EFFECTIVENESS OF N-N-BIS-P-CHLOROPHENYL-3P-TOLYL GLUTARIC ACID DIAMIDE (SRC-3605) AS A HYPOCHOLESTEROLAEMIC COMPOUND IN HYPERCHOLESTEROLAEMIC FEMALE WEANLING AND ADULT RATS

SUNDAR GUJRAL, MRUNALINI PUAR AND NEELAM RANKA

Department of Foods and Nutrition,
Faculty of Home Science, Maharaja Sayajirao University of Baroda, Baroda-390002

Summary : SRC-3605, N-N-bis-P-chlorophenyl 3-p-tolyl glutaric acid diamide, was studied for its hypocholesterolaemic effect on serum and liver cholesterol in hypercholesterolaemic weanling and adult female rats. Weanlings were administered doses of SRC-3605 ranging from 100 to 300 *mg/kg* body weight for 4 or 8 consecutive days. The greatest hypocholesterolaemic effect was observed with doses of 150, 200 and 250 *mg*, although a progressive decreases in serum cholesterol was noted with increasing doses. Hepatic cholesterol decreases supported the serum data, but were inconsistent. Hypercholesterolaemic adult animals received 50, 100, 150 or 200 *mg/kg* body weight of either SRC-3605 or clofibrinic acid for 4 days. A decrease in serum cholesterol levels was observed only with the 200 *mg* SRC-3605. No clear-cut influence of the either compounds was found on hepatic cholesterol. The results indicated that SRC-3605 possesses the property to reduce both serum and liver cholesterol in hypocholesterolaemic weanling female rats.

Key words: SRC-3605 hypocholesterolaemic female weanling rats

INTRODUCTION

During the past several years many hypocholesterolaemic agents have been discovered. Clofibrate (2p-chlorophenoxy, 2 methyl propionic acid ethyl ester, Atromid-S) and other related compounds, have been found to decrease serum lipid levels in animals and man; therefore, they are used clinically for this purpose. The mechanism by which these compounds act is not well understood. It has been postulated that clofibrate decreases serum cholesterol by inhibiting the conversion of acetyl CoA to mevalonate in the hepatic cholesterol synthesis process (6,8). Krishnakantha and Kurup (10) also observed a significant decrease in serum cholesterol with an increase in liver weights in rats fed a 0.5% clofibrate diet.

Another hypocholesterolaemic compound commonly employed is Probucol (DH-581). Barnhart and his co-workers (2,3) have reported 20 to 60% decreases in serum cholesterol in rats, mice, dogs and monkeys but no significant changes in hepatic cholesterol contents. The results of their animal studies on the incorporation of labelled acetate and mevalonate into cholesterol led the authors to suggest that DH-581 inhibited cholesterol biosynthesis in the early stages.

Many other compounds such as Scholex and Clofenate (8,10) have also been tried and found to possess the same utility as Clofibrate and Probucol, but a compound which has a

cholesterol-lowering effect on both serum and hepatic tissues has yet to be discovered. We report here our results with N-N-bis-p-chlorophenyl-3-p-tolyl glutaric acid (SRC-3605), a compound synthesized at Sarabhai Research Centre, Baroda.

MATERIALS AND METHODS

Seventy-eight female weanling (body weights 30 to 60 g) and 60 adult female rats (200 to 250 g) were used in this study. On arrival, 6 animals from each age group were randomly selected to form the normal control groups. They were fed the basal diet for 14 days, after which they were autopsied to get the zero-time control values. The remaining weanling and adult rats were fed a cholesterogenic diet for 14 days (pre-experimental period). The weanling cholesterogenic diet contained 1.0% cholesterol and the adult diet 1.5%. On day 15 the weanlings were divided into 12 equal groups - 1 each 4- and 8-day experimental controls plus 10 compound-fed experimental groups. The hypercholesterolaemic adult rats were divided into 9 equal groups - 1 control plus 8 experimental groups. SRC-3605 in a 1% tragacanth solution or clofibrate was then administered intragastrically in varying doses to the animals in the experimental groups. Weanling were given 100, 150, 200, 250 or 300 *mg* SRC-3605/*kg* body weight daily for 4 or 8 days, while adult animals received 50, 100, 150, or 200 *mg* SRC-3605 or clofibrate/*kg* body weight for 4 days. During this period (experimental), all the animals continued to get the cholesterogenic diet as before.

Autopsy was performed under ether anaesthesia. Blood was collected from the carotid artery and livers were removed and stored frozen until analysed. Serum cholesterol was determined by the method of Sperry and Webb (11) and liver cholesterol by the method of Bowan and Wolf (4).

The data presnted in the Tables include means of 6 animals $\pm$ standard errors of the means, unless otherwise stated. Identification of significant differences between means ($P < 0.05$) was determined by the Student's test and was limited to comparisons of experimental groups with their respective hypercholesterolaemic control group.

RESULTS AND DISCUSSION

Serum cholesterol levels in the weanling rats fed the cholesterogenic diet for 18 or 22 days rose from the basal value of 71.5 to 133.5 and 160.8 *mg*/100 *ml* respectively. In the groups administered the compound, cholesterol concentrations decreased progressively with increasing doses of SRC-3605 (Table I). Percent decreases in the experimental groups as compared to their respective control groups, ranged from 61 to 81 and 73 to 94% in the 4- and 8-day groups respectively. This indicated that an increase in the duration and/or the dosage of the compound augmented its hypocholesterolaemic effect. The enhanced cholesterol accumulation seen in the livers of the weanlings fed only the cholesterogenic diet seemed to be effectively

restrained by the administration of SRC-3605. Hepatic cholesterol decreases in these groups were also noted. They ranged from 10 to 32% in 4 days and 14 to 43% in 8 days.

TABLE I: Serum and hepatic cholesterol concentrations in female weanling hypercholesterolaemic rats after administration of SRC-3605 for 4 and 8 days.

Group	*Daily dose of compound in mg*	*Cholesterol (mean ± S.E.M.)*	
		Serum (mg/100 ml)	*Lever (mg/g wet wt.)*
Normal control		71.5±1.48	2.64±0.14
4-day experimental control		133.5±6.39	7.72±0.21
4-day SRC-3605	100	52.2±1.07**	8.37±0.35
	150	50.5±0.99**	6.93±0.88
	200	43.2±0.80**	6.36±0.16
	250	31.3±0.77**	5.66±0.07**
	300	25.7±0.53**	3.28±0.13**
8-days experimental control		160.8±5.36	17.71±0.31
8-day SRC-3605	100	42.1±2.34**	10.11±0.96**
	150	25.4±0.89**	15.29±2.11
	200	17.2±0.49**	13.95±4.49*
	250	12.4±0.32**	15.19±1.17
	300	9.6'	13.25±2.03

'Mean of two assays due to sample insufficiency.
* $P<0.05$; ** $P<.01$

In normocholesterolaemic rats clofibrate fed for 6 to 8 days (0.3%) in diet) has been found to decrease serum and hepatic cholesterol by 10 and 30% respectively (1). It has also been reported that feeding 250 *mg* clofibrate/*kg* body weight to young normal rats for 14 days caused a decrease in serum and hepatic cholesterol by 57 and 10% respectively (5). Furthermore, Susuki *et al.* (12) have reported that a compound structurally related to clofibrate when fed to normocholesterolaemic rats at levels ranging from 100 to 300 *mg/kg* decreased hepatic cholesterol concentrations by 15 to 20%.

In adult female rats on cholesterogenic diet, serum cholesterol levels increased from the mean basal value of 71.6 to 267.7 *mg*/100 *ml* in 18 days. However, the hypocholesterolaemic effect of SRC-3605 observed in weanlings was not evident in the hypercholesterolaemic adult rats (Table II). It could be that the doses administered were inadequate, as a 20% non-significant decrease in serum cholesterol level was seen only in the group fed high dose (200 *mg*) of SRC-3605. Clofibrate therapy had no beneficial effect on hypercholesterolaemic adult female rats. The effects of clofibrinic acid and SRC-3605 on hepatic cholesterol were inconsistent.

TABLE II : Effect of SRC-3605 or clofibrinic acid on serum and hepatic cholesterol levels in female adult rats fed a cholesterogenic diet.

Group	*Daily dose of compund in mg*	*Cholesterol (mean ±S.E.M.)*	
		Serum (mg/100 ml)	*Liver (mg/g wet wt.)*
Normal control		71.6±12.83	5.99±0.25
Experimental control		267.7±49.24	39.86±3.16
SRC-3605	50	267.0±15.66	46.21±6.84
	100	279.0±54.92	30.88±1.92*
	150	275.0±29.48	51.75±5.10
	200	214.2±35.24	43.21±5.24
Clofibrinic acid	50	291.3±44.48	33.25±5.27
	100	328.3±25.93	30.93±3.03
	150	276.3±48.86	50.10±4.81
	200	324.8±39.71	34.66±3.06

* $P<0.05$

Table III compares data culled from literature with that obtained in the present study. Thus the following possibilities could account for the lack of effect of clofibrinic acid obtained in the present study: (a) hypercholesterolaemic rats were used in thiss tudy; (b) the cholesterogenic diet was continued during the experimental period; (c) the clofibrate dose was much lower than the ones used in the studies reported (1, 5) and (d) the duration of clofibrate feeding was short.

TABLE III: The effects of variation in the dose and duration of clofibrate feeding on serum and hepatic cholesterol levels.

	Source								
	Avoy et al. (1)				*Cenedella & Crouthamel*(5)		*Present study*		
Diet	fat free				normal		normal	cholesterogenic	
Experimental period, days	6		8		14		4		
Group	C	E	C	E	C	E	C	HC	E
Clofibrate dose, *mg/kg*	0	300	0	300	0	250	0	0	200
Cholesterol									
Serum *mg/100 ml*	70.0	48.0	65.0	45.0	96.6	41.1	71.6	267.7	324.8
Liver *mg/g*	2.44	2.30	2.76	2.06	2.16	1.87	5.99	39.86	34.66

C = control ; E = experimental ; HC = hypercholesterolaemic control

Howard *et al.* (8) fed hyperlipidemic humans 1.5 g clofibrate for 8 weeks and observed no significant changes in serum cholesterol.

The authors concluded that clofibrate was not effective in reducing serum cholesterol in hyperlipidemia. However, in hypercholesterolaemic patients clofibrate fed at a level of 500 *mg* thrice daily had reduced serum cholesterol level but only by 7% in two weeks (9). Also in a double blind clinical trial of clofibrate (1.5 - 2 g/day) in the treatment of ischemic heart disease, it was reported that after 6 months of clofibrate therapy, serum cholesterol levels decreased by 12 and 15% in males and females respectively but these levels remained higher than the normal standards (7).

It has been suggested that decreasing serum cholesterol by increasing cholesterol deposition into hepatic and extrahepatic tissues may lead to cardiovascular disorders. Therefore, a compound which without permitting the accumulation of cholesterol in the tissues decreases serum cholesterol levels may be considered as the ideal one. Since SRC-3605 significantly reduced serum and hepatic cholesterol in weanling rats and also serum cholesterol (20% non-significant decrease) in adult rats fed 200 *mg*/*kg*/day for 4 days, the drug seems to be an effective cholesterol lowering agent in hypercholesterolaemia.

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