability of transplanted rat hepatocytes to respond in an almost identical quantitative fashion to a peroxisome proliferator, as compared to hepatocytes in the liver, strongly supports the contention that transplanted rat hepatocytes retain cytological and functional control mechanisms at these extrahepatic locations.

The results obtained from the studies with feline hepatocytes heterotransplanted into athymic nude mice are of potential interest. The transplanted cat liver cells exhibited a remarkable peroxisome-proliferative response when the nude mouse host was fed a hypolipidemic peroxisome proliferator. These results do point out interesting similarities between rodent and nonrodent hepatocytes in their response to peroxisome proliferators. Further studies, however, are necessary to establish whether heterologous (i.e., cat) and homologous (nude mouse) hepatocytes transplanted into nude mice respond to the administered chemicals in a quantitatively similar manner. This can be accomplished by transplanting hepatocytes of human, primates, cat, or any other nonmurine species at one location and nude mouse hepatocytes at a second location in the interscapular fat pads of the same recipient. Quantitative analysis of peroxisome proliferation in these hepatocytes should provide clues pertaining to the role of absorption, metabolism, pharmacokinetics, and receptor content in determining the response to peroxisome proliferators. Likewise, this hepatocyte heterotransplantation system should prove to be of considerable value in evaluating the comparative toxic effects of a variety of xenobiotic chemicals. This approach, as pointed out elsewhere, may obviate the logistic difficulties in the use of an intact human liver (23), nonhuman primates, or...
other nonmurine species.

In summary, the hepatocyte transplantation system can be utilized to study host factors affecting hepatocyte functions. Since the transplanted cells retain structural and functional integrity, they offer an important model system to investigate quantitatively the factors influencing initiation and promotion of hepatocarcinogenesis. Carcinogenic potency of chemicals can be determined by exposing hepatocytes in vitro and monitoring their response following transplantation in the subcapsular fat or at other extrahaematopoietic locations. Similarly, clonalogenic survival of initiated hepatocytes (i.e., dispersed cells obtained from enzyme-altered foci in the liver) at these transplantation sites as well as their response to a variety of tumour promoters can be accurately assayed.

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REFERENCES

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