J. Pharm. Pharmacol. 1985, 37: 906–909
Communicate May 24, 1985

Further assessment of glunicate hypolipidaemic activity in the rat

ANNA RITA RENZETTI, MARCO CRISCUOLI*, ALESSANDRO SUBISSI, *Department of Pharmacology, Research Division, Laboratori Guidotti S.p.A., via Livornese 402, 56010 S. Piero a Grado, Pisa, Italy*

The hypolipidaemic activity of glunicate administered orally to rats has been further evaluated. In the fasted normolipidaemic rat, glunicate, in single doses of 10–300 mg kg^{-1} (as nicotinic acid equivalents), exerted a dose-dependent reduction of plasma triglycerides and cholesterol. At the lower doses, the HDL : total cholesterol ratio was enhanced, but at higher doses it was depressed. Similar results were obtained in the fasted obese Zucker rat, but the acute effects of glunicate seemed less marked in this strain. When the same doses of glunicate were given for 7–8 consecutive days no alteration of the plasma lipid pattern was observed, the reason being that the hypocholesterolaemic action of single doses of glunicate is reduced or abolished in fed animals; this was not due to pharmacokinetic factors depending on feeding. The same lack of hypocholesterolaemic action in fed animals occurred with nicotinic acid.

Glunicate (2-deoxy-2-nicotinamide-β-D-glucopyranose 1,3,4,6-tetranicotinate; LG 13979) is a new derivative of nicotinic acid, which, after oral administration provides low, constant and lasting concentrations of plasma nicotinic acid and thereby showing a more potent and lasting hypolipidaemic action in the rat (Subissi et al 1983). Moreover, glunicate protected the rabbit arterial wall from cholesterol-induced atherosclerotic changes (Criscuoli et al 1984), lowered plasma triglycerides, circulating immune complex levels and liver lysosomal membrane permeability in rats kept on an atherogenic diet (Feher et al 1985), and, in preliminary trials, lowered plasma cholesterol in hyperlipoproteinaemic patients (Subissi 1984).

In view of these properties of glunicate, we have further examined its hypolipidaemic action after single or repeated oral doses in rats of two strains, the normolipidaemic Sprague-Dawley and the hyperlipidaemic obese Zucker rat.

Materials and methods

Animals and drugs

Male Sprague-Dawley rats, 8–12 weeks, and female obese (fa/fa) Zucker rats, 36–40 weeks (from our own breeding station), freely fed Altromin-MT (Rieper, Vandoies, BZ, Italy) and water and kept in constant environmental conditions (temperature 20–22 °C, relative humidity 60–70% and 12 h light/12 h dark), were used.

Drugs used were: glunicate (Laboratori Guidotti, Pisa, Italy) and nicotinic acid (Merck, Darmstadt, FGR). Both drugs were dissolved in water acidified with HCl and dosed by gastric tube in a volume of 10 ml kg^{-1} (pH 1·5–2·5). The controls received an equal volume of acidified vehicle. Solutions were administered within 30 min of preparation. All the doses are reported in terms of nicotinic acid equivalents.

Experimental procedure

Single dose experiments. Rats, fasted for 24 h unless otherwise stated, were dosed orally with the test drugs. At different times thereafter, the thorax was opened under ether anaesthesia and the rats bled by intracardiac puncture with heparinized syringes; blood was centrifuged at 1500*g* and the plasma was stored at −20 °C until analysis.

Repeated dose experiments. The test drugs were administered orally, once a day, for 7 or 8 consecutive days, between 0900 and 1100h. The animals, fasted for 16 h, were killed and bled as above 24 h after the last treatment.

Methods

Plasma free fatty acids were determined according to Dole & Meinertz (1960), plasma triglycerides according to a modified version (Da Fonseca-Wollheim 1973) of the method of Eggstein & Kreutz (1966) and plasma total cholesterol according to Roeschlau et al (1974). Plasma HDL cholesterol was determined with the same method after precipitation of LDL and VLDL with phosphotungstic acid and $MgCl_2$ (Burstein et al 1970). Plasma free nicotinic acid was determined according to Carlson (1966) and blood glucose according to Werner et al (1970). The statistical comparisons were made using Student's *t*-test.

Results

Effects of a single administration in the fasted normolipidaemic rat. Table 1 shows a dose response study of glunicate on plasma lipids. At 12 h the lowering effects on triglycerides (27–77%) and total cholesterol (22–54%) were dose-related and significant from the dose of 10 mg kg^{-1}. Only the two lower doses increased HDL : total cholesterol ratio, while the higher doses had no effect. At 24 h the higher doses were still effective on triglycerides and on total cholesterol, and also depressed the HDL : total cholesterol ratio, the lower doses being ineffective.

Effects of a single administration in the fasted Zucker obese rat. As seen in Table 2, a single administration of glunicate, at 300 mg kg^{-1}, exerted a considerable hypo-

* Correspondence.

Table 1. Effects of a single oral administration of glunicate on plasma lipids in the fasted rat. Doses are expressed in terms of nicotinic acid. Mean values ± s.e.m. are reported (n = 6–7). Significance levels vs controls are denoted by: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

	Time from administration (h)									
	12					24				
Dose mg kg^{-1} p.o.	0	10	30	100	300	0	10	30	100	300
Triglycerides (mg dl^{-1})	44±3·5	32±2·0†	22±3·3†	22±4·6†	10±1·0‡	59±6·5	54±6·5	50±3·8	41±6·6	28±3·9†
Total cholesterol (mg dl^{-1})	46±2·2	36±3·5*	30±2·2‡	27±2·9‡	21±3·2‡	42±3·2	39±2·1	34±3·2	25±3·9†	16±2·2‡
HDL cholesterol (mg dl^{-1})	31±2·2	31±3·0	25±2·0	18±3·2†	15±2·2‡	28±2·3	24±1·9	20±2·3*	11±1·6‡	7·0±1·0‡
HDL : total cholesterol (%)	69±3·2	84±2·4†	82±1·9†	67±9·1	71±3·5	66±2·7	62±3·2	58±6·3	41±2·9‡	43±4·2†

lipidaemic action in the obese Zucker rat, generally comparable to that of an equivalent dose of nicotinic acid. The action of glunicate on triglycerides and cholesterol was more rapid in onset and raised HDL : total cholesterol ratio, while nicotinic acid depressed it. Moreover glunicate did not influence blood glucose, while nicotinic acid had a biphasic effect, an initial increase being followed by a decrease.

Effects of repeated administration. When glunicate was administered once daily to normolipidaemic rats for 8 consecutive days at 100 and 300 mg kg^{-1} (doses which were active acutely), none of the observed plasma lipid parameters was affected significantly. A slight non-significant reduction (20%) of plasma triglycerides was seen at the two higher doses (Table 3).

Both glunicate and nicotinic acid were also tested in obese Zucker rats, at 100 mg kg^{-1}, for 7 consecutive days; neither drug influenced the plasma lipids while nicotinic acid, but not glunicate, raised blood glucose by 21% ($P < 0.05$) (Table 3).

Influence of feeding on the hypolipidaemic effect of a single dose of glunicate in the rat. As shown in Table 4, glunicate 300 mg kg^{-1} lowered plasma FFA, triglycerides, cholesterol and blood glucose in the fasted rat. In the fed animal, the hypocholesterolaemic action of glunicate was completely lost at 4 and 8 h and greatly diminished at 24 h (from 65 to 20%), also the hypoglycaemic effect was not observed. The effects of feeding on the lowering action of glunicate on plasma FFA and triglycerides were less clear, since there were large differences between the basal values of these parameters in the fasted and in the fed state. A loss of this lowering activity, however, did take place at least at the longer times. The effects of feeding on the hypocholesterolaemic effects of nicotinic acid were also studied. In this experiment nicotinic acid, tested in fasted rats at the oral dose of 300 mg kg^{-1}, did not influence plasma cholesterol at 2, 4 and 8 h, but lowered it at 24 h (26%, $P < 0.05$). In fed animals, plasma cholesterol was not reduced by the same dose of nicotinic acid at any of the four observation times (data not shown). Fig. 1 shows that feeding slowed the absorption of glunicate (peak of nicotinic acid at 4 rather than at 2 h). However, the area under the curve between 0 and 24 h is practically identical in the two experimental conditions.

Table 5 deals more extensively with the effects of feeding on the hypocholesterolaemic action of glunicate. In animals fasted before and after administration, glunicate 100 mg kg^{-1} lowered plasma cholesterol by 49%. Feeding the animals either before and after administration, or just after administration, completely antagonized the hypocholesterolaemic action of glunicate, while in animals fed before administration and fasted thereafter, the lowering effect was reduced to 30%.

Table 2. Effects of a single oral administration of glunicate and nicotinic acid 300 mg kg^{-1} (as nicotinic acid equivalents) on plasma lipids and blood glucose of the fasted obese Zucker rat. Mean values ± s.e.m. are reported (n = 5). Significance levels vs controls are denoted by: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

	FFA mequiv litre^{-1}				Triglycerides mg dl^{-1}				Total cholesterol mg dl^{-1}				HDL cholesterol mg dl^{-1}				HDL/total cholesterol %				Blood glucose mg dl^{-1}			
	Time from administration (h)																							
Treatment	2	4	8	24	2	4	8	24	2	4	8	24	2	4	8	24	2	4	8	24	2	4	8	24
Vehicle	0·95± 0·06	1·01± 0·08	0·76± 0·07	0·96± 0·12	226± 40	355± 42	344± 41	242± 12	50± 4·4	60± 3·5	64± 6·2	60± 2·2	43± 4·4	42± 3·6	45± 3·9	48± 2·6	84± 2·7	71± 3·8	71± 3·3	80± 3·5	77± 4·0	95± 6·9	96± 2·3	71± 3·9
Glunicate	0·35± 0·09*	0·52± 0·07†	0·43± 0·03†	1·14± 0·11	79± 9†	59± 14‡	56± 14‡	155± 41	50± 1·6	52± 4·8	45± 2·0*	47± 3·7*	49± 3·5	43± 5·3	41± 3·5	35± 4·2*	92± 2·9	83± 2·3*	88± 1·7†	74± 6·1	91± 4·3	84± 7·6	83± 5·6	59± 3·2
Nicotinic acid	0·37± 0·03‡	0·52± 0·02‡	0·45± 0·05†	1·30± 0·15	212± 23	283± 83	66± 16‡	250± 31	60± 10·4	68± 6·0	49± 3·3	50± 3·2*	50± 7·1	34± 7·5	42± 2·8	30± 3·5†	79± 2·8	52± 13·7	80± 3·5	59± 4·9†	114± 10·9*	101± 14·6	79± 3·4†	62± 5·5

Table 3. Effects of repeated oral administrations of glunicate and nicotinic acid on plasma lipids and blood glucose in the Sprague Dawley and in the obese Zucker rat. Doses are expressed in terms of nicotinic acid equivalents. Mean values ± s.e.m. are reported. Significance levels vs controls are denoted by: $*P < 0.05$.

Rats	Treatment	Dose mg kg^{-1}	n	Triglycerides mg dl^{-1}	Total cholesterol mg dl^{-1}	HDL cholesterol mg dl^{-1}	HDL : total cholesterol %	Blood glucose mg dl^{-1}
Sprague Dawley	Vehicle		10	77·3 ± 7·1	51·7 ± 1·6	42·8 ± 1·2	83·1 ± 2·3	—
	Glunicate	100	10	60·7 ± 4·4	49·2 ± 2·7	39·0 ± 2·0	79·1 ± 1·4	—
	Glunicate	300	10	62·3 ± 5·2	52·1 ± 2·2	40·6 ± 1·6	78·2 ± 4·9	—
Obese Zucker	Vehicle		7	276·7 ± 16·3	105·7 ± 3·8	75·0 ± 2·0	71·0 ± 1·4	72·8 ± 3·6
	Glunicate	100	7	329·2 ± 34·0	101·8 ± 2·9	76·3 ± 2·5	74·9 ± 1·4	76·7 ± 5·2
	Nicotinic acid	100	7	338·2 ± 44·5	99·2 ± 3·6	72·0 ± 3·9	72·1 ± 1·5	87·9 ± 5·2*

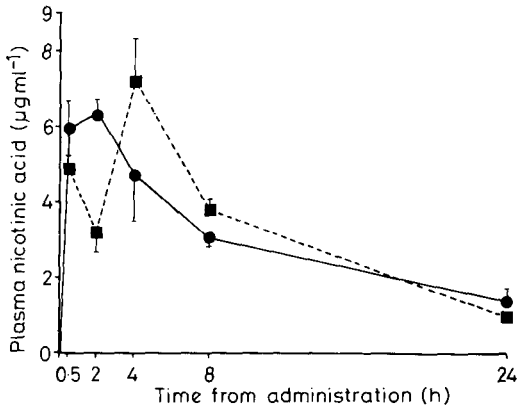

FIG. 1. Plasma levels of free nicotinic acid following a single oral dose of glunicate 30 mg kg^{-1} (as nicotinic acid equivalents) in the fasted (●) and in the fed (■) rat. Mean values ± s.e.m. are reported (n = 100).

Discussion

Apart from confirming the previously described (Subissi et al 1983) potent and long-lasting hypolipidaemic effects of glunicate, the present experiments in the fasted normolipidaemic rat show its biphasic effect on the HDL : total cholesterol ratio. This parameter seemed to be increased by the lower doses of glunicate, close to the anticipated human therapeutic dose, and decreased by the higher doses. An increase of the HDL : total cholesterol ratio is generally believed to be a beneficial effect, since plasma HDL levels in man are inversely correlated to coronary heart disease (Prugh et al 1983).

Also, when tested in the fasted obese Zucker rat, a genetic model of obesity and hyperlipoproteinaemia (Bray 1977), glunicate, at a high single dose, exerted hypolipidaemic effects, generally more marked than the parent compound, but not as large as in the normolipidaemic rat. An increase of the HDL : total cholesterol ratio was found again with glunicate, while a decrease was induced by nicotinic acid. A hyperglycaemic effect in this animal strain, also characterized by hyperinsulinaemia, insulin resistance and mild glucose intolerance (Hayek & Woodside 1980), was observed after both single and repeated doses of nicotinic acid and is reminiscent of the well-known diabetogenic effects of this drug after prolonged administration in man (Hueller & Glende 1984). Glunicate was devoid of such an effect.

In sharp contrast to the results in single dose experiments, repeated doses of glunicate, administered either to normolipidaemic or to obese Zucker rats, had practically no influence on plasma lipid pattern. As tachyphylaxis has been reported not to occur with this kind of compound (Gey et al 1971; Bizzi & Garattini 1971) and feeding was shown to interfere only on the peak-time of oral absorption (Fig. 1) the lack of hypocholesterolaemic action of glunicate and nicotinic acid in repeated dose experiments was possibly related to metabolic factors depending on feeding. This was confirmed in the single dose trials with fed animals. Our data are consistent with previously published data

Table 4. Effects of a single oral administration of glunicate 300 mg kg^{-1} (as nicotinic acid equivalents) on plasma lipids and blood glucose in fasted and fed rats. Mean values ± s.e.m. are reported (n = 5). Significance levels vs controls are denoted by: $*P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.001$.

		FFA mEq litre^{-1}				Triglycerides mg dl^{-1}				Total cholesterol mg dl^{-1}				Glucose mg dl^{-1}			
		Time from administration (h)															
Treatment	Feeding	2	4	8	24	2	4	8	24	2	4	8	24	2	4	8	24
Vehicle	Fasted	0·86± 0·073	0·65± 0·071	0·81± 0·047	1·07± 0·050	15± 2·9	38± 3·1	24± 2·0	40± 6·0	45± 2·6	48± 2·9	47± 6·4	48± 2·7	74± 5·9	83± 5·7	61± 5·9	76± 5·3
Glunicate	Fasted	0·40± 0·09†	0 23± 0·032‡	0·48± 0·034‡	0·73± 0·159*	18± 4·5	18± 5·2*	11± 0·5‡	18± 2·8*	42± 2·4	39± 2·2*	39± 4·4	17± 2·8‡	57± 5·6	64± 4·3*	65± 4·3	62± 3·7*
Vehicle	Fed	0·22± 0·008	0·31± 0·077	0·25± 0·058	0·24± 0·035	145± 20·4	203± 5·0	121± 26·0	198± 18·0	49± 1·9	57± 6·0	51± 2·2	59± 2·6	101± 4·8	102± 4·1	105± 3·9	98± 5·4
Glunicate	Fed	0·17± 0·011*	0·30± 0·081	0·16± 0·010	0·28± 0·069	104± 10·2	65± 10·6‡	78± 9·0	235± 30·7	52± 4·2	45± 3·8	50± 3·2	47± 2·5*	122± 4·8	101± 2·8	99± 3·0	101± 3·0

Table 5. Effect on plasma total cholesterol of rats 24 h after a single oral administration of glunicate 100 mg kg^{-1} (as nicotinic acid equivalents) in different feeding conditions. Mean values ± s.e.m. are reported (n = 5–8). Significance levels vs controls are denoted by: *$P < 0.02$; †$P < 0.01$.

Treatment	Feeding Before admin.	Feeding After admin.	Plasma total cholesterol mg dl^{-1}
Vehicle	Fasted	Fasted	51 ± 4·1
Glunicate			26 ± 3·6†
Vehicle	Fed	Fed	53 ± 3·9
Glunicate			48 ± 3·1
Vehicle	Fasted	Fed	53 ± 1·0
Glunicate			49 ± 3·2
Vehicle	Fed	Fasted	51 ± 1·7
Glunicate			36 ± 4·3*

showing that nicotinic acid does not alter plasma cholesterol after repeated oral administration in the rat (Kritchevsky et al 1960; Priego et al 1979). The reason(s) for this are not clear (as well as many other aspects of the nicotinic acid's mechanism of action) and still open to speculation. Nicotinic acid, with its definite antilipolytic activity, might control cholesterol biosynthesis when acetyl-CoA production depends mainly on fatty acids released from tissues (as in the case of prolonged fasting), but not when it depends on glycolysis of alimentary carbohydrates. Moreover, Hamprecht et al (1971) reported that the depressive action of nicotinic acid on liver hydroxymethylglutaryl-CoA-reductase (the key enzyme in cholesterol biosynthesis) is more important in the fasted than in the fed rat. Alternatively, the sources of plasma cholesterol in fed and fasted animals may be different (Myant 1971) and nicotinic acid and its derivatives might influence mainly the sources that are not depressed during fasting (e.g. intestinal wall).

From our results, it is impossible to validate fully any of these mechanisms. On the other hand, this feature of nicotinic acid and its derivatives seems to be restricted to rats, as the substances have long been used successfully in long-term treatment of human dyslipoproteinaemias. Glunicate itself, in preliminary clinical trials, lowered plasma cholesterol in patients with familial hyperlipoproteinaemia type IIa and IIb, treated for 4 weeks with 2–3 g daily (Subissi 1984).

REFERENCES

Bizzi, A., Garattini, S. (1971) in: Gey, K. F., Carlson, L. A. (eds) Metabolic effects of nicotinic acid and its derivatives. Hans Huber Publ. Bern, pp. 207–220

Bray, G. A. (1977) Federation Proc. 36: 148–153

Burstein, M., Scholnick, H. R., Morfin, R. (1970) J. Lip. Res: 583–595

Carlson, L. A. (1966) Clin. Chim. Acta 13: 349–351

Criscuoli, M., Renzetti, A. R., Subissi, A. (1984) Atherosclerosis 53: 59–68

Da Fonseca-Wollheim, F. (1973) Aertzl. Lab. 19: 65–74

Dole, V. P., Meinertz, H. (1960) J. Biol. Chem. 235: 2595–2599

Eggstein, M., Kreutz, F. H. (1966) Klin. Wschr. 44: 262–267

Feher, J., Kiss, A., Blazovics, A., Szondy, E., Toncsey, H., Mathiasz, D., Geroe, S. (1985) Drugs Exptl. Clin. Res. 11: 413–419

Gey, F. K., Lengsfeld, H., Lorch, E. (1971) in: Gey, K. F., Carlson, L. A. (eds) Metabolic effects of nicotinic acid and its derivatives, Hans Huber Publ., Bern, pp 567–580

Hamprecht, B., Nuessler, C., Waltinger, G., Lynen, F. (1971) in: Gey, K. F., Carlson, L. A. (eds) Metabolic effects of nicotinic acid and its derivatives, Hans Huber Publ., Bern, pp 625–632

Hayek, A., Woodside, W. F. (1980) Metabolism 29: 120–124

Hueller, H., Glende, M. (1984) in: Dukes, M. N. G. (ed.) Meyler's side effects of drugs, Tenth Edition, Elsevier, Amsterdam, pp 817–828

Kritchevsky, D., Whitehouse, M. W., Staple, E. (1960) J. Lip. Res. 1: 154–158

Myant, N. B. (1971) in: Gey, K. F., Carlson, L. A. (eds) Metabolic effects of nicotinic acid and its derivatives. Hans Huber Publ., Bern, p. 634

Priego, J. D., Maroto, M. L., Pina, M., Catalan, R. E. (1979) Gen. Pharmacol. 40: 215–219

Prugh, J. D., Rooney, C. S., Smith, R. L. (1983) in: Hess, H. J. (ed.) Annual Reports in Medicinal Chemistry, vol. 18. Academic Press, New York, pp. 161–170

Roeschlau, P., Bernt, E., Gruver, W. (1974) Z. Klin. Chem. Klin. Biochem. 12: 403–407

Subissi, A. (1984) Drugs of the Future 9: 898–899

Subissi, A., Criscuoli, M., Biagi, M., Murmann, W. (1983) J. Pharm. Pharmacol. 35: 571–575

Werner, W., Rey, H. G., Wielinger, H. (1970) Z. Analyt. Chem. 252: 224–228