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Acute effects on plasma lipids in the rat of a new long-acting nicotinic acid derivative: LG 13979

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The effects on plasma lipids and nicotinic acid concentrations of a single dose of 2-(3-pyridinecarbonylamino)-2-deoxy-1,3,4,6 dihydrogen-D-glucose tetra-3-pyridinecarboxylate (LG 13979) compared with the effects of nicotinic acid and of its known derivatives niceritrol and sorbinicate, at the same doses, were studied in the fasted rat. Results show that LG 13979 has more prolonged activity on plasma free fatty acids and triglycerides, with longer lasting and more intense activity on plasma cholesterol than these three reference standards. Free fatty acid rebound occurs after administration of nicotinic acid and niceritrol, but not after LG 13979. This pharmacodynamic profile may be explained on the basis of the kinetics of nicotinic acid plasma concentrations, which are low, constant and lasting after LG 13979 administration.

Despite recent pharmacological research efforts to identify new hypolipaemic agents (Cayen & Kallai-Sanfacon 1980; Sirtori et al 1980), nicotinic acid still remains one of the most used as it is safe in long-term therapy. Since absorption and excretion of nicotinic acid are very rapid, massive doses are needed to achieve hypolipaemic action so its potential as a drug cannot be fully exploited, as such doses induce elevated blood concentrations that are of no therapeutic benefit and cause some of the drug's side effects i.e. cutaneous flush and pruritus, gastrointestinal disturbances, abnormalities of liver function and glucose tolerance and hyperuricaemia (Levy 1980).

With this in mind, we first developed D-glucitol hexanicotinate (sorbinicate), a derivative that gives lower and constant plasma concentrations of nicotinic acid (Subissi et al 1980a), with hypolipaemic and antiatherogenic activity equal to or greater than that of nicotinic acid in laboratory animals (Subissi & Murmann 1978; Subissi et al 1980b). In clinical trials this derivative exhibits hypolipaemic properties (Avogaro et al 1977; Magnati et al 1978; Vergani et al 1980; Zuliani et al 1981) without nicotinic acid's side effects (Avogaro et al 1980).

Through investigating nicotinic acid derivatives, we came upon a compound, LG 13979 (Murmann & Ponchiroli 1981; Fig. 1), which in the rat produces plasma nicotinic acid concentrations that are pharmacologically active far longer than those of nicotinic acid itself and of other known nicotinic derivatives. We report here the effects of a single dose of LG 13979 on lipids, and on the nicotinic acid plasma concentrations in the normolipaemic rat.

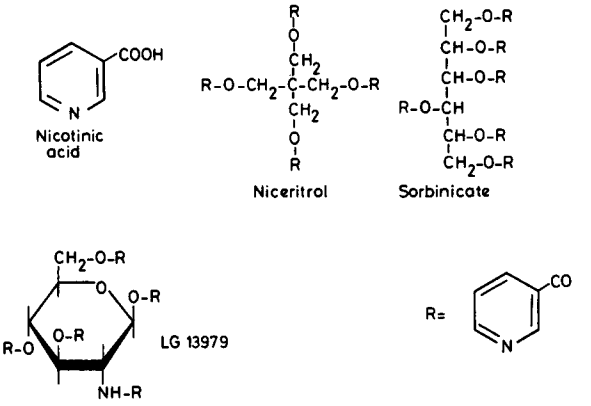

FIG. 1. Structures of nicotinic acid, niceritrol, sorbinicate and LG 13979.

MATERIALS AND METHODS

Animals and drugs

Male Sprague Dawley rats, aged 45–60 days (180–330 g) from our 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: nicotinic acid (Merck, Darmstadt, GFR), niceritrol (Bofors Nobel Kemi, Sweden), sorbinicate and LG 13979 (Laboratori Guidotti, Pisa). The drugs were dissolved in water by salification with HCl and dosed by gastric tube in a

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volume of 10 ml kg^{-1} (pH 2·0–2·5). 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.

Plasma concentrations of nicotinic acid

Animals, fasted for 24 h, were dosed with vehicle or one of the test drugs at the dose of 100 mg kg^{-1}. At different times thereafter the animals, under ether anaesthesia, were thoracotomized and bled by intracardiac puncture with heparinized syringes; blood was kept on ice until centrifugation and the plasma was stored at −20 °C to prevent hydrolysis of any nicotinic esters.

Effects on plasma lipids

24 h before the experiment the animals were fasted and kept in a quiet room as already described. Most experiments were carried out on five groups of animals receiving vehicle or one of the four test drugs. The order of treatments was randomized, since it has been shown that drawing blood from a group of animals may affect the free fatty acid levels in subsequent groups (Barrett 1964). The blood was drawn at various times after treatment, as described above, and in Table 2.

Methods

Free nicotinic acid was determined in plasma according to Carlson (1966). Total nicotinic acid was determined by the same method after hydrolysis in an alkaline medium for about 24 h at 22 °C (Svedmyr et al 1969b). The optical density of blanks (from vehicle-treated animals) was subtracted from all the samples at each time. Preliminary tests showed that LG 13979 does not interfere with the determination of free nicotinic acid, as it has been already demonstrated for sorbinicate (Subissi et al 1980a) and niceritrol (Svedmyr et al 1969b) and under these experimental conditions, hydrolysis of the three nicotinic esters is practically complete. Plasma free fatty acids were determined according to Dole & Meinertz (1960), plasma glycerol and triglycerides according to a modified version (Da Fonseca-Wollheim 1973) of the method of Eggstein & Kreutz (1966) and plasma cholesterol according to Roesch-

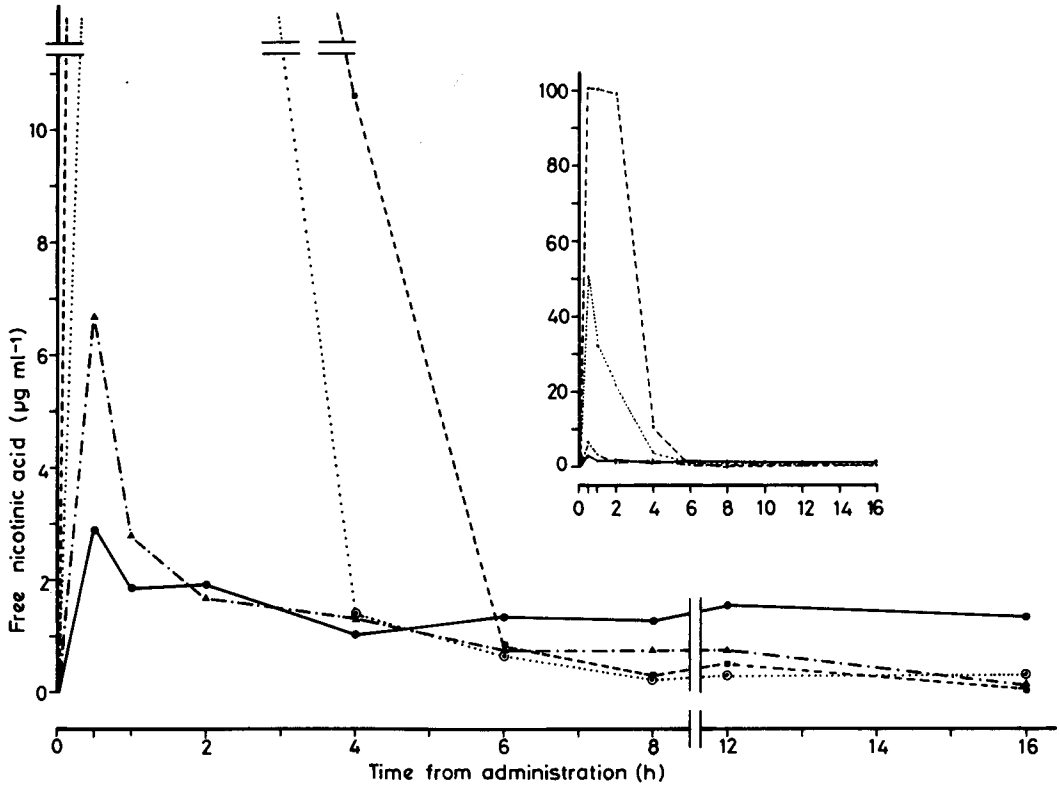

FIG. 2. Plasma concentrations of free nicotinic acid after a single oral dose of nicotinic acid (– – – ■ – – –), niceritrol (· · · · ○ · · · ·), sorbinicate (— · — · — ▲ — · — · —) and LG 13979 (—— ● ——) 100 mg kg^{-1} in terms of nicotinic acid in the rat, mean values (n = 4).

Table 1. Pharmacokinetic constants obtained from plasma concentrations of free nicotinic acid depicted in Fig. 2.

Treatment	Peak Concn μg ml^{-1}	Peak Time (h)	AUC μg h^{-1} ml^{-1} 0→4 h	AUC 4→16 h	AUC 0→16 h
Nic. acid	108	0·5	236·8	15·0	251·8
Niceritrol	50	0·5	83·3	5·0	88·3
Sorbinicate	7	0·5	9·3	8·1	17·4
LG 13979	3	0·5	5·1	17·7	22·8

lau et al (1974). The statistical comparisons were made using Student's *t*-test. The areas under the concentration/time curves (AUC) were calculated by the trapezoidal rule.

RESULTS

Plasma kinetics of nicotinic acid

Fig. 2 shows the kinetics of free nicotinic acid after oral administration of all four substances at 100 mg kg^{-1}, and Table 1 shows maximum concentration, peak time and AUC. The initial phase of the four curves in Fig. 2 is similar in that each shows a peak at 30 min, but the maximum free nicotinic acid levels reached after nicotinic acid are about 2, 15, 35 times higher than those after niceritrol, sorbinicate and LG 13979, respectively. Subsequently, the levels rapidly fall after all but LG 13979 with which there is a plateau, between 1 and 2 μg ml^{-1}, lasting for more than 12 h. Comparison of the AUCs of free nicotinic acid after administration of the test drugs shows that the AUC after nicotinic acid is about 3 times greater than that of niceritrol, 14 times that of sorbinicate and 11 times that of LG 13979, with the greater part of the AUCs lying between 0 and 4 h for the three standards (94% after nicotinic acid and niceritrol, 53% after sorbinicate), whilst for LG 13979 it is between 4 and 16 h (78%).

The free and total nicotinic acid concentrations were determined separately to find out whether appreciable quantities of the acid in the esterified form would be found after administration of the esters. This possibility could be excluded for LG 13979, as for niceritrol and sorbinicate, given that at the shorter times free and total nicotinic acid are identical. From hour 4, however, the total values are higher than the free nicotinic acid values for all the substances tested, including nicotinic acid (data not shown): this is attributable to the formation of one or more metabolites of nicotinic acid, determined by the method of Carlson only after alkaline hydrolysis. Moreover, preliminary t.l.c. tests revealed a large quantity of nicotinamide in the plasma from hour 2 after treatment with LG 13979, but no trace of the ester at any time.

Effects on plasma lipids

Table 2 shows the effects of the four drugs at 100 mg kg^{-1}.

All drugs show a remarkable free fatty acid lowering effect, which is similar in intensity but different in duration: 2 h for niceritrol and sorbinicate, 4 h for nicotinic acid and 10 h for LG 13979. After niceritrol and nicotinic acid, respectively at hours 6 and 8, the free fatty acids rise to values higher than the corresponding control group. No such effect occurs up to 16–24 h after administration of sorbinicate and LG 13979. Also triglycerides are lowered by all four drugs. In this case however the effect of LG

Table 2. Effects of a single oral dose of nicotinic acid, niceritrol, sorbinicate and LG 13979 (100 mg kg^{-1} in terms of nicotinic acid) on plasma free fatty acids, triglycerides and cholesterol in the fasted rat.

Treatment	Free fatty acids (μ equiv litre^{-1}) Time from administration (h) 2	4	6	8	10	16	24	Triglycerides (mg dl^{-1}) Time from administration (h) 2	4	6	8	10	16	24	Cholesterol (mg dl^{-1}) Time from administration (h) 4	6	8	10	16	24	32	48
Vehicle	753 ±91	774 ±72	937 ±73	774 ±42	894 ±48	967 ±90	861 ±32	60·1 ±7·8	42·8 ±4·2	54·9 ±2·2	43·0 ±6·6	33·1 ±3·3	49·4 ±7·9	50·7 ±2·2	49·6 ±2·5	49·9 ±3·5	49·2 ±2·0	49·0 ±2·3	48·5 ±4·8	49·1 ±1·3	51·1 ±4·3	52·8 ±4·6
Nicotinic acid	175 ±13 c	249 ±42 c	1156 ±74	950 ±17 b	1008 ±74	885 ±63		26·1 ±2·6 b	17·9 ±4·3 b	22·5 ±2·8 c	26·5 ±2·2 a	34·4 ±3·6	60·0 ±6·2		43·6 ±2·3	44·0 ±2·6	43·0 ±0·9 a	40·9 ±1·7 a	41·8 ±2·7			
Niceritrol	230 ±47 c	445 ±139	1194 ±71 a	852 ±35	873 ±73	1024 ±56		35·7 ±8·7	18·8 ±2·8 b	20·3 ±3·6 c	30·2 ±3·4	36·1 ±2·8	47·9 ±6·1		48·4 ±3·7	46·2 ±3·0	49·1 ±2·6	43·9 ±2·4	40·2 ±2·0			
Sorbinicate	209 ±41 c	767 ±130	998 ±75	807 ±52	847 ±72	1023 ±64		23·6 ±0·9 b	23·1 ±2·3 b	24·5 ±2·7 c	40·7 ±5·4	36·1 ±2·5	50·9 ±6·3		42·7 ±5·4	42·2 ±6·1	45·9 ±2·6	41·7 ±1·8 a	42·3 ±2·8			
LG 13979	198 ±28 c	456 ±59 b	672 ±80 a	439 ±27 c	564 ±113 a	901 ±126	844 ±49	28·7 ±5·1 b	15·2 ±1·5 c	15·4 ±3·0 c	10·7 ±1·6 c	13·3 ±2·4 c	16·2 ±3·0 b	49·4 ±6·8	53·9 ±3·2	47·7 ±2·5	42·8 ±3·1	35·8 ±2·6 b	22·8 ±2·4 c	30·9 ±2·5 c	29·5 ±3·0 b	37·5 ±2·3 a

Mean values ± s.e.m. (n = 10; n = 5 at 2, 4 and 6 h).
Significance levels vs controls are denoted by: a $P < 0·05$; b $P < 0·01$; c $P < 0·001$.

13979 appears slightly more intense, reaching 75%, while that of the others is around 60% at its peak. Duration of effect roughly parallels that described above for free fatty acids: 6 h for niceritrol and sorbinicate, 8 h for nicotinic acid and 16 h for LG 13979.

Their effects on cholesterol differ in both intensity and duration. Niceritrol never reduces it significantly, sorbinicate reduces it at hour 10, nicotinic acid from hour 8 to 10 and LG 13979 from hour 10 to 48. In addition to being longer lasting, the effect of LG 13979 is markedly more intense than that of nicotinic acid and sorbinicate, the peak effect of the three drugs being respectively 53, 17 and 15%. The effects of the test drugs on plasma lipids were assayed also at a lower dose, i.e. 30 mg kg^{-1} p.o. (data not shown). At this dose LG 13979 lowers free fatty acids up to hour 2, triglycerides up to hour 12 and cholesterol up to hour 24, while the effects of the other test drugs are less intense and/or lasting, especially on triglycerides and cholesterol. In this case free fatty acid rebound occurs only after administration of nicotinic acid. In further experiments LG 13979 showed significant and dose-dependent cholesterol lowering activity starting from the dose of 10 mg kg^{-1}.

DISCUSSION

Nicotinic acid and the derivatives investigated show different pharmacokinetic profiles in the rat. Nicotinic acid was the product absorbed in the greatest quantity, with a peak of over 100 μg ml^{-1}, but its plasma levels were negligible as from hour 6. Although niceritrol is absorbed less, its pharmacokinetic profile is quite similar to that of nicotinic acid. Sorbinicate presents much the same profile although it induces a far lower peak and somewhat more sustained levels even after 6 h. The profile of free nicotinic acid concentrations after treatment with LG 13979 is therefore unique for its plateau, reached immediately after a peak concentration not greater than 3 μg kg^{-1} and lasting more than 12 h after treatment. We do not yet have sufficient data to establish if this is due to factors of absorption, distribution, metabolism or excretion. However, this pharmacokinetic difference of LG 13979 explains why its antilipolytic and hypocholesterolaemic activities are more lasting than that of the three reference standards at both doses tested. High levels of nicotinic acid are not necessary to achieve a maximum effect on the plasma lipids (Svedmyr et al 1969a), but what is important therapeutically is that the concentrations should be constant and long-lasting, without the early peaks in nicotinic acid that are the cause of some of its side effects and to eliminate the drawback of its rapid absorption and elimination. The relatively low levels of plasma nicotinic acid may also account for the absence of free fatty acid rebound once the antilipolytic effect of LG 13979 is exhausted. Given the drug's slow disappearance from the bloodstream, the mechanisms, most probably hormonal (Pereira 1967), which return the free fatty acid concentrations to above-normal levels when the action of nicotinic acid and niceritrol has worn off, are presumably not triggered. Our experiments demonstrated that prolonged plasma levels of nicotinic acid ensure more intense as well as longer action on the cholesterol levels. At the dose of 100 mg kg^{-1}, nicotinic acid induces less than 20% lowering of cholesterol while the effect of LG 13979 is constantly around or above 30% and at its peak activity exceeds 50%.

The normolipaemic rat model is perhaps not the most suitable for evaluating the hypolipaemic effects of certain nicotinic derivatives such as niceritrol and sorbinicate, for in other laboratory animals (rabbits on an atherogenic diet) these derivatives exhibited an activity equal to or greater than that of nicotinic acid itself (Subissi et al 1980b; Brattsand & Lundholm 1971), whilst in our experiments they proved less active. This may be due in part to major pharmacokinetic differences of niceritrol (Hattori et al 1975; Harthon & Brattsand 1979) and sorbinicate (Subissi et al 1980a) in the various animal species .

In conclusion, in our experimental model, LG 13979 exhibited hypolipaemic properties superior to those of nicotinic acid and of its derivatives niceritrol and sorbinicate and its pharmacokinetic and pharmacodynamic characteristics are worthy of further investigation.

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