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EFFECT OF MEVINOLIN ON CHOLESTEROL METABOLISM IN OBESE AND LEAN ZUCKER RATS

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Abstract—Mevinolin is a potent competitive inhibitor of HMG-CoA reductase, the enzyme catalyzing the major rate-limiting step in cholesterol synthesis. In this study the drug was administered as an intragastric dose at 2.5 mg/kg/day to 10 to 12-week-old lean and obese Zucker female rats over a 5-day period. Mevinolin showed no effect on plasma cholesterol levels in the lean rat; however, in the obese rat there was a significant decrease in plasma cholesterol (about a 40% decrease from initial levels). Although there was a difference in effect on plasma cholesterol levels in obese and lean rats, hepatocytes isolated from both fed lean and obese rats incubated with various concentrations of mevinolin exhibited similar levels of inhibition of cholesterol synthesis and showed no effects on the other metabolic processes studied. These results indicate that the drug was effective acutely on cholesterol synthesis in hepatocytes isolated from both lean and obese rats, but on a chronic treatment basis the hypocholesterolemic effect was observed only in the obese Zucker rat. This study supports the idea that the naturally occurring hypercholesterolemic obese Zucker rat may be a good model for testing potential new cholesterol lowering agents.

Mevinolin is a potent competitive inhibitor of HMG-CoA reductase [1], the enzyme catalyzing the major rate-limiting step in cholesterol synthesis. It has been used to lower serum cholesterol in dogs [1], rabbits [2] and humans [3]. It has been shown to cause acute inhibition of cholesterol synthesis in rats; however, it does not cause significant drop in serum cholesterol in normal rats when given over a number of days [1].

Another potent inhibitor of HMG-CoA reductase, ML-236B or compactin, has also been shown to inhibit cholesterol synthesis in acutely treated rats [4], but it does not lower cholesterol levels in rats chronically treated with levels up to 500 mg/kg [5, 6]. In rats made hypercholesterolemic by treatment with Triton WR-1339, ML-236B (80 mg/kg) lowers plasma cholesterol levels by 28% at 24 hr after Triton treatment [7]. ML-236B also effectively reduces plasma cholesterol levels in dogs [8], monkeys [9] and humans [10].

Since rats, made hypercholesterolemic by Triton treatment, show some decrease in plasma cholesterol levels, and since no decrease has been observed in normal rats with these type of compounds, it is possible that a naturally occurring hypercholesterolemic rat may be a good model to test potential cholesterol-reducing agents. The obese Zucker rat has a naturally elevated plasma cholesterol level [11, 12]; it, therefore, was used in these studies.

In the present study, mevinolin was administered to lean and obese female Zucker rats over a 5-day period, and plasma cholesterol levels were measured before and after drug. The results indicate that the drug caused quite an effective lowering of cholesterol levels in the obese Zucker rat but not in the lean rat. To determine if part of the effect may have been due to resistance of the obese rat liver cells to inhibition by mevinolin, the acute effects of mevinolin on cholesterol synthesis and general metabolism in hepatocytes isolated from both lean and obese rats were also studied. However, the degree of inhibition of cholesterol synthesis by mevinolin added to hepatocytes from either obese or lean rats was found to be very similar. This study was presented in part at the meeting of the American Societies for Experimental Biology in St. Louis [13].

MATERIALS AND METHODS

Materials. Collagenase was obtained from the Worthington Biochemical Corp. (Malvern, PA, U.S.A.); other enzymes and most reagent grade biochemicals were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Radioisotopes were from Research Products International (Mount Prospect, IL, U.S.A.).

Animals. Female obese (fa/fa) and lean (Fa/?) Zucker rats were obtained from Dr. Julia Clark, Indiana University Medical Center, Indianapolis, IN. The animals were kept on a 12 hr (8:00 am. to 8:00 p.m.) light cycle and given free access to Purina Rat Chow pellets and water. The animals were used when their ages were 11 ± 1 weeks of age.

Dosage schedule. The level of cholesterol in the obese rats can be quite variable from rat to rat; therefore, before the drug study was started initial cholesterol values were obtained. Three days prior to the drug feeding, all rats were fasted for 16 hr (overnight), and the tail of each animal was bled to obtain a pretreatment sample of plasma. The animals were then divided into groups such that each group had a similar initial range of cholesterol values (shown in Table 1).

The rats were given an appropriate dose of drug or carrier each day for 5 days at 3.30 p.m. Mevinolin

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(obtained from A. W. Alberts of the Merck Co.) at a dosage of 2.5 mg/kg/day (dissolved in 100% propylene glycol) was given intragastrically to the rats. The control rats received propylene glycol (0.1 ml/100 g body weight). The doses were given such that 1 ml of liquid per kg body weight was administered with each dose, i.e. for the 15 mg/kg dose a solution was made with a concentration of 15 mg/ml. After the fifth dose the rats were once again fasted overnight and bled from their tails to obtain a post-treatment sample of plasma.

Plasma cholesterol and triglyceride determinations. The cholesterol assay was performed by the method of Allain *et al.* [14]. Triglycerides were measured by the method of Bucolo and David [15].

Isolation and incubation of hepatocytes. Hepatocytes are prepared from *ad lib.* fed rats by the method of Berry and Friend [16] with minor modifications [17]. We also preincubated the cells at 37° for 10 min prior to the first centrifugal washing. The cells were isolated between 9:00 and 10:00 a.m. to compensate for any diurnal rhythms such as glycogen depletion.

The cells were suspended in 2 ml of Krebs–Henseleit buffer supplemented with 2% bovine serum albumin (essentially fatty acid free and dialyzed) under an atmosphere of 95% O_2, 5% CO_2 in stoppered 25-ml Erlenmeyer flasks. Incubations with 23–30 mg wet weight of cells per ml (about 5×10^6 cells/ml) were conducted in a shaking water bath at 37° for appropriate times. Cells for fatty acid and cholesterol synthesis were treated with 50 μl of ^3H_2O (10 mCi/ml) after 30 min of preincubation and stopped at 60 min.

Assay of metabolites. Incubations were terminated with $HClO_4$ (0.1 ml of 60%) and treated as described previously [17]. The metabolites in the extracts were measured spectrophotometrically by enzymic methods, according to Hohorst *et al.* [18] for pyruvate and lactate, Williamson *et al.* [19] for acetoacetate and beta-hydroxybutyrate, Slein [20] for glucose, and Lamprecht and Trautschold [21] for ATP.

Determination of fatty acid and cholesterol synthesis. The rates of fatty acid synthesis and cholesterol synthesis, expressed as moles of acetate equivalents/g wet wt of hepatocytes, were determined by the incorporation of ^3H_2O into total lipid and extracted by the methods of Kates [22] and Harris [23]. Calculations were done according to Jungas [24].

RESULTS

Effect of mevinolin on plasma cholesterol, triglycerides and glucose levels. Because of the variability of the initial plasma cholesterol levels in the obese rats, there was a pretreatment measurement of cholesterol, each animal could then act as its own control. The initial groups were very close in their range of cholesterol (Table 1). Figure 1 shows the lack of any consistent effect by mevinolin or the vehicle on cholesterol levels in the lean rats. Figure 2 shows the dramatic effects of mevinolin at lowering plasma cholesterol in the obese rats. It was also noted that with the higher initial cholesterol levels

Table 1. Initial body weights and cholesterol levels of lean and obese female Zucker rats

Rat group	Initial body wt (g)	Initial serum cholesterol (mg/dl)
Control		
Lean	175 ± 5	29.7 ± 2.0
Obese	289 ± 7	178.9 ± 25.4
Mevinolin		
Lean	182 ± 6	29.8 ± 1.2
Obese	297 ± 5	218.4 ± 40.2

Cholesterol values were determined on blood samples taken from rats fasted for 16 hr. Results are mean ± SEM for ten rats in each group.

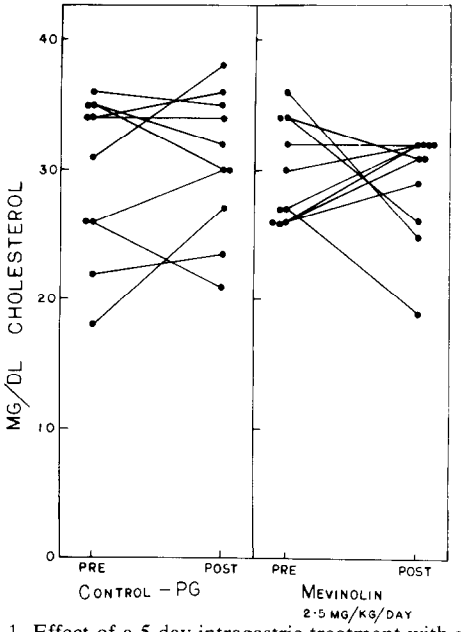

Fig. 1. Effect of a 5-day intragastric treatment with mevinolin (2.5 mg/kg/day; dissolved in propylene glycol) or propylene glycol (PG) on plasma cholesterol levels in lean female Zucker rats. Each line represents a single animal pre- and post-treatment.

there was a greater decrease in the level with the 5-day treatment.

When the data were calculated as percentage change of the post-treatment serum value compared to the initial cholesterol value, mevinolin had no effect on cholesterol levels in the lean rat (propylene glycol 105.3 ± 6.4%; mevinolin 99.1 ± 6.7%). In the obese rat there was a major reduction in just the 5-day treatment with mevinolin (57.9 ± 5.7%).

The 100% propylene glycol given to the control obese rats caused an unexplained increase in plasma cholesterol in the obese rats (177.7 ± 28.9%) but only a small change in the lean rats. Part of this effect in the obese rat may have been due to the fact that at 11 weeks of age many of the rats are still increasing their plasma cholesterol levels. Some of the animals which showed the most dramatic increases were those with very low initial cholesterol levels. Another reason may be that the propylene glycol is a detergent-like compound and may cause a greater release of lipids in the hyperlipidemic obese rat.

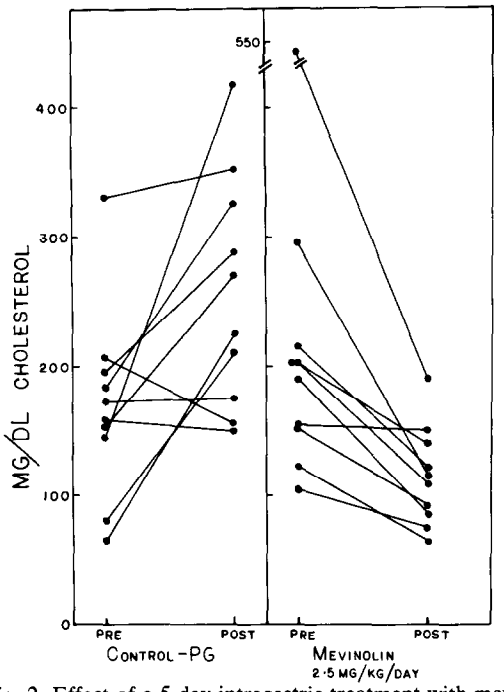

Fig. 2. Effect of a 5-day intragastric treatment with mevinolin (2.5 mg/kg/day; dissolved in propylene glycol) or propylene glycol (PG) on plasma cholesterol levels in obese female Zucker rats. Each line represents a single animal pre- and post-treatment.

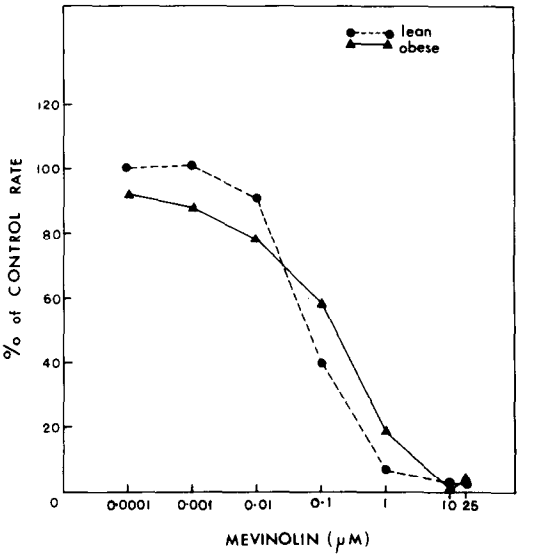

Fig. 3. Effects of various concentrations of mevinolin on cholesterol synthesis in hepatocytes isolated from lean and obese Zucker rats. The rate of cholesterol synthesis was determined by ^3H_2O incorporation between 30 and 60 min of incubation. The data shown are from a representative experiment from an obese and lean rat. The same experiment was repeated at least three times in hepatocytes isolated from obese and lean rats, and the same trends were observed. The control rates of cholesterol synthesis (100% value) in these experiments were 0.025 μmol/min/g wet wt for hepatocytes isolated from the lean rat and 0.018 μmole/min/g wet wt for the hepatocytes isolated from the obese rat.

Plasma triglyceride levels in the obese rat were elevated compared to those in the lean rat; however, there was no consistent change with mevinolin treatment (data not shown). There was no difference in fasting blood glucose levels between the obese and the lean rats, and no change in these concentrations was observed when mevinolin was administered (data not shown).

Effect of mevinolin in hepatocytes. Mevinolin was a very effective inhibitor of cholesterol synthesis in hepatocytes isolated from both lean and obese Zucker rats (Fig. 3). At various concentrations of mevinolin (0.0001 to 25 μM), there was no significant difference in the inhibition of cholesterol synthesis regardless of the source of the hepatocytes. At the lowest dose the obese rats exhibited slightly less inhibition.

When mevinolin was added to hepatocytes isolated from either obese or lean Zucker rats, there was a decrease in cholesterol synthesis but not much change in any other metabolites or processes studied (Table 2). The cells isolated from the obese Zucker rat had higher rates of fatty acid synthesis and increased lactate and pyruvate levels when compared to cells isolated from lean rats as we observed in earlier studies [25]. In addition to those metabolites or processes listed in Table 2, ATP and ketone body production in cells treated with mevinolin were not altered from untreated control levels.

DISCUSSION

This study demonstrates how effective mevinolin was in lowering plasma cholesterol in obese Zucker rats. The data from the lean rat support the findings of others [1, 5, 6], that cholesterol synthesis inhibitors are not very effective in lowering plasma cholesterol in normal lean rats. One of the possible explanations is that the mevinolin may not have been absorbed by the lean rats but was taken up from the diet in the obese Zucker rat. Alberts *et al.* [1] demonstrated in an acute assay with normal rats that orally administered mevinolin is an active inhibitor of cholesterol synthesis; this is evidence that the lean rat does absorb the compound.

A reason why the lean rat did not show reduction in cholesterol levels with chronic drug treatment may be that there is a very tight control of the level of plasma cholesterol in the normal rat. In other studies of rats treated for a period of time with mevinolin or the related compound, compactin, an increase was demonstrated in the amount of HMG-CoA reductase and smooth endoplasmic reticulum in the liver [7, 26, 27]. The liver of the rat seems to adjust to these type of inhibitors such that normal amounts of cholesterol are synthesized, with no overproduction. The obese Zucker rat may represent a defect in the feedback control of the HMG-CoA reductase gene since the liver of the obese rat apparently fails to compensate as efficiently as that of the lean rats for the mevinolin inhibition of HMG-CoA reductase activity. Further studies should be done to determine if there is any compensation once the cholesterol levels reach the normal range.

In the obese rat the cholesterol level is elevated; therefore, the drug may cause an inhibition of cholesterol synthesis only to the extent that normal blood

Table 2. Effect of mevinolin on metabolism in hepatocytes isolated from lean and obese female Zucker rats

Hepatocytes isolated from:	Metabolite or process measured				
	Fatty acid synthesis (μmoles/min/ g wet wt)	Cholesterol synthesis (μmoles/min/ g wet wt)	Pyruvate (μmoles/ml incubation medium)	Lactate (μmoles/ml incubation medium)	Glucose released (μmoles/ min/ g wet wt)
Lean rats					
Control	0.19 ± 0.08	0.024 ± 0.006	0.31 ± 0.02	0.64 ± 0.13	0.59 ± 0.03
Mevinolin	0.18 ± 0.08	0.001 ± 0.001*	0.31 ± 0.01	0.64 ± 0.12	0.63 ± 0.02
Obese rats					
Control	0.37 ± 0.13	0.020 ± 0.004	0.37 ± 0.02	0.82 ± 0.08	0.51 ± 0.06
Mevinolin	0.35 ± 0.13	0.002 ± 0.001*	0.37 ± 0.02	0.88 ± 0.09	0.46 ± 0.03

For these experiments, mevinolin was added at the final concentration of 1 μM. The rates of fatty acid and cholesterol syntheses were determined by ^3H_2O incorporation between 30 and 60 min of incubation. The other metabolites were determined after a 60-min incubation period from neutralized perchloric acid extracts via the spectrophotometric methods described in Materials and Methods. Results are the mean ± SEM for at least four hepatocyte preparations.

* $P < 0.05$, Student's *t*-test for paired samples.

concentrations are maintained. The data seem to indicate that this may be the case, since the obese rats with the highest level of cholesterol showed the greatest lowering of cholesterol. None of the cholesterol levels was reduced below the normal range and cholesterol of the lean rats was not lowered. These results are similar to results using the compactin-resistant cell lines which only make enough HMG-CoA reductase to overcome the inhibition [28, 29].

What is clear from these studies is that the obese Zucker rat may be a good model for testing cholesterol-lowering drugs even though the normal lean rat is not. In preliminary studies, two other drugs that inhibit cholesterol synthesis in hepatocytes (via different mechanisms than mevinolin) also lowered plasma levels of cholesterol in the obese, but not the lean rat (McCune *et al.*, unpublished data). The methods currently used to make normal rats hypercholesterolemic are: (1) the use of the detergent Triton-WR1339, which causes changes in lipid levels in several stages and only lasts a short time [5, 30], or (2) the feeding of special diets or drugs for several weeks or months [6, 31]. The obese Zucker has the advantage in that it becomes hypercholesterolemic naturally on regular chow diet.

Other animal models that have been used to test hypocholesterolemic drugs are dogs [1, 8], monkeys [9], chickens [6], and rabbits, both diet-induced [2] hypercholesterolemic and the genetically-induced Watanabe type [32]. A rat model has the following advantages compared to others: less drug is required, no special diet is necessary, it is more economical in initial cost and in upkeep, and the rat is easier to handle than many of the other animals.

The hepatocyte studies indicate that the difference in the effect on plasma cholesterol was not a function of the liver of the obese rat being less sensitive to inhibition by the drug. The levels of inhibition of cholesterol synthesis were within the ranges found by others in hepatocytes with compactin [33, 34] and in cultured hepatocytes with mevinolin [35]. Since no other hepatic metabolites were altered during the short incubation time, it indicates how specific mevinolin is to inhibition of HMG-CoA reductase and cholesterol synthesis.

The effectiveness of new cholesterol-lowering drugs in reducing atherosclerosis depends not only on the drug lowering total cholesterol levels but also on specific changes in lipoprotein patterns. Mevinolin has been shown to lower total cholesterol and LDL cholesterol in dogs [1] and humans [3, 36]. In fact, these studies have established that interference with cholesterol synthesis by a competitive inhibitor of HMG-CoA reductase can reduce LDL levels in plasma without depleting vital body stores of cholesterol [29]. The obese Zucker rat has elevated LDL cholesterol, and it is possible that mevinolin also lowers it in these animals (studies to determine this are under way). If these studies are positive, these naturally occurring hypercholesterolemic rats will be an even better model for testing cholesterol reducing compounds.

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