The Use of d-Limonene Preparation as a Dissolving Agent of Gallstones

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The dissolving mixture is administered through a choledochal drain to treat postoperatively retained cholesterol gallstones. It is prepared by mixing 97.0 parts of d-limonene with 2.1 parts of polysorbate 80 and 0.9 part of sorbitan monooleate, a mixture of which may easily reach the surface of the gallstones which are wetted by bile. The d-limonene preparation was found to be safe both in laboratory experiments and clinical trials. Before applying the preparation, the usual choledochal drain must be replaced with a recently developed catheter made from epichlorohydrine rubber, which is chemically resistant to the preparation. Three cases of retained gallstones are described where the preparation was successfully used. In the fourth case treatment with the preparation was tried in lieu of surgery but was not successful due to other complications. However, some dissolution of retained stones was observed. There were no postoperative complaints in the long-term follow-up of some cases for more than 2 yr after treatment with the preparation. This procedure promises to be of value because retained cholesterol stones may be dissolved without the necessity of further surgery.

At present most cases of cholelithiasis are treated by surgery, and it is not always possible to remove all the stones present. This is particularly true of those in the common bile duct or secondary biliary radicals. Mechanical methods (1, 2) for removing gallstones have been employed, but the size and hardness of the stones may limit the usefulness of these methods. On the other hand, direct dissolution of gallstones was also attempted years ago by Pribram (3) and Best (4). For this purpose they used ether, chloroform, or a mixture of both which they introduced with a choledochal drain; unfortunately, severe side effects occurred. Other works (5) introduced a solution of bile salts or a mixed micellar solution containing bile salts and lecithin into the biliary system, reasoning that cholesterol in bile is held in solution by lecithin–bile salts micelles. These trials achieved some solubilization, but undesirable side effects due to the irritating action of the free bile salts limited their usefulness (6–8).

We have been interested in identifying agents which would solubilize or dissolve gallstones more effectively and which could be administered using a relatively safe technique. In our search for solubilizers or dissolving agents for cholesterol stones, terpene compounds (monoterpenes or sesquiterpenes) present in essential oils were found to have the desired physicochemical properties (9, 10). More than 30 terpenes were selected and examined both for their dissolving effect on cholesterol and their acute toxicity. The d-p-mentha-1,8-diene, carrying the generic name of d-limonene, was found to be distinctly superior to the others (Fig. 1). A solution comprising 97.0 parts of d-limonene, 2.1 parts of polysorbate 80, and 0.9 part of sorbitan monooleate, when added to human common duct bile, was miscible with the bile. This preparation was used as a dissolving agent.

We here report solubility studies in vitro and in situ with d-limonene preparation to determine its
suitability for use as a dissolving agent of gallstone. Furthermore, we report clinical studies, which were conducted after the safety of d-limonene preparation was confirmed in the preclinical studies including toxicities (acute, subacute, and chronic) (11–13), pharmacodynamics (14, 15), and metabolism (absorption, distribution, metabolism, excretion, as well as enzyme induction) (16–20) studies. The results indicate that d-limonene merits more extensive clinical study as a potentially useful dissolving agent for cholesterol gallstones.

**SOLUBILITY EXPERIMENTS**

**Materials and Methods**

*d*-Limonene (*d*-p-mentha-1,8-diene) \( (D)_{25}^\infty = 0.8444, n_{20}^\infty = 1.4733, [\alpha]_{D}^\infty = +126^\circ \text{C} \) was obtained from Nagaoa Perfumery Co., Ltd. Osaka, and polysorbate 80 (USP grade) and sorbitan monoooleate (NF grade) from Nikko Chemicals Co., Ltd., Tokyo. The dissolving mixture (d-limonene preparation) was prepared by adding 2.1 parts of polysorbate 80 and 0.9 part of sorbitan monoooleate to 97.0 parts of d-limonene.

**Experiment (1): Dissolving Effects in vitro of d-Limonene Preparation on Various Human Gallstones.** Five kinds of human gallstones (one pure cholesterol, two cholesterol pigment-calcium, one bile pigment-calcium, and one fatty acid–calcium gallstones) obtained at surgery in this department were vacuum-dried until each reached constant weight. These stones were immersed in 100 ml of d-limonene preparation in separate Erlenmeyer flasks which were maintained at a temperature of 37°C, and their dissolution and fragmentation were observed. An insoluble residue was collected by filtration and dried *in vacuo* at 85°C for 3 hr to measure its weight. Five other stones separately obtained from the same patients were chosen randomly for determination of cholesterol, ash, and bile pigment contents.

**Experiment (2): Dissolving Effects *in situ* of d-Limonene Preparation on Human Cholesterol Pigment-Calcium Stones.** Three male adult pigs (Mini Japan) weighing approximately 25 kg were anesthetized with thiopental sodium. Laparotomy was carried out in the midline and each cholesterol stone was placed in the gallbladder. One terminal end of a silicone tube (3 mm in diameter) was inserted in the gallbladder for infusion of d-limonene preparation and the other end was fixed subcutaneously to avoid bacterial infection. On day 6 after operation, the fixed end of the tube was reopened, and d-limonene preparation which was previously sterilized through a millipore filter (0.45 μm pore size) was infused into the gallbladder. One animal was given d-limonene preparation at a volume of 20 ml, once daily, for 2 days and another in a volume of 40 ml. The third was used as a control. The animals were killed 24 hr after the last infusion, and dissolving effects of the d-limonene preparation on human gallstones were examined. Liver tests were carried out both immediately before operation and immediately before sacrifice. Multiple organs were carefully examined for gross pathological changes and histological examination of gallbladder, common bile duct, duodenum, small and large intestines, liver, kidneys, adrenal glands, and sternal marrow was undertaken.

**Experiment (3): Solubility of Cholesterol in Various Solvents and Solubilizers.** Cholesterol (USP grade; Wako Pure Chemical Industries, Osaka) was purified by recrystallization to have a melting point of 147–148°C. Dissolution tests of cholesterol were carried out at either 25°C or 37°C according to the dissolution test defined in NF XIII. Amounts of cholesterol dissolved in various solvents and solubilizers were determined by gas chromatography on a column of 1.5% silicone GE SE-52 60-80 mesh Chromosorb W, using a Shimadzu gas chromatograph (model 4BM).

**Experiment (4): Solubility of Artificial Cholesterol Stones in d-Limonene-Water System.** Cholesterol was processed

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**Table 1. Dissolving Effects of d-Limonene Preparation on Various Human Gallstones**

<table>
<thead>
<tr>
<th>Gallstone Type</th>
<th>Weight (g)</th>
<th>Free Cholesterol (%)</th>
<th>Ash (%)</th>
<th>Bile Pigment (%)</th>
<th>Dissolution (%)</th>
<th>Time for dissolution and fragmentation (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure cholesterol stone</td>
<td>1.412</td>
<td>98.1</td>
<td>0.12</td>
<td>trace</td>
<td>100</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Cholesterol pigment–calcium stone</td>
<td>3.250</td>
<td>74.0</td>
<td>1.48</td>
<td>5.9</td>
<td>85.4</td>
<td>2</td>
</tr>
<tr>
<td>Cholesterol pigment–calcium stone</td>
<td>1.673</td>
<td>78.0</td>
<td>1.51</td>
<td>2.8</td>
<td>90.1</td>
<td>2</td>
</tr>
<tr>
<td>Bile pigment–calcium stone</td>
<td>1.952</td>
<td>11.2</td>
<td>9.84</td>
<td>20.1</td>
<td>16.8</td>
<td>&lt; 150</td>
</tr>
<tr>
<td>Fatty acid–calcium stone</td>
<td>1.059</td>
<td>13.1</td>
<td>22.01</td>
<td>–</td>
<td>25.2</td>
<td>ca. 6</td>
</tr>
</tbody>
</table>

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