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A COMPARISON OF HYPOLIPIDEMIC DRUGS IN THE PREVENTION OF AN OROTIC ACID FATTY LIVER*

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Abstract—The prevention of a fatty liver by adding test substances to a 1 per cent orotic acid diet was used to obtain assay curves for various hypolipidemic drugs. The following drugs had approximately the same activity in this test: ethyl *p*-chlorophenoxyisobutyric acid (PCIB); *p*-phenoxyphenyl methanesulfonate and *N,N*-dimethyl-*N'*-(4-phenoxyphenyl) sulfamide (Upjohn U 22105 and U 25030); and 2,2'-[(1-methyl-4,4'-diphenylbutylidine)-bis-(*p*-phenyleneoxy)] bis-triethylamine-citrate (Squibb SQ 11071). 1-Methyl-4-piperidyl-bis-(*p*-chloro-phenoxy) acetate (Sandoz Salt 42348) was two to three times as active, and 2-methyl-2-(*p*-1,2,3,4-tetrahydro-1-napthylphenoxy) propionic acid (Ciba Su 13437) was ten times as active as CPIB. On the same weight basis, choloxon (D-thyroxine; D-T_4) was 80–90 times as active. Dilantin, L-thyropine (L-T_4), Dow DH 581, nicotinic acid, and cholestyramine had little or no effect. Adenine, allopurinol [4-hydroxytyrazolo (3,4-*d*) pyrimidine] and 5,5'-diphenyl-2-thiohydantoin (DPTH) were as active as most of the drugs in preventing triglyceride deposition. The only substances which increased liver α-glycerophosphate dehydrogenase were: CPIB, Su 13437, SaH 42348, D-T_4 and L-T_4. The lipotropic effect of these drugs was not mediated through this enzyme. Feeding orotic acid alone practically eliminated the pre-β and β-lipoprotein (βLP) band from the serum gel electrophoresis, but the intensity of the β band was restored by the active drugs in proportion to their dosages; inactive substances did not restore the βLP band. A quick screening procedure was developed for substances which intensify the serum βLP band.

TWO HYPOLIPIDEMIC drugs, CPIB† and Su 13437, prevented the formation of a fatty liver when fed simultaneously with orotic acid.[1] They also were characterized by the appearance of a strong β-lipoprotein (βLP) band in serum gel electrophoresis, and this was easily detected in orotic acid-fed rats because the latter had little or no pre-β or βLP in their sera in the absence of the hypolipidemic drugs.[1] These observations suggested that the effects of these drugs on lipid metabolism might be mediated through a βLP response, and this raised several other questions which are the subject of this report: (1) do all of the hypolipidemic drugs which are currently being developed have a similar effect on an orotic acid fatty liver and the serum β-lipoproteins; and (2) can these responses be used to compare different drugs quantitatively?

The drugs were supplied by the various companies listed.† The structures and other

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† The drugs used in this study were obtained from the companies listed in parentheses and have the following chemical structures: CPIB, ethyl *p*-chlorophenoxyisobutyric acid (Ayerst); choloxon, D-thyroxine (D-T_4) (Flint); U 22105, *p*-phenoxyphenyl methanesulfonate (Upjohn); U 25030, *N,N* dimethyl-*N'*-(4-phenoxyphenyl) sulfamide (Upjohn); SaH 42348, 1-methyl-4-piperidyl-bis-(*p*-chlorophenoxy) acetate (Sandoz); Su 13437, 2-methyl-2-(*p*-1,2,3,4-tetrahydro-1-naphthylphenoxy) propionic acid (Ciba); SQ 11071, 2,2'-[(1-methyl-4,4'-diphenylbutylidine)-bis-(*p*-phenyleneoxy)] bis-triethylamine-citrate (Squibb); allopurinol, 4-hydroxytyrazolo (3,4-*d*) pyrimidine (Burroughs-Wellcome); L-T_4, L-thyroxine; GPD, α-glycerophosphate dehydrogenase; βLP, β-lipoprotein; DPTH, 5,5'-diphenyl-2-thiohydantoin.

studies on CPIB, Su 13437, SaH 42348 and DH 581 have been reviewed.[2] The Upjohn[3,4] and Squibb[5] drugs have been reported to be hypolipidemic or inhibitors of cholesterol synthesis. Other substances which prevent an orotic acid fatty liver—adenine,[6,7] allopurinol[8] and DPTH[9]—have been included in this study for comparative purposes. Since DPTH is the thiol analog of Dilantin, the latter was also studied. D-T_4 was compared with L-T_4.

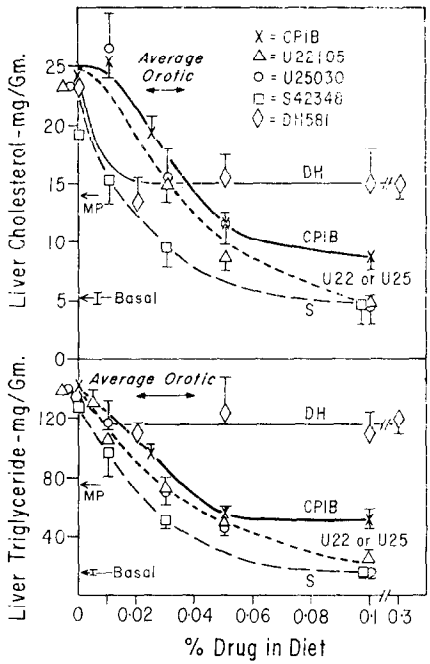

FIG. 1. Dose–response curves of some hypolipidemic drugs in the prevention of an orotic acid fatty liver. Weanling male rats were fed 1 per cent orotic acid ± the test drug at the dietary concentrations indicated for 2 weeks before the liver was analyzed for triglycerides and cholesterol. Mean ± S.E. for 15–25 rats per point at drug concentrations of 0·02–0·05 per cent; five to ten rats per point at other concentrations; 15 rats per point for each orotic acid alone; 60 rats for the basal diet; ten rats per point for DH 581 at 0·1–0·3 per cent (five rats for 0·05 + 0·02 per cent). MP = midpoint or one-half the distance between the orotic acid and basal levels.

METHODS

Weanling male Holtzman rats were fed a basal diet (28 per cent casein, 10 per cent corn oil, 58 per cent glucose, 4 per cent salts, and a vitamin mixture) containing 1 per cent orotic acid and an appropriate quantity of the test substance for 14 days. Food consumption was measured daily and averaged for the 2-week period. The rats were sacrificed by decapitation, and the blood serum was used for gel electrophoresis (Quick-Disc electrophoresis of lipoproteins, Canalco Diagnostic Products, Rockville, Md.). Livers were analyzed for triglycerides,[10] cholesterol[11] and GPD activity.[12] When the proper dose range was established, the experiments were repeated often enough to provide a reliable assay curve.

RESULTS

The assay curves for the various hypolipidemic drugs and the other test substances are shown in Figs. 1–3. Individual experiments with orotic acid alone illustrate the relatively small variability of this point (zero dosage) on the assay curve in spite of the fact that liver lipids were still increasing after 2 weeks of orotic acid feeding.[1] All of the drugs tested in this series effectively prevented the orotic acid fatty liver, except for small effects from DH 581, L-T_4 and Dilantin. The addition of 0·1 to 0·3 per cent nicotinic acid or 0·5–1 per cent cholestyramine or 0·3 per cent pyrazoinic acid to the

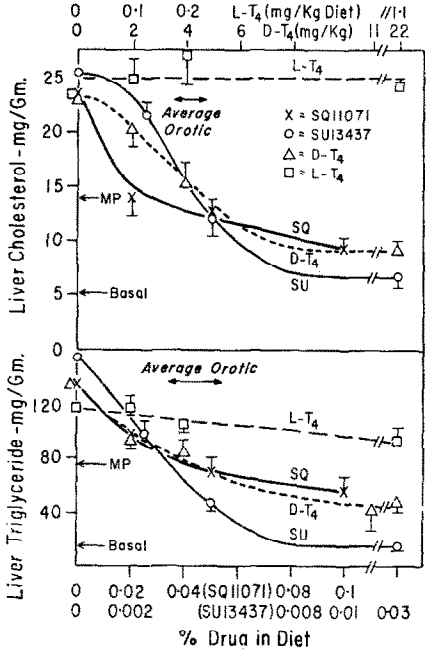

FIG. 2. Dose–response curves for hypolipidemic drugs. Procedures used were the same as those described in Fig. 1; ten rats per point for SQ 11071 and L-T_4; 15 and 20 rats per group for two low dosages of Su 13437 and D-T_4 (five to ten rats for higher doses); ten rats per group for all orotic acid only values, except for 24–30 rats per group for D-T_4 experiments.

diet had no effect on the orotic acid fatty liver. Some of the drugs and all three of the other test substances seemed to be more effective in preventing triglyceride than cholesterol deposition in the liver, since they showed a "lag" in the cholesterol assay curve at low dosages, while the liver triglyceride decreased immediately with small doses of all drugs.

An exact comparison of such diverse curves for the purpose of evaluating relative activities is difficult at best, but an approximation has been obtained by comparing the dosages required to reduce the liver lipids halfway from the average orotic acid levels to the basal levels. Such a comparison ignores the fact that some drugs were more effective than others in completely restoring normal values at higher dosages. The results in Table 1 show that U 22105, U 25030 and SQ 11071 had approximately the

same or only slightly more activity than CPIB. SaH 42348 was two to three times more active, while Su 13437 was ten times as active. D-T_4 was clearly effective in this test, while an equivalent amount of L-T_4 on a calorigenic basis (1/20) was relatively inactive. It should be emphasized that the relative activities in this test have not been correlated with their relative hypolipidemic activities, but Su 13437 is considered by the manufacturer to be about ten times as active as CPIB. Adenine, allopurinol and DPTH were as effective as most of the drugs in preventing triglyceride deposition, but appreciably higher concentrations of these substances were needed to prevent cholesterol deposition; i.e. the lag in the cholesterol vs. triglyceride reduction in the assay curves was especially apparent with adenine.

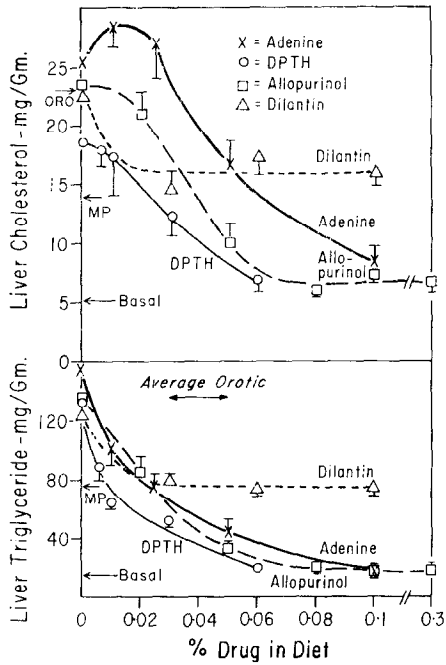

FIG. 3. Dose–response curves for other substances which prevent an orotic acid fatty liver. Procedures used were the same as those described in Fig. 1; 10–15 rats per point for adenine and DPTH; 20 rats per point for allopurinol and Dilantin.

Food consumption. Rats on the basal diet ate an average of 11·5 ± 0·36 g of food/day over the 2-week period and gained 84 ± 2·3 g in body weight. On the 1 per cent orotic acid diet, they ate 10·7 ± 0·30 g (93 per cent) and gained 59 ± 1·9 g (70 per cent). At the midpoint of each triglyceride assay curve, the food consumption with all these drugs was ± 12 per cent of that obtained with orotic acid alone or with the reference compound, CPIB (Table 1). Similar results were obtained with DH-581, L-T_4 and Dilantin. At 0·1 per cent of the diet, most of the drugs also had no effect on food consumption, but SaH 42348 and SQ 11071 were 80 and 60 per cent, respectively, of their corresponding midpoint values.

When compared at a diet concentration equal to the midpoint of each triglyceride assay curve, the only drugs which inhibited growth were SaH 42348 and D-T_4; these

TABLE 1. RELATIVE ACTIVITIES OF VARIOUS HYPOLIPIDEMIC DRUGS IN THE PREVENTION OF AN OROTIC ACID FATTY LIVER

Company	Drug No.	Midpoint*		Relative activities†		Food consumption‡	Liver wt.§
		TG	Chol.	TG	Chol.		
Ayerst	CPIB	0·036	0·042	1·0	1·0	9·9	5·9
Upjohn	U 22105	0·028	0·024	1·3	1·7	10·5	6·5
	U 25030					11·3	6·1
Sandoz	SaH 42348	0·018	0·014	2·0	3·0	9·5	6·8
Ciba	Su 13437	0·0035	0·0044	10·3	9·6	11·1	5·8
Squibb	SQ 11071	0·038	0·027	1·0	1·6	10·4	5·1
Flint	Choloxon ($D\text{-}T_4$)	4·3*	4·6*	83·0	91·0	11·1	5·6
Nutritional Biochemicals	Adenine	0·023	0·060	1·0	1·0	11·5	4·5
Aldrich Chemical	DPTH	0·010	0·024	2·3	2·5	10·3	6·3
Burroughs-Wellcome	Allopurinol	0·024	0·039	1·0	1·5	11·4	4·4

* Dietary concentrations required to reduce liver lipids half-way from the average orotic acid level (TG = 136; cholesterol = 23) to the basal level (TG = 16; cholesterol = 5); midpoints: TG = 76; cholesterol = 14. All values are given as per cent of the diet, except for $D\text{-}T_4$ which is given as milligrams per kilogram of diet, TG = triglyceride; Chol. = cholesterol.

† CPIB and adenine were arbitrarily given a value of 1·0 for comparison with the other compounds in each series. Adenine was 1·6 times as active as CPIB in preventing TG deposition, but only 0·7 as active for cholesterol.

‡ Food consumption in grams per day at drug concentrations equal to the midpoint of each TG assay curve. Basal diet = 11·5 g/day; orotic acid alone = 10·7.

§ Liver weight as per cent of body weight when drug concentration = 0·1% of the diet, except: Su 13437 = 0·01%; DPTH = 0·06%; $D\text{-}T_4$ = 11 mg/kg of diet. Basal liver weight = 4·1%; orotic acid alone = 5·5%; S.E. = approximately ± 3·5% of the mean for groups of 15 rats.

gave body weight increases equal to 75 per cent of the growth rate obtained with orotic acid alone. Most of the drugs also did not inhibit growth at the higher diet concentrations tested; the exceptions were: SaH 42348, 17 per cent and SQ 11071, 46 per cent (at 0·1 per cent of the diet); Su 13437, 66 per cent (at 0·01 per cent); D-T_4, 75 per cent (at 11 mg/kg of diet); all are given as per cent of the growth rate obtained with orotic acid alone. Dilantin, DH 581 and L-T_4 also did not increase the growth rate below that obtained with orotic acid alone. None of these drugs increased the growth rate above the orotic acid level to the basal level.

When the orotic acid diet was supplemented with 0·02–0·1 per cent adenine or allopurinol, the food consumption increased to the basal level, and the body weight gain increased above the orotic acid level to 82 and 89 per cent, respectively, of the basal level. Food consumption and weight gain remained at the orotic acid level when the orotic acid diet was supplemented with 0·01% DPTH, but both fell to about 85 per cent of this value at 0·06 per cent.

Liver GPD. Liver GPD was 22 $\pm$ 1·1 and 18 $\pm$ 0·7 units, respectively, in rats fed the basal diet or 1 per cent orotic acid. Three of the drugs (CPIB, Su 13437, SaH 42348) increased this enzyme activity to 25–30 units (S.E. = approximately 12 per cent of the mean) at diet concentrations equivalent to the midpoint of each triglyceride assay curve; much higher GPD values were obtained at higher concentrations. At the midpoint of the D-T_4 curve, the liver GPD was 60 units, while an "equivalent" amount of L-T_4 gave 30 units. The liver GPD was, therefore, slightly elevated by these drugs at concentrations which partially prevented an orotic acid fatty liver, but a similar elevation of this enzyme by L-T_4 had no effect on liver lipids. Hence, the lipotropic effect of the drugs was not exerted through an increased liver GPD, and the effect of D-T_4 does not appear to be due to a metabolic conversion to L-T_4.

The following substances (all tested in the presence of orotic acid) had no effect on liver GPD (15–21 units) at relatively high concentrations: U 22105, U 25030, DH 581, nicotinic acid and allopurinol at 0·3 per cent of the diet; SQ 11071, Dilantin and adenine at 0·1 per cent; cholestyramine at 1 per cent. Liver GPD was decreased to 8 units by 0·03–0·06% DPTH. Such studies provided additional evidence that an elevated GPD is not essential for the lipotropic effect of the active drugs.

Hepatomegaly. Orotic acid alone increased liver weight from the basal 4·1 per cent of the body weight to 5·5 per cent. When compared at the midpoint of each triglyceride assay curve, all of the substances tested gave liver weights which were in the 5·4–5·6 per cent range, except Su 13437 = 5·8, DPTH = 5·9, SQ 11071 = 5·0, U 25030 = 4·9 and allopurinol = 4·7 per cent. The corresponding values for 0·1 per cent of the diet are given in Table 1 and they show an additional hepatomegalic effect from most of the hypolipidemic drugs. SQ 11071 and DH 581 (liver weight = 5·1 per cent) were the only drugs which decreased the orotic acid liver weight slightly. Both adenine and allopurinol not only prevented the orotic acid fatty liver, but also largely prevented the associated hepatomegaly. Dilantin and L-T_4 (1·1 mg/kg) had no effect on the orotic acid liver weight.

Lipoproteins. In young Holtzman rats fed the basal diet for 2 weeks, the pre-β band was absent in one-half the rats and was weak in the others. There was a good β band in all. After 2 weeks on the 1 per cent orotic acid diet, the pre-β band was absent in all rats; the β band also disappeared from three-fourths of the samples and was weak in the remaining. When the hypolipidemic drugs were added to the orotic acid diet, the

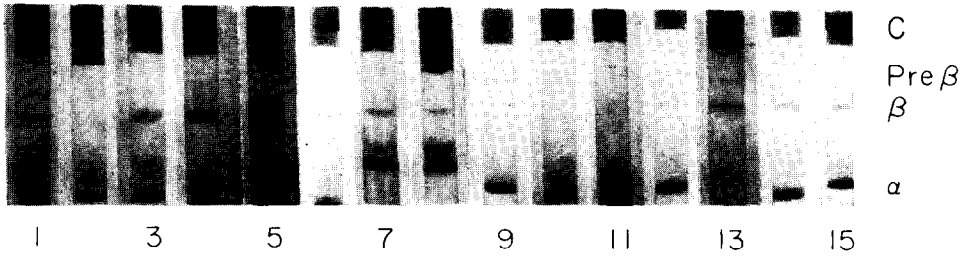

FIG. 4. Gel lipoprotein electrophoresis patterns of sera from weanling male rats fed 1 per cent orotic acid plus the drug for 2 weeks. Differences in the heights of the columns are due to variations in filling the tubes. They have been lined up in relation to the β-bands. On the arbitrary evaluation scale: tube 2 = 0; tube 9 = 1; tube 1 = 4; tubes 4 and 5 exceeded a rating of 4 and could not be evaluated on this scale. All drugs were added to the 1 per cent orotic acid diet at a concentration of 0·1 per cent unless noted otherwise. (1) Basal diet (no orotic acid); (2) orotic acid alone; (3) CPIB; (4) SaH 42348; (5) SQ 11071; (6) U 25030; (7) U 22105; (8) SU 13437 (0·01%); (9) D-T_4 (4–22 mg/kg of diet); (10) L-T_4 (0·2–1·1 mg/kg of diet); (11) DH 581 (0·3%); (12) Dilantin (0·06–0·1%); (13) allopurinol; (14) DPTH (0·06%); (15) adenine.

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pre-β band continued to be absent, while the β-band was restored in relation to the dosage. Since the density of the β band could not be measured quantitatively, an attempt was made to rate the intensity of the bands visually on an arbitrary scale of 1–4. On this scale, the average rat on the basal diet rated 3·3 and the average orotic acid-fed rat was 0·4. At dietary concentrations equal to the midpoint of the triglyceride assay curve for each active drug, this βLP score was approximately 1·5 for all; this was a detectable difference visually, but not a strong β band.

At higher concentrations, the active drugs produced strong β bands, and typical results are shown in Fig. 4. SaH 42348 and SQ 11071 gave unusually strong β bands at 0·1 per cent of the diet. D-T_4 (4–22 mg/kg of diet) had a slight effect (rating = 1), while an equivalent amount of L-T_4 had no detectable effect. Allopurinol, adenine and DPTH produced βLP bands in the same way that the active drugs did. DH 581 was relatively inactive in preventing an orotic acid fatty liver and also had little or no effect in restoring the βLP. The addition of 0·5–1 per cent cholestyramine, 0·1–0·3 per cent nicotinic acid or 0·3 per cent pyrazoinic acid to the orotic acid diet had no effect on the serum βLP.

βLP test. Drugs like CPIB, which stimulate the production of βLP or otherwise intensify the βLP band, can be screened readily by determining their effect on the βLP band in the presence of orotic acid. Weanling and adult (200 g) Holtzman and Miller male rats were fed the basal diet $\pm$ 0·3% CPIB or 0·25 per cent adenine and the 1 per cent orotic acid diet with or without CPIB or adenine. Post-orbital blood samples were taken after 3, 5, 7 and 10 days for serum gel electrophoresis. With all rats on the basal diet, the serum gel electrophoresis was characterized by a strong β band and a weak or absent pre-β band. The pre-β band was also weak or absent in all other groups, except that a moderate to good pre-β band appeared after feeding adenine (basal or orotic acid diets) for 7–10 days. On orotic acid, the β band became very weak after 5 days in weanlings and after 5–7 days in adults; this was prevented by adding CPIB or adenine to the orotic acid diet. Since the β band was already strong on the basal diet, the addition of CPIB or adenine to the basal diet did not produce enough of a difference to be detected with confidence. The effect of CPIB on the β band was easily detected after 5–7 days of feeding with orotic acid. In these experiments, the CPIB response could be differentiated from the adenine effect because only the latter also restored a strong pre-β band.

DISCUSSION

Essentially all of the drugs currently under investigation as hypolipidemic drugs prevented an orotic acid fatty liver, and they did so in association with an increased intensity of the serum β-lipoprotein band on gel electrophoresis. It is tempting to believe that this effect is related to their hypolipidemic action, since both phenomena are concerned with serum lipids, but such a relationship has not yet been established. Relative activities of the various drugs can be approximated by this test, but there is no assurance that their relative hypolipidemic activities parallel their effectiveness in this test. If the phenomena are related, the hypolipidemic effect of these drugs would seem to be mediated via the serum β-lipoproteins, and all of the active drugs do have this effect on βLP in common.

The interrelationship of pre-β and βLP is not clear. Orotic acid clearly eliminated the pre-β band from all gel electrophoresis patterns, but it also eliminated or greatly

reduced the β band when that was the predominant lipoprotein in serum. βLP appeared independently of the pre-β, since that was what the hypolipidemic drugs did in the presence of orotic acid. Adenine not only reversed the orotic acid effect as it related to either the pre-β or βLP, but it seemed to have a special relationship to the pre-β-lipoproteins. It intensified the pre-β band even when given to normal rats on a basal diet.

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