COLCHICINE INHIBITS HEPATIC CHOLESTEROL SYNTHESIS AND MICROSOMAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG CoA REDUCTASE) ACTIVITY

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1. Introduction

Colchicine has been shown rapidly to block the secretion of serum proteins, including VLDL [1–4] and albumin [4]. The VLDL accumulates in Golgi-derived secretory vesicles [1], possibly from interference with the microtubular system [2] and causes fatty liver [1–4]. Since cholesterol is an important component of lipoproteins, the inhibition of VLDL release provides an interesting model for studying the role of hepatic cholesterol synthesis in serum lipoprotein formation. Other drugs which lower serum VLDL levels and induce fatty liver differ from each other in their effects on cholesterogenesis. Thus, carbon tetrachloride inhibits [5], but orotic acid stimulates cholesterol synthesis [6]. Accordingly, since the effect of colchicine was unpredictable, we established experimental conditions to test the opposite hypotheses that the drug has a stimulatory or inhibitory effect on the sterol's production and on HMG CoA reductase (mevalonate: NADP oxidoreductase (acylating CoA) EC 1.1.1.34), the rate-controlling enzyme for cholesterol synthesis.

2. Materials and methods

2.1. *Treatment schedules (fig. 1)*

Male or female Holtzman rats weighing between 180 and 250 g and housed one to a cage were fed according to our previously described controlled lighting and feeding schedule [7]. Lights were automatically turned off at 9:00 a.m. and on at 9:00 p.m. daily and food was placed in cages at 9:00 a.m. and removed at 5:00 p.m. Since rats are nocturnal feeders, reversal of the lighting provides optimal conditions for adapting the feeding pattern to daylight hours. The rats were cyclically fed according to this 8 h feeding–16 h fasting schedule for at least one week, but food was withheld on the day of sacrifice.

In one set of experiments, cholesterol synthesis was determined in rats fed a previously described diet, containing 0% fat, 20% protein, and 75% dextrose, that was shown to induce low levels of HMG CoA reductase activity [7]. Groups of rats were injected with three separate doses of either 0.1 mg (Experiment IA) or 0.125 mg (Experiment IB) of colchicine i.p. and subsequently injected with 10 mCi of ^3H_2O i.p. 90 min prior to sacrifice. The amount of tritium incorporated into digitonin-precipitated sterol recovered from livers under these conditions is considered a valid measurement of in vivo hepatic cholesterol synthesis [8,9].

In a second series of experiments (IIA,B,C), rats were cyclically fed a diet containing 20% corn oil, 2% cholestyramine, 20% protein, 31% dextrose, and 22% cellulose [7]. This diet was previously shown to induce a high level of HMG CoA reductase activity [7]. In order to determine the effect of colchicine on this induction and on the synthesis of cholesterol, a single dose of 0.2 mg colchicine was injected at midnight and rats were then sacrificed at 8:00 a.m. or 9:30 a.m. (fig.1). When cholesterol synthesis was determined, rats were injected with 10 mCi of ^3H_2O i.p. 90 min prior to sacrifice. Rats used as controls in all studies were injected with volumes of isotonic saline equivalent to the volumes of colchicine solution.

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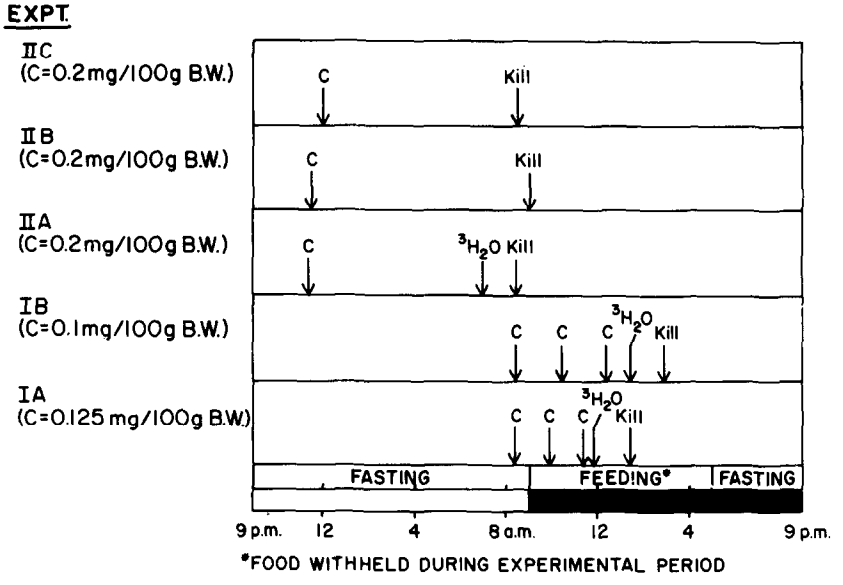

Fig.1. Treatment schedule of rats in various experiments. Female Holtzman rats fed the 0% fat diet were used in Exp. IA and IB and male Holtzman rats were used in Exp. IIA, IIB and IIC. Lighting and prior feeding–fasting schedules are indicated in the lower part of the chart. Colchicine (C) was prepared as a 0.5% solution in isotonic saline and injected i.p. at the indicated dosages and times. 10 mCi ^3H_2O were injected i.p. 90 min prior to sacrifice in the indicated experiments.

2.2. *Lipid assay methods*

In the earlier experiments (IA and IB) non-saponifiable lipids were extracted by heating the liver in alcoholic KOH, extracting three times with chloroform, evaporating the solvent, suspending the lipid residue in water, and re-extracting with petroleum ether [10]. In the later experiments, lipids were separated from 1 g aliquots of liver by the method of Folch et al. [11]. Complete removal of ^3H_2O from the chloroform–methanol extracts was insured by repeated washing of the 'Folch lower phase' with 1:1 mixtures of CH_3OH:0.76% KCl solution. Measured aliquots were taken for triglyceride [12] and cholesterol determinations. The latter were dried under nitrogen and the residues saponified in alcoholic KOH. The non-saponifiable lipid was extracted with petroleum ether. Digitonides were prepared from the petroleum ether extracts by the method of Sperry and Webb [13]. The precipitated digitonide was washed and resuspended in glacial acetic acid [13]. For radioactivity determination, 1 cc. samples were dissolved in 10 cc. Tulene PPO. In some studies, samples in glacial acetic acid were taken for cholesterol determination by the method of Zak [14].

HMG CoA reductase activity was determined by a modification [7] of our previously described method [15]. The specific activity of the [^{14}C]HMG CoA substrate was 3.7×10^6 dpm/μmole.

3. Results and discussion

Nine hours after a single i.p. injection of 0.2 mg colchicine into rats fed the hypercholesterogenic diet (Exp. IIA), the hepatic triglyceride level had increased more than 2.5-fold (table 1). However, the level of hepatic cholesterol had not changed. In this same experiment cholesterol synthesis, measured by incorporation of ^3H_2O into digitonin precipitated sterol, was 57% lower than in controls (table 2). The level of HMG CoA reductase activity was decreased by 75% and 68% respectively in rats injected with colchicine 9.5 and 8 h prior to sacrifice. Rats fed the fat-free diet and injected with three doses of colchicine totalling 3.75 mg/100 g body weight and sacrificed after 5 h (Exp. IA) and those injected with doses of colchicine totalling 3.0 mg and sacrificed after 6.5 h (Exp. IB) respectively showed 97% and 95% reduction in cholesterogenesis (table 2).

Table 1
Hepatic triglyceride and cholesterol in rats fed 2% cholestyramine and 20% corn oil diet (Experiment IIA) and injected with 0.2 mg of colchicine or saline i.p. and sacrificed after 8 h.

	Colchicine (5)	Saline (5)	Significance
Cholesterol mg/g liver	2.3 ± 0.4[a]	2.1 ± 0.1	N.S.
Triglyceride mg/g liver	11.1 ± 1.6	4.5 ± 1.4	$P < 0.001$

The number of rats in each group is indicated in parentheses.

[a] Standard deviation.

In this study the inhibition of cholesterol synthesis by colchicine was demonstrated under two entirely different kinds of experimental conditions. In the earlier experiments, rats fed a fat-free diet were injected with repeated small doses of colchicine. The drug reduced the already low fasting level of cholesterogenesis to a striking degree. Since it was not possible accurately to measure changes in the low level of HMG CoA reductase activity under these conditions, we then studied the inhibitory effect in rats fed a high fat diet containing cholestyramine. Here, too, the level of cholesterogenesis was significantly reduced – this time after a single dose of 0.2 mg colchicine/100 g body weight. The almost proportionate reduction of HMG CoA reductase activity in rats fed this hypercholesterogenic diet suggests that the colchicine effect is at the level of the rate controlling enzyme.

All experiments were purposely carried out late in the fasting period in order to avoid any effect that colchicine might have on feeding behavior. This is important since the feeding pattern affects the diurnal rhythm of cholesterogenesis and HMG CoA reductase activity [16,17]. We have shown that HMG CoA reductase activity doubles between midnight and 8 a.m. in rats fed the diet containing 20% corn oil and 2% cholestyramine [7]. Colchicine injection effectively blocked this induction since experimental rats showed less than 50% of the level of HMG CoA reductase activity and cholesterogenesis noted in control rats. Previously we speculated that the induction of HMG CoA reductase activity during this fasting period reflected an adaptive increase in bile acid synthesis for the subsequent high fat meal [7]. However, since colchicine injection caused a 250% increase in the level of hepatic triglyceride, a block in excretion of nascent VLDL might have caused the accumulation of a small, but critically located, pool of cholesterol that prevented the induction of HMG CoA reductase activity [18]. Accordingly, we suggest that the enzyme increase induced by dietary lipid reflects a need for newly synthesized cholesterol for VLDL production. The hypothesis is consistent with the observations that VLDL is the primary lipoprotein secreted by the liver and that it is much more rapidly

Table 2
Tritium incorporation into digitonin-precipitated sterol of liver and hepatic microsomal HMG CoA reductase activity in control and experimental animals on the different treatment schedules shown in fig.1.

Digitonin-pptd. sterol dpm/g liver				HMG CoA reductase activity (μmole mevalonate/mg microsomal protein/h)				
Exp.	Colchicine		Saline	Colchicine		Saline		Significance
IIC	–		–	10.7 ± 2.1	(5)	33.6 ± 8.7	(5)	$P < 0.001$
IIB	–		–	9.7 ± 3.2	(5)	38.6 ± 4.9	(5)	$P < 0.001$
IIA	10 356 ± 2363	(6)	24 130 ± 6716 (6)	–		–		$P < 0.01$
IB	98 ± 41	(3)	1647 ± 467 (3)	–		–		$P < 0.02$
IA	97 ± 30	(2)	3886 ± 530 (2)	–		–		$P < 0.01$

metabolized than the other smaller serum lipoproteins [19]. It is also unlikely that the reduction of cholesterol synthesis by colchicine reflects a decrease in excretion of biliary sterols. This is suggested since colchicine does not affect biliary cholesterol and bile flow [3]. Since bile acid excretion is the major determinant of bile flow [20], it is therefore unlikely that bile acid excretion is affected by colchicine.

Colchicine was selected for this study because it is rather specific in the way in which it blocks the release of serum proteins. In contrast to ethionine [21], and carbon tetrachloride [22] which also decrease the level of circulating VLDL, it does not interfere with protein synthesis at the levels used in this experiment [3]. In addition it does not block the early steps in the secretory process nor, as in the case of orotic acid [2], does it alter the size of the intrahepatic nucleotide pool [4]. However, the drug does affect nucleoside transport [23]. Therefore, in order to further validate the use of colchicine as a tool for studying the integration of cholesterol and serum lipoprotein synthesis, its effect on cholesterol synthesis should be compared with that of lumicolchicine, a structural analog [24]. This comparison might be valuable since lumicolchicine has only a minimal effect on secretory protein release [4] but it does show some of the other effects of colchicine including the decrease in nucleoside transport [25].

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