Effect of High Molecular Weight Dextran on Experimental Hypercholesterolemia

By Damodor M. Brahmannar, Ph.D., and William E. Connor, M.D.

ABSTRACT

Thirty ml of 6% dextran (mol wt 70,000) was given intravenously daily to rabbits that were hypercholesterolemic from the ingestion of a 0.5%-cholesterol diet. Serum cholesterol concentrations were reduced from 990 ± 103 mg/100 ml to 676 ± 79 mg/100 ml after 3 days and to 331 ± 35 mg/100 ml after 7 days of dextran treatment. With the cessation of dextran infusions, serum cholesterol levels rose promptly above the pretreatment levels reaching 1346 ± 156 mg/100 ml by the seventh day after treatment. Similar changes occurred in serum phospholipid and triglyceride values. The chief reason for the reduction of serum lipids was the expansion of plasma volume as a result of the dextran treatment. Other factors had a less significant role. Dextran did not change the cholesterol content of most tissues including aortic cholesterol. Liver cholesterol content actually increased. The effects of dextran were only temporary and were not accompanied by cholesterol mobilization from the tissues.

ADDITIONAL KEY WORDS

serum cholesterol serum phospholipids tissue cholesterol plasma volume expansion dextran infusion serum triglycerides rabbits

Dextran1 (mol wt 70,000) has been used extensively as a plasma expander. It has also been suggested for the treatment of hypercholesterolemia and atherosclerosis (2). Flotte and Buxton (3, 4) have reported that the intravenous administration of high molecular weight dextran to patients with hypercholesterolemia and to animals fed cholesterol greatly reduced serum lipid concentrations. Treated animals had less atherosclerosis. The lipid levels in both humans and animals remained lowered for as long as 4 to 5 weeks after the cessation of dextran treatment when they gradually returned to pretreatment levels. These reports seemed to offer promise for the use of dextran in the treatment of atherosclerosis. The present investigation was undertaken to explore further the action of dextran on hypercholesterolemia and upon tissue cholesterol. We wished also to study the duration of any effects.

Methods

Forty-five New Zealand white male rabbits weighing 2.3 to 3.2 kg were caged individually and fed the experimental diet prepared to contain 0.5% cholesterol and 2.5% peanut oil by weight. The crystalline cholesterol and peanut oil were dissolved in ether. This combination was mixed with commercial rabbit chow2 and the ether was evaporated off completely. The rabbits had free access to food and water throughout the entire period of the study.

After 15 days of this cholesterol-enriched diet, 25 rabbits were infused with 30 ml of a 6% solution of dextran in saline at a constant rate over a 30-min period. These infusions were given into the marginal ear vein of each rabbit daily for 7

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1Dextran is a branched polymer of 1-6, 1-4, and 1-3 D-glucopyranose units formed by the action of various strains of bacterium Leuconostoc mesenteroides on sucrose (1).

Purina Chow, Ralston Purina Co.
days. Fifteen rabbits did not receive any treatment and served as controls. Five rabbits were infused with 30 ml of isotonic saline each day for 7 days.

Fourteen rabbits from the dextran group were used to determine the duration of the dextran effect. Blood was drawn periodically from the central ear artery on the third and seventh day, and 1 day, 3 days and 7 days after the dextran infusions had been completed. Six control rabbits had similar withdrawals of blood. Eleven dextran-treated and 5 saline-treated rabbits were killed for tissue analysis 4 hr after the completion of the 7th infusion (the 22nd day of the experiment). Nine control rabbits were also killed at the same time for tissue analysis. Bile was obtained by gall bladder aspiration.

The serum and bile cholesterol concentrations were estimated by the method of Abell et al. (5) as modified by Anderson and Keys (6). The serum triglyceride was measured by the method of Van Handel and Zilversmit (7) and the serum phospholipid by the method of Dryer et al. (8). For the determination of tissue cholesterol content, samples of various tissues were dried at 90°C in a vacuum oven until they reached constant weights and then they were ground into a fine powder. The tissue powder was extracted in 20 parts of 2:1 chloroform-methanol mixture for 24 hr and heated gently. The extraction was repeated a second time to ensure completeness. Aliquots of the extracts were used for cholesterol determinations as described above. The hematocrit changes were determined by the capillary hematocrit method. In some rabbits the net volume of plasma was measured before and after dextran treatment by employing 131I radioiodinated serum albumin (9).

Results

The dextran infusions reduced the serum cholesterol level of each rabbit so treated (Fig. 1 and Table 1). The serum cholesterol concentration of each rabbit rebounded rapidly after the cessation of dextran treatment; the mean serum cholesterol level increased within a day after the dextran treatment was stopped and within 1 week it had exceeded the initial level with an increase to 1346 mg/100 ml.

Table 2 presents the data for the control rabbits and those that received saline and dextran infusions. Values for the mean serum triglyceride concentration and mean serum phospholipid are presented in Table 1. The

changes in circulating red cell mass followed the same pattern as the serum lipids. The hematocrit fell significantly from 43.7 to 36.2% on the third day and to 29.1% on the seventh day of dextran treatment. It returned gradually to the pretreatment range by 7 days after dextran treatment (Table 1).

The effects of dextran and saline infusions on serum, bile and tissue cholesterol are contrasted in Table 2. The dextran-induced lowered serum cholesterol concentrations were not accompanied by depletion of tissue cholesterol. Liver cholesterol was higher in dextran-treated rabbits versus the saline-treated rabbits (77 vs. 53 mg/g of liver). Bile cholesterol in dextran-treated rabbits was significantly lower than control rabbits but not from the rabbits infused with saline.

In 4 dextran-treated rabbits, the total plasma volume was measured before and after...
TABLE 1

Effect of Dextran upon the Mean Serum Cholesterol, Phospholipid, Triglyceride and Hematocrit Levels in Rabbits

<table>
<thead>
<tr>
<th></th>
<th>No. of rabbits</th>
<th>Before treatment</th>
<th>During treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 Days</td>
<td>7 Days</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>14</td>
<td>990 ± 103 (SE)</td>
<td>676 ± 79*</td>
<td>331 ± 35*</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td></td>
<td>570 ± 81†</td>
<td>586 ± 119‡</td>
<td>1346 ± 156‡</td>
</tr>
<tr>
<td>Serum phospholipid</td>
<td>6</td>
<td>276 ± 39</td>
<td>161 ± 13§</td>
<td>335 ± 39‡</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td></td>
<td>196 ± 29</td>
<td>145 ± 12</td>
<td>197 ± 25</td>
</tr>
<tr>
<td>Serum triglyceride</td>
<td>6</td>
<td>43.7 ± 0.88</td>
<td>36.2 ± 0.74</td>
<td>29.1 ± 0.86*</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td></td>
<td>32.6 ± 1.33†</td>
<td>37.7‡</td>
<td>40.9‡</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>14</td>
<td>92.0 ± 2.39</td>
<td>77.0 ± 2.40*</td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake</td>
<td>14</td>
<td>920 ± 80 SE</td>
<td>770 ± 2.40*</td>
<td>90 ± 3</td>
</tr>
</tbody>
</table>

SE = Standard error of mean.
* = Different from pretreatment, P < .01.
† = Different from 7-day treatment value, P < .05.
‡ = Different from 7-day treatment value, P < .01.
§ = Different from pretreatment, P < .02.

TABLE 2

Cholesterol Content of Serum, Tissues and Gall Bladder Bile of Control, Dextran-Treated and Saline-Treated Rabbits

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rabbits</th>
<th>Serum (mg/100 ml)</th>
<th>Bile (mg/100 ml)</th>
<th>Liver (mg/g of dry weight)</th>
<th>Intestine (mg/g of dry weight)</th>
<th>Muscle (mg/g of dry weight)</th>
<th>Kidney (mg/g of dry weight)</th>
<th>Spleen (mg/g of dry weight)</th>
<th>Aorta (mg/g of dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (untreated)</td>
<td>9</td>
<td>882 ± 80 SE</td>
<td>176 ± 19</td>
<td>67 ± 1.53</td>
<td>19.9 ± 1.06</td>
<td>2.62 ± .11</td>
<td>21.6 ± .55</td>
<td>41.4 ± 3.49</td>
<td>4.86 ± 0.12</td>
</tr>
<tr>
<td>Dextran-treated</td>
<td>11</td>
<td>308 ± 27*</td>
<td>110 ± 21†</td>
<td>77 ± 5.5‡</td>
<td>20.5 ± 1.61</td>
<td>2.86 ± .12</td>
<td>20.2 ± .68</td>
<td>37.0 ± 5.46</td>
<td>4.15 ± 0.23</td>
</tr>
<tr>
<td>Saline-treated</td>
<td>5</td>
<td>1005 ± 67§</td>
<td>136 ± 16</td>
<td>53 ± 9</td>
<td>19.8 ± 0.35</td>
<td>2.72 ± .18</td>
<td>20.7 ± .94</td>
<td>31.0 ± 3.0</td>
<td>5.60 ± 0.25</td>
</tr>
</tbody>
</table>

Blood and tissues obtained 4 hr after completion of the 7th infusion (22nd day).

SE = Standard error of mean.
* = Significantly lower than saline-treated and control group (P < 0.01).
† = Significantly lower than control (P < 0.05) but not different from the saline-treated group.
‡ = Significantly higher than saline-treated group (P < 0.05).
§ = Not significantly different from control group.
TABLE 3

Changes in the Plasma Volume in Dextran-Treated Rabbits and Correction in Serum Cholesterol Values

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Plasma volume (ml)</th>
<th>Serum cholesterol (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>7-day treatment</td>
</tr>
<tr>
<td>1</td>
<td>82</td>
<td>151</td>
</tr>
<tr>
<td>2</td>
<td>106</td>
<td>173</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>218</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
<td>159</td>
</tr>
<tr>
<td>Mean</td>
<td>92 ± 8 SE</td>
<td>185 ± 13*</td>
</tr>
</tbody>
</table>

SE = Standard error of the mean.
* = Significance of difference from pretreatment value, P < .001.

the seventh infusion. In these animals, the plasma volume was increased greatly by the dextran treatment, from a pretreatment mean value of 92 to 185 ml (Table 3). From this dilution alone, the mean serum cholesterol level would have been lowered from 982 to 525 mg/100 ml. This corrected value was somewhat higher than the observed value of 341 mg/100 ml.

The food intake, including the intake of cholesterol, was reduced from 92 to 77 g/day per rabbit during dextran treatment (Table 1). All rabbits appeared in the same excellent condition at the beginning and end of the study. Dextran-treated rabbits tolerated the infusions well. They gained slightly less weight (36 g) than did the control rabbits (40 g) during the 7-day treatment.

Discussion

These results indicate that high molecular weight dextran did produce significant lowering of serum lipid concentrations in cholesterol-fed rabbits. However, the decrease in serum cholesterol level was only temporary. The reversal of this hypocholesterolemic response began promptly within 1 day after treatment was stopped. The 7-day increase of serum cholesterol during the postdextran period was noteworthy. There was no indication from our studies to suggest that dextran had a prolonged effect as had been suggested by Flotte and Buxton (3, 4). Similarly, in the studies of Heyman et al. (10) in nephrotic rats, and in those of Rothschild et al. (11) with cortisone-induced lipemia in rabbits, it is noteworthy that reelevation of serum lipids followed the cessation of the dextran treatment. Heyman noted the return of lipid levels to pretreatment values within a week and Rothschild within 10 days after dextran infusions had been discontinued.

The chief reason for the serum lipid lowering effect of dextran appeared to be the expansion of the plasma volume. The 7-day infusion of dextran caused about twofold dilution of plasma volume which decreased the serum cholesterol to about one-half of the pretreatment level. After the dextran treatment was stopped and the hemodilution disappeared, as evident from the significant rise in hematocrit of dextran group, the serum lipids promptly increased. The pattern of changes in serum lipids agreed with that of the changes in hematocrit.

Cholesterol mobilization from the tissues did not occur. The possible mechanism for the increase in liver cholesterol might be a shift of plasma cholesterol to the liver as a result of osmotic activity of dextran or possible phagocytosis of the complex which dextran forms with beta lipoproteins (12-14). The reticuloendothelial cells of the liver might remove dextran-coated lipoproteins from plasma. However, other reticuloendothelial organs such as the spleen did not have increased cholesterol concentrations after dextran treatment. Biliary stasis might have been
an alternative possibility for the increase of liver cholesterol. The bile cholesterol was significantly lower in dextran-treated rabbits from the controls but not significantly different from the saline-treated animals.

The possibility of dextran acting on lipemia through the activation of lipoprotein lipase seems remote. Allen et al. (15) assayed the lipoprotein lipase activity in normal and nephrotic rats following the intravenous injection of dextran. They reported that dextran, unlike heparin, neither stimulated the lipoprotein lipase activity nor possessed the lipid clearing action.

The intake of food (including cholesterol) was diminished in some rabbits, but those in which dietary intake was not much affected also showed profound decline in serum cholesterol during dextran treatment. A reduction in the intake of dietary cholesterol might have contributed somewhat to the reduced serum cholesterol levels.

The results of this study do not lend support to the thesis that high molecular weight dextran would provide a useful or practical method for the treatment of hypercholesterolemia. Any serum cholesterol lowering effect is at most temporary and begins to disappear immediately after the daily dextran infusions are discontinued and the dextran is metabolized. Although we have no direct evidence concerning its action on atherosclerosis, the fact that dextran did not reduce tissue cholesterol, including aortic cholesterol, casts some doubt upon its ability to lessen established atherosclerotic lesions. Apparently more experimental evidence about the efficacy of dextran should be available before this treatment is considered for use in human beings with hypercholesterolemia and atherosclerosis.

Acknowledgment

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References

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