Spontaneous Multiple Focal to Massive Hepatic Necrosis in Guinea Pigs

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Abstract: One hundred and eleven cases of multiple focal to massive hepatic necrosis were noted out of 202 guinea pigs, aged between 4 and 23 weeks, purchased from 3 different Japanese breeding colonies. Guinea pigs with hepatic lesions showed no abnormal clinical symptoms. The occurrence of lesions peaked at 5 weeks of age in males, and 10 weeks of age in females. Grossly, yellowish white lesions, up to 10 mm in diameter, were observed singly or scattered in the liver. Histopathologically, the commonest lesions consisted of coagulative necrosis or vacuolation of hepatocytes in the central area, and macrophage/lymphocyte infiltration and regenerative bile ducts around the peripheral area. The cytoplasm of the necrotic hepatocytes and macrophages often contained mineral granules, which were thought to be dystrophic mineralization and associated with a concomitant occurrence of chronic nephropathy. The other organs examined revealed no significant lesions related to the hepatic lesions. Light and electron microscopic examinations failed to uncover causal organisms; an experimental infection by inoculation of extract from the guinea pig hepatic lesions to immuno-suppressed mice failed to cause hepatic lesions. (J Toxicol Pathol 2000; 13: 207–212)

Keywords: Guinea Pigs, Necrosis, Calcinosis, Liver

Introduction

Guinea pigs have been widely used as an experimental animal. In 1977 in Argentina, out of 149 clinically normal guinea pigs, ninety-eight cases of focal hepatic necrosis were recorded as an incidental finding. In these cases, hepatic lesions were observed across a wide range of ages (from suckling young to aged animals), although their occurrence was more prevalent in older animals. Histopathologically, coagulative necrosis and subsequent bile duct proliferation were the characteristic findings. Causal organisms could not be detected by light or electron microscopic examination, and experimental infection failed to cause hepatic lesions in mice. Since then, there have been neither further reports nor any detailed examination of this phenomenon.

Here we report 111 cases of focal to massive hepatic necrosis observed in guinea pigs from 3 breeding colonies in Japan. We describe the morphological characteristics of hepatic necrosis, and discuss differences from the previously reported cases in Argentina. Furthermore, we describe an attempted experimental infection by inoculation of the hepatic extract to immuno-suppressed mice.

Materials and Methods

One hundred and fourteen male and 88 female (total: 202) guinea pigs (SPF Hartley) were purchased from 3 different breeding colonies in Japan. Males were aged 4 to 14 weeks and females were aged 10 to 23 weeks at the time of necropsy. Animals were housed, 1 or 2 per cage, in aluminum cages equipped with water bottles, and fed a cube diet (CG-7, Clea Japan Inc., Tokyo, Japan). All animals were reared and treated in accordance with the Guidelines for Animal Experimentation published by the Japanese Association for Laboratory Animal Science. Animal rooms were maintained at 22 ± 2°C with a relative humidity of 50 ± 10%. At a series of time points, including a point immediately after arrival, animals were euthanized by exsanguination under barbitol anesthesia and necropsy. Systemic organs and tissues were removed and fixed in 10% neutral buffered formalin. Tissue blocks from the liver and gallbladder of all animals, and the heart, aorta, thymus, spleen, bone marrow, mesenteric and submandibular lymph nodes, lung, trachea, tongue, esophagus, stomach, small intestine, large intestine, pancreas, kidney, urinary bladder, ureter, urethra, ovary, uterus, vagina, pituitary, thyroid, parathyroid, adrenal, brain, spinal cord, sciatic nerve, eyeball, lacrimal gland, submandibular gland, skin, skeletal muscle, mammary gland, femur, and sternum of 113 animals were embedded in paraffin with an automated tissue processor, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). All liver and gallbladder sections were stained with periodic acid-Schiff, Giemsa, Ziehl Neelsen and von Kossa to detect bacteria and fungi. In order to determine the presence of virus particles, freshly prepared tissue fragments of the liver, including grossly visible hepatic lesions, from 1 male and 6 females at 5 weeks of age were fixed in 2.5% glutaraldehyde in phosphate-buffer (0.1 M, pH 7.4) overnight, post-fixed in 1% osmium tetroxide in phosphate-buffer (0.1 M, pH 7.4) for 1 hour, and then dehydrated in a series of graded ethanol and embedded.
in Epon 812. At least 5 ultrathin sections were cut per animal, stained with uranyl acetate and lead citrate, and examined with an electron microscope (JEM-1200EX, JEOL, Tokyo, Japan).

An experimental infection study by inoculation of the fresh hepatic lesions to immuno-suppressed mice was also performed in accordance with previously reported methods. Eleven 6-week-old female ICR (Crl: CD-1) mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan). Housing conditions were the same as for the guinea pigs, except for cage type (suspended stainless steel cage) and number of animals per cage (3 to 5 animals per cage). Each animal was fed a cube diet (CE-2, Clea Japan, Inc., Tokyo, Japan). After a 1-week quarantine period, mice were divided into 2 inoculation groups and 1 control group. Freshly prepared hepatic lesions removed under sterile conditions from 6 male guinea pigs at 4 weeks of age were homogenized with saline and centrifuged. The supernatant of the homogenate was diluted to obtain a 10% saline extract. In the first group, 4 mice were inoculated intravenously with 0.2 ml of the 10% saline extract. In the second group, 4 mice received a single subcutaneous injection of 0.2 ml of hydrocortisone sodium succinate (Solu-Cortef, Upjohn, USA) to suppress the immune system, and then intravenously inoculated with the 10% saline extract. Three control mice received saline intravenously in the same manner. Fourteen days after inoculation, all mice were euthanized, necropsied, and processed for HE staining.

Results

Clinical Signs

All 111 animals in which hepatic lesions were observed showed no abnormal clinical symptoms after arrival from the breeding colonies.

Gross Autopsy Findings

Yellowish white, round or linear, discrete lesions about 1 to 5 mm in diameter were noted singly or scattered in the liver (Fig. 1). The lesions tended to be superficial, and their distributions were random in the lobules. Some lesions contained red spots, while others had fused together to form extensive irregularly shaped lesions, approximately 10 mm in diameter.

Histopathological Findings

Necrotic lesions were noted in the liver of 78 of the 114 males (68%) and 33 of the 88 females (38%). In males, the occurrence of lesions peaked at 5 weeks of age and tended to decrease thereafter. In females, the occurrence of lesions peaked at 10 weeks of age and also tended to decrease thereafter (Table 1). No sexual or inter-breeder differences in the occurrence of lesions was apparent.

Grossly visible discolored hepatic foci microscopically corresponded to clearly defined necrotic lesions with a random lobular distribution. The hepatic lesions showed various characteristics, and could be classified into 3 types: early phase, typical phase, and healing phase.

In the early phase, necrotic or vacuolated hepatocytes were observed, sometimes accompanied by hemorrhage and neutrophil infiltration (Fig. 2).

The typical phase, which was the commonest and largest type, consisted of coagulative necrosis or vacuolation of hepatocytes in the central area, and macrophage/lymphocyte infiltration and regeneration of bile ducts around the periphery (Fig. 3). A small number of neutrophils and hemorrhage were occasionally observed in the necrotic foci.

In the healing phase, lesions consisting solely of regenerated bile ducts, macrophage/lymphocyte infiltration, and fibrosis were observed (Fig. 4).

In the typical phase, the cytoplasm of the necrotic hepatocytes and macrophages observed around the periphery was frequently filled with large numbers of basophilic granules. As these granules were stained positive by Kossa (Fig. 5) and negative by Giemsa, PAS, and Ziehl Neelsen stains, they were identified as mineral deposits. In the early and healing phases of necrosis, the lesions contained relatively fewer mineral granules.

Chronic nephropathy, characterized by regeneration of urinary tubules, interstitial fibrosis, and massive mineralization in the cortical and papillary tubules, was evident in all animals examined (Fig. 6). The incidence of renal lesions did not differ between hepatic necrosis-affected and unaffected animals. No significant lesions related to the hepatic lesions were noted in any of the other organs examined.

Electron Microscopic Findings

Swollen, dilated and degenerated mitochondria and endoplasmic reticulum were observed. Causal organisms were not noted in the nucleus and cytoplasm including phagolysosomes of necrotic and degenerated hepatocytes or macrophages (Fig. 7).

Experimental Infection Study

Inoculation of freshly prepared extract of the guinea pig hepatic lesions to intact or immuno-suppressed mice failed to cause hepatic necrosis. Those lesions observed, including microgranuloma, mononuclear cell infiltration, and bile duct proliferation in the inoculated mice, were similar to those in the control mice, and therefore considered to be spontaneous.

Fig. 1. Macrophotograph of an affected guinea pig liver. Male, 4 weeks of age. Yellowish white, round (arrow) or irregularly shaped (arrowhead) lesions are noted. Measure = 1 mm/unit
### Table 1. Histopathological Findings of the Liver in 202 Guinea Pigs

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 Week</td>
<td>5 Week</td>
</tr>
<tr>
<td>Necrosis of hepatocyte</td>
<td>8 (100%)*</td>
<td>30 (81%)</td>
</tr>
<tr>
<td>Vacuolation of hepatocyte</td>
<td>3 (38%)</td>
<td>16 (43%)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>4 (50%)</td>
<td>25 (68%)</td>
</tr>
<tr>
<td>Neutrophil infiltration</td>
<td>7 (88%)</td>
<td>27 (73%)</td>
</tr>
<tr>
<td>Mineralization</td>
<td>7 (88%)</td>
<td>21 (57%)</td>
</tr>
<tr>
<td>Macrophage/lymphocyte infiltration, fibrosis</td>
<td>0 (0%)</td>
<td>15 (41%)</td>
</tr>
<tr>
<td>Regeneration of bile duct</td>
<td>3 (38%)</td>
<td>15 (41%)</td>
</tr>
<tr>
<td>Number of affected animals</td>
<td>8 (62%)**</td>
<td>37 (88%)</td>
</tr>
<tr>
<td>Number of examined animals</td>
<td>13</td>
<td>42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>10 Week</th>
<th>19 Week</th>
<th>23 Week</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis of hepatocyte</td>
<td>3 (93%)</td>
<td>10 (71%)</td>
<td>4 (80%)</td>
<td>27 (82%)</td>
</tr>
<tr>
<td>Vacuolation of hepatocyte</td>
<td>9 (64%)</td>
<td>7 (50%)</td>
<td>2 (40%)</td>
<td>18 (55%)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>6 (43%)</td>
<td>2 (14%)</td>
<td>2 (40%)</td>
<td>10 (30%)</td>
</tr>
<tr>
<td>Neutrophil infiltration</td>
<td>2 (86%)</td>
<td>10 (71%)</td>
<td>4 (80%)</td>
<td>26 (79%)</td>
</tr>
<tr>
<td>Mineralization</td>
<td>10 (71%)</td>
<td>6 (43%)</td>
<td>2 (40%)</td>
<td>18 (55%)</td>
</tr>
<tr>
<td>Macrophage/lymphocyte infiltration, fibrosis</td>
<td>4 (29%)</td>
<td>6 (43%)</td>
<td>2 (40%)</td>
<td>12 (36%)</td>
</tr>
<tr>
<td>Regeneration of bile duct</td>
<td>7 (50%)</td>
<td>2 (14%)</td>
<td>0 (0%)</td>
<td>9 (27%)</td>
</tr>
<tr>
<td>Number of affected animals</td>
<td>14 (70%)</td>
<td>14 (32%)</td>
<td>5 (21%)</td>
<td>33 (38%)</td>
</tr>
<tr>
<td>Number of examined animals</td>
<td>20</td>
<td>44</td>
<td>24</td>
<td>88</td>
</tr>
</tbody>
</table>

*1: (Number of animals showing finding/Number of affected animals) × 100 (%)
*2: (Number of affected animals/Number of examined animals) × 100 (%)

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Fig. 2. Necrosis or vacuolation of hepatocytes with numerous neutrophil infiltration. Male, 14 weeks of age. Liver, HE, ×320.

Fig. 3. Accumulation of macrophages and lymphocytes, and regenerated bile ducts with mitosis (arrow) surrounding the coagulative necrosis. Male, 5 weeks of age. Liver, HE, ×320.
Discussion

Clearly defined coagulative necrosis with mineralization was found in 111 clinically normal guinea pigs. The occurrence of these lesions was much higher in young animals and tended to decrease thereafter, whereas it was much higher in older animals in the Argentina cases. No sexual or interbreeder differences in the occurrence of lesions were apparent.
In those cases reported in Argentina by Cuba-Caparo et al., two types of lesions were described; necrosis without macrophage/lymphocyte infiltration and regeneration hepatocytes, and necrosis with subsequent regeneration of bile ducts. In our cases, necrosis without macrophage/lymphocyte infiltration and regeneration, which resembled the former mentioned above, was thought to be the early phase of the focal hepatic necrosis, as the size of the lesions was relatively small and they were accompanied solely by acute inflammation, i.e., hemorrhage and neutrophil infiltration. The typical type lesions reported here, necrosis accompanied by regeneration of bile ducts, resembled the latter type of lesions in the Argentinean report. In our cases, however, these lesions were accompanied by macrophage/lymphocyte infiltration, which was not described by Cuba-Caparo et al. The differences in inflammatory findings between the present and reported cases may reflect differences in the interval between the initiation of the lesions and necrosis. Chronic inflammation and repair, i.e., macrophage/lymphocyte infiltration and regeneration of bile ducts designated above as the healing phase, may take place in response to the necrotic hepatocytes. Finally chronic inflammation indicative of fibrosis may entirely replace the necrotic areas.

Mineralization of the necrotic hepatocytes and macrophages was a unique and striking feature of our cases, and not described by Cuba-Caparo et al. Dystrophic mineralization is a localized phenomenon observed in various organs and tissues that develops as a result of tissue damage, caused by excess leakage of extracellular calcium into the cytosol due to membrane damage. Hepatocellular mineralization, however, is rare, although such lesions have been reported to be associated with carbon tetrachloride intoxication in lambs, viral infectious disease in the European brown hare. In the rare event of human hepatocellular mineralization related to cirrhotic necrosis secondary to cor pulmonale, hyperphosphatemia due to renal insufficiency is thought to be an exacerbating factor. Systemic metabolic mineralization including the liver and kidneys, which was suspected to be caused by dietary insufficiency, has been reported in guinea pigs over 1 year of age. However, the cases reported here were observed in much younger animals, and nor were they accompanied by systemic mineralization in organs other than the liver and kidney. Mineralization of the hepatocytes, which occurred secondary to necrosis, was dystrophic in type. Whether the chronic nephropathy characterized by regeneration of tubules and interstitial fibrosis was the consequence, or the cause, of mineralization in the kidney, could not be determined, but renal insufficiency may exacerbate the dystrophic mineralization of the hepatocytes. The mineral granules present in the macrophage were thought to be the engulfed debris of the mineralized hepatocytes.

In the previous report of focal hepatic necrosis in guinea pigs, a causal organism was not determined, although the presence of virus-like particles was demonstrated. In this study, special staining for bacteria and fungi, and ultrastructural examination for viral particles failed to reveal a causal organism. In rodents, several viruses are associated with focal hepatic necrosis. In guinea pigs, cytomegalovirus has been shown to cause multiple focal necrosis with intranuclear and intracytoplasmic inclusion bodies in numerous organs, including the liver. However, in those animals examined here, neither necrosis nor inclusion bodies were noted in other organs. In other rodents with hepatic necrosis, the macroscopic and microscopic features of the liver in murine poxvirus infection mimic the focal hepatic necrosis in guinea pigs reported here. However, the present cases were not accompanied by intracytoplasmic inclusion bodies corresponding to virus particles, or necrosis of lymphatic tissues such as the spleen, lymph nodes, and Peyer's patches, that is notable in typical cases of mouse pox virus infection. Reovirus has also been shown to cause focal necrosis, not only in the liver but also other organs.

Since no causal organisms were detected and experimental inoculation of hepatic extract to mice was unsuccessful, further study is needed to clarify the cause of hepatic necrosis and subsequent mineralization.

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References
