Effect of Interferon on Plasma Lipoproteins and on the Activity of Postheparin Plasma Lipases

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The effect of Interferon administration on the concentration of plasma lipoproteins and on the activity of postheparin plasma lipoprotein lipase and hepatic lipase was studied in six healthy men. Daily injection of human leukocyte Interferon for 1 week lowered the plasma level of total cholesterol, very low density lipoprotein + low density lipoprotein cholesterol, high density lipoprotein cholesterol, and apolipoprotein A-I in all subjects. Simultaneously, the activity of postheparin plasma hepatic lipase and lipoprotein lipase decreased by 20% to 50%. These observations may be of importance in the interpretation of lipoprotein changes seen in acute and chronic infections and should be borne in mind when prolonged treatment with Interferon is considered. (Arteriosclerosis 2:68-73, January/February 1982)

Interferons, cellular proteins synthesized in response to viral infections and various nonviral inducers, were originally described to be mediators of viral interference. Since their discovery they have been shown to have an increasing array of actions including inhibition of viral replication, modification of immunological functions, and depression of cell division. Human leukocyte interferons are effective in the prevention and treatment of experimental viral infections and tumors. Promising results have been obtained also in clinical trials.

Acute and chronic infections are associated with changes in serum lipids and lipoproteins, but the mechanism underlying these changes is unknown. We recently reported that administration of human leukocyte interferon lowers the plasma concentration of high density lipoprotein (HDL) cholesterol in man. We have now carried out a detailed study on the effects of interferon administration on plasma lipoproteins and on the activity of postheparin plasma lipoprotein lipase and hepatic lipase, two enzymes with a central role in lipoprotein metabolism.

Methods

Subjects
Six healthy men, 30 to 48 years of age, were studied. All gave a negative history of bleeding tendency, peptic ulcer disease, hypertension, and recent acute illness. Laboratory examination showed no evidence of disturbances in liver function. The subjects were ambulatory, and the diets and body weights were stable during the study. Informed consent was obtained from all subjects.

Interferon
Human leukocyte interferon was produced and purified as described earlier. Two preparations with specific activities of $0.9 \times 10^6$ and $8 \times$
10⁶ international units (IU) per milligram of protein were used. No difference was observed between the effects of these two preparations used in the experiments.

**Experimental Design**

The study lasted 4 weeks, beginning with a control period of 1 week. On each day of the second week, the subjects received an intramuscular injection of 3 × 10⁶ IU interferon. During the first 3 weeks, plasma cholesterol, triglycerides, HDL cholesterol, and γ-glutamyl transpeptidase were determined five times per week, and at the end of the 4th week. Also during the first 3 weeks, the activity of postheparin plasma triglyceride lipases was determined three times a week, and at the end of the study. Ultracentrifuge fractionation of plasma lipoproteins was carried out immediately before the beginning of interferon administration, on the day after the last interferon injection, and at the end of the experiment.

**Analytical Procedures**

Ultracentrifugal fractionation of serum lipoproteins was carried out using a Beckman L 5-50 centrifuge according to the procedure recommended in the Lipid Research Clinic Manual. Cholesterol and triglyceride concentrations were analyzed by enzymatic methods with Boehringer Mannheim reagent kits using continuous flow technique (AutoAnalyzer II, Technicon, Tarrytown, New York). HDL cholesterol was determined after precipitation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) with dextran sulphate-magnesium chloride. The activities of aspartate-glutamyl-transferase, alkaline phosphatase, and γ-glutamyl-transferase were measured according to the Scandinavian recommendations. Concentrations of apolipoproteins (apo) A-I and A-II were measured by radial immunodiffusion, as described earlier. The activity of postheparin plasma hepatic lipase and lipoprotein lipase was measured 15 minutes after intravenous injection of 1 mg (100 IU) sodium heparin per kilogram of body weight, as previously described.

**Results**

All six subjects became pyrexial after interferon treatment, as reported previously. The temperature began to rise 4 hours after interferon administration and reached a peak 6 to 10 hours later; it returned to normal within 24 hours. Tolerance to fever developed within 2 to 4 days. No changes were observed in liver function tests during or after interferon treatment.

Interferon administration lowered plasma total cholesterol and HDL cholesterol in all six subjects studied (table 1 and figure 1). The changes in plasma triglycerides were not consistent. The reduction in HDL cholesterol was accompanied by a parallel decrease in the serum concentration of apo A-I, the major protein component of the HDL fraction (figure 2). On the other hand, no change was seen in the level of apo A-II, another peptide present in HDL. The results of ultracentrifuge fractionation of plasma lipoproteins in two participants with the most pronounced changes in total and HDL cholesterol are shown in figure 3. The decrease in total cholesterol is illustrated in figure 1.

**Figure 1.** Effect of interferon administration on the concentration of plasma HDL cholesterol. I = mean and range of the values during the control week preceding interferon treatment. II = immediately after the end of the 1-week interferon treatment. III = 2 weeks after the end of interferon treatment. A different symbol represents each of the six subjects studied. (The same symbols are used in figure 4.) The equivalent values for 1 mmole/liter in mg/dl for cholesterol and triglycerides are 38.7 and 88.0 mg/dl, respectively.
Table 1. Effect of Interferon Administration on the Concentration of Plasma Lipids and on the Activity of Postheparin Plasma Lipases

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Immediately before 1st injection</th>
<th>1 day after last of 7 injections</th>
<th>2 weeks after last injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmole/liter)</td>
<td>6.6 ± 0.4</td>
<td>5.7 ± 0.3*</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>VLDL + LDL cholesterol (mmole/liter)</td>
<td>5.90 ± 0.38</td>
<td>4.52 ± 0.26*</td>
<td>4.99 ± 0.46</td>
</tr>
<tr>
<td>HDL cholesterol (mmole/liter)</td>
<td>1.33 ± 0.13</td>
<td>1.14 ± 0.13*</td>
<td>1.28 ± 0.11</td>
</tr>
<tr>
<td>Triglycerides (mmole/liter)</td>
<td>1.8 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>Lipoprotein lipase (µmole FFA/ml/hr)</td>
<td>15.6 ± 1.7</td>
<td>9.7 ± 0.7*</td>
<td>13.1 ± 1.8</td>
</tr>
<tr>
<td>Hepatic lipase (µmole FFA/ml/hr)</td>
<td>33.9 ± 6.9</td>
<td>20.8 ± 3.8*</td>
<td>35.1 ± 7.3</td>
</tr>
</tbody>
</table>

*Significant difference from the initial value, p < 0.01 (paired t test).

Values are mean ± SEM. The equivalent values for 1 mmole/liter in mg/dl for cholesterol and triglycerides are 38.7 and 88.0 mg/dl respectively.

FFA = free fatty acid; VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.

serum cholesterol in these two subjects was due to reductions of cholesterol in all lipoprotein fractions including VLDL, LDL, HDL₂, and HDL₃.

The effect of interferon administration on the activity of postheparin plasma lipoprotein lipase and hepatic lipase is shown in figure 4. The activity of both lipases fell in all six subjects during interferon treatment. The mean activity of hepatic lipase returned to the pretreatment level within 2 weeks after the end of interferon injections. In contrast, the activity of postheparin plasma lipoprotein lipase came back to the original level only in one of the six subjects before the end of the observation period. To exclude the possibility that the changes in postheparin plasma lipases were due to repeated heparin injections, we followed the activity of the two enzymes in four volunteers who received two heparin injections (1 mg per kg body weight) weekly for 4 weeks (data not shown). In accordance with our earlier observations, no changes were seen.

Figure 2. Effect of interferon administration on the plasma concentration of apolipoprotein (apo) A-I (solid line) and apo A-II (dashed line) in two participants of the study. The period of interferon treatment is indicated by the horizontal line in the panel.
Discussion

The present results confirm our preliminary observations on the decrease of serum HDL cholesterol during interferon administration and further demonstrate that interferon also reduces the concentrations of VLDL + LDL cholesterol, and the level of apo A-I, the major protein component of the HDL fraction. Simultaneous changes in several lipoprotein fractions suggest that interferon affects one or several basic mechanisms regulating lipoprotein metabolism. Parallel decreases in LDL + VLDL cholesterol and HDL cholesterol could ensue from reduced synthesis of the lipoprotein molecules, or their accelerated breakdown, or a combination of both. There is evidence that interferon exerts its action by influencing the control mechanisms of protein synthesis. Furthermore, interferon inducers have been shown to suppress the cytochrome P-450 system in the liver. In view of these observations, it is interesting to note that a close association has also been described between the hepatic cytochrome P-450 and the serum level of HDL cholesterol and apo A-I. Thus, the first step in the sequence of events by which interferon affects plasma HDL level might be the inhibition of hepatic cytochrome P-450. This would in turn lead to changes in the production or secretion of HDL and possibly of other serum lipoproteins.

A significant decrease was observed during interferon treatment in the activity of both post-heparin plasma lipoprotein lipase and hepatic lipase. A positive correlation has earlier been observed between the hepatic cytochrome P-450 and the serum level of HDL cholesterol and apo A-I. Thus, the first step in the sequence of events by which interferon affects plasma HDL level might be the inhibition of hepatic cytochrome P-450. This would in turn lead to changes in the production or secretion of HDL and possibly of other serum lipoproteins.

Figure 3. Effect of interferon administration on the total plasma cholesterol concentration and on the concentration of cholesterol in various lipoprotein fractions in two subjects with the most pronounced changes in total plasma cholesterol. A = before the beginning of interferon treatment; B = immediately after the end of the 1-week interferon treatment; C = 2 weeks after the end of interferon treatment. The equivalent values for 1 mmole/liter in mg/dl for cholesterol and triglycerides are 38.7 and 88.0 mg/dl respectively.

Figure 4. Effect of interferon administration on the activity of postheparin plasma lipoprotein lipase and hepatic lipase. I = before the beginning of interferon treatment; II = immediately after the end of the 1-week interferon treatment; III = 2 weeks after the end of interferon treatment. Values are expressed as percent of the activity at time point I. Absolute activities are given in table 1. The symbols represent the six subjects studied, as in figure 1.
described between the serum HDL level and the activity of both adipose tissue and postheparin plasma lipoprotein lipase. There is also evidence that a part of serum HDL is generated in the lipoprotein lipase-catalyzed degradation of triglyceride-rich lipoproteins. Thus, one explanation for the decrease in HDL and its subfractions during interferon treatment might be the inhibition of the lipoprotein lipase system and subsequent decrease in the production of HDL from triglyceride-rich VLDL. No consistent change was observed in serum triglycerides during interferon treatment, however. In fact, a constant triglyceride level in the presence of low activity of lipoprotein lipase speaks strongly in favor of a simultaneous decrease both in the synthesis and the breakdown of serum triglycerides.

Inhibition of hepatic lipase by a specific anti-serum raises the level of HDL cholesterol in the rat. Furthermore, a negative correlation has recently been reported between the activity of postheparin plasma hepatic lipase and HDL in our present study, we observed a decrease both in plasma HDL level and in the activity of postheparin hepatic lipase during interferon treatment. Thus, it is not likely that the altered hepatic lipase activity is directly contributing to the changes in the lipoprotein pattern induced by interferon administration.

The lipoprotein pattern seen during interferon treatment bears some resemblance to the pattern observed in acute and chronic infections. Similar "nonspecific" changes are also seen in patients with acute myocardial infarction and other disease states characterized by tissue necrosis. Viruses and many other microorganisms can induce the production of interferon in experimental animals. Thus, interferon might well be the factor responsible for changes in lipoprotein levels during viral and nonviral infectious diseases. The connections between interferon and the lipid changes in disease states involving sterile tissue damage are hypothetical. However, it is not impossible that even sterile tissue necrosis may trigger the synthesis of interferons or some closely related substances, which in turn may affect the metabolism of lipoproteins.

Two precautions should be taken in the interpretation of our results. First, the leukocyte interferon preparations are not pure and may contain other leukocyte factors influencing lipoprotein metabolism. Second, some of the changes in serum lipids observed here resemble the effects seen after injection of endotoxin. The contribution of endotoxin to the present results is, however, highly unlikely as no endotoxin was detected in our interferon preparations with the extremely sensitive limulus assay. On the other hand, endotoxins are known to be potent inducers of interferon in vivo. Therefore, the possibility cannot be excluded that the changes in serum lipids after administration of endotoxin are also mediated by interferons.

In summary, our results show that administration of human leukocyte interferon preparations has profound effects on the metabolism of plasma lipoproteins. Further studies on the clinical significance of these changes are imperative in the light of the potential usefulness of long-term interferon administration in the therapy of various chronic disorders.

Acknowledgments

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References

1. Stewart WE Jr. The interferon system. Vienna: Springer Verlag, 1979
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Kekkilä M. Lipoprotein-lipase action determining plasma high density lipoprotein cholesterol in adult normolipemics. Atherosclerosis 1980;37:143-150


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