GANGLIOSIDE TREATMENT IN DIABETIC PERIPHERAL NEUROPATHY: A MULTICENTER TRIAL

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Peripheral neuropathy is the earliest and most frequent complication of diabetes mellitus. While opinions differ considerably as to the frequency of the symptomatic form, many Authors agree on the presence of one electrophysiological impairment already in the early stages of the disease[2, 3, 4, 6, 14, 18, 36, 48, 50]. The role of metabolic control in determining the onset, evolution, and improvement of diabetic neuropathy is still under discussion, even though some Authors have recently pointed out the relationship between metabolic improvement and amelioration of both motor and sensitive nerve conduction velocities[27, 28, 40, 41, 46] and some cardiovascular autonomic reflexes[8, 12].

After the negative and uncertain results of treatment with neurotrophic vitamins[36, 47, 49, 54], aldose reductase inhibitors[10, 19, 31] and myoinositol[7], at present no specific treatment exists.

Key-words: Diabetic neuropathy; Gangliosides; Nerve conduction velocity.

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Many investigators have recently focused their attention on brain gangliosides, a class of molecules pharmacologically active in enhancing the physiological reinnervation processes by stimulating sprouting mechanisms[5, 22, 23, 26, 37]. These observations represented the starting point for investigating the therapeutic effects of gangliosides in different forms of peripheral neuropathies[1, 35].

As for diabetic neuropathy, ganglioside treatment[21, 24, 38] was found to improve neuropathy in mice with congenital and alloxan-induced diabetes. Preliminary investigations in man[1, 42] have confirmed the positive effect of gangliosides on diabetic peripheral neuropathy. These results prompted us to evaluate on a statistical basis the efficacy of ganglioside administration in both subclinical and clinical neuropathy.

MATERIALS

The study was carried out in four Centers (Padova, Milano, Torino and Mestre), in the period January 1979 - February 1980, during which the standardization of metabolic and electrophysiological analyses was assessed.

Criteria for the inclusion of patients were:

- age between 18 and 55 years;
- no pregnancy;
- diabetes treated with insulin for at least 1 year;
- alcohol intake less than 500 ml of wine a day;
- cigarette smoking less than 10 a day;
- no other causes of peripheral neuropathy;
- no other significant disease besides diabetes;
- electroneurological impairment in at least two nerves out of the four evaluated.

Electrophysiological parameters were considered impaired when differing from the normal values by at least twice the standard deviation. Tab. 1 shows the normal reference values found in 60 healthy volunteers, 30 between 15-35 years and 30 between 36-55 years.

According to these criteria, 151 patients were recruited in the four Centers. The drop-outs were 11; thus a total of 140 patients, 91 males and 49 females, completed the study.

According to the severity of neurological symptoms, the patients were allocated to two protocols: *protocol I*: patients with no or mild symptoms ($n = 97$); *protocol II*: patients with frequent and severe symptoms ($n = 43$).

The patients' clinical and metabolic baseline data are reported in tab. 2. The 43 subjects of protocol II differed from those of protocol I in age, 43 ± 9 *vs* 34 ± 11 years (mean $\pm$ SD) ($p < 0.001$), duration of diabetes, 16 ± 8.6 *vs* 9.4 ± 4 years (mean $\pm$ SD) ($p < 0.001$), and for a higher incidence of ketosis, 21% *vs* 14% ($p < 0.01$). On the other hand, metabolic control did not differ in the two groups.

METHODS

Clinical and metabolic parameters - The following clinical and metabolic parameters were assessed both at baseline and during the study: body weight, heart rate, blood pressure, daily insulin requirement, plasma glucose levels

	age	
	15-35 years	36-55 years
median nerve:		
* SCV a) finger-wrist	> 46.3 m/sec	> 44.3 m/sec
* SCV b) wrist-elbow	> 57.1 m/sec	> 54.2 m/sec
sural nerve:		
* SCV malleolus-sura	> 45.2 m/sec	> 42.8 m/sec
ulnar nerve:		
** MCV	> 53.1 m/sec	> 51.2 m/sec
*** DL	< 3.7 msec	< 3.9 msec
MAP amplitude	> 7 mV	> 7 mV
peroneal nerve:		
** MCV	> 43.6 m/sec	> 41.2 m/sec
*** DL	< 6.4 msec	< 6.7 msec
MAP amplitude	> 5 mV	> 5 mV

* SCV = sensory conduction velocity; ** MCV = motor conduction velocity; *** DL = distal latency

Tab. 1 - Normal values of electroneurographic parameters in 60 healthy volunteers: mean values −2 SD (+ for latencies).

		protocol I (n = 97)	protocol II (n = 43)
age	(years)	34 ± 11	43 ± 9**
sex	M	65	26
	F	32	17
duration of diabetes	(years)	9.4 ± 4	16.0 ± 8.6**
insulin requirement	(U/day)	51 ± 16	45 ± 20
glycosylated hemoglobin	(%)	11.1 ± 2.2	11.1 ± 2.3
mean daily plasma glucose	(mg/dl)	215 ± 72	232 ± 79
glycosuria	(g/day)	17 ± 20	15 ± 16
urine ketone bodies	(0-4+)	14%	21%*

* $p<0.01$; ** $p<0.001$

Tab. 2 - Basal clinical and metabolic data ($\bar{x} \pm SD$) in 140 subjects with diabetic peripheral neuropathy subdivided into two groups according to presence or absence of rare (protocol I) and frequent or severe neurological symptoms (protocol II).

fasting and 2-h after breakfast and lunch, daily glycosuria, urinary ketone bodies, glycosylated hemoglobin (GHb). The mean of the plasma glucose values was considered as mean daily plasma glucose. These parameters were assessed in the four centers by the same methods: plasma glucose with glucose-oxidase method[30], urinary ketone bodies with Ketostix, and GHb with the Bio-Rad microcolumns at constant temperature of 21-23 °C[22,45]. The mean intra-assay variability of GHb was 2.07%[13].

Electrophysiological parameters - Sensory Conduction Velocity (SCV) of median (finger-wrist and wrist-elbow) and sural nerve; Motor Conduction Velocity (MCV), mean Distal Latency (DL) and Motor Action Potential (MAP) amplitude of ulnar and peroneal nerve were recorded in all subjects. The evaluation was carried out my means of MEDELEC MS6. Recorded SCV was always antidromic; DL was measured from the first sensory potential reduction assessed with a digital averager; stimulation applied with surface electrodes was always supramaximal.

SCV of median nerve was measured in the segments 2nd finger-wrist, wrist-elbow; recording was performed by means of two loose electrodes. As for sural nerve, the malleolus-sura segment was considered; recording was performed by surface electrodes, consisting of two silver chloride contact plates. MCV evaluation took into account DL and MAP amplitude; recording was performed by means of silver chloride disc electrodes. Skin temperature, strictly controlled with DISA equipment, was kept at 35 °C. All centers used comparable techniques; random controls were also performed to ensure comparability. For the same reason, during the study copies of each record were collected. A double evaluation (basal A and basal B) was performed at baseline.

Neurological symptoms - Paresthesias, nocturnal pain, and cramps were evaluated as to their frequency and severity by means of a suitable questionnaire. The answer to each question was recorded on a 1 to 4 score, where 1 represented no symptoms, 2 mild symptoms, 3 frequent symptoms, and 4 severe and constant symptoms (every day). Ninety-seven patients, rated 1 and 2, were included in protocol I; 43 patients, rated 3 and 4, were included in protocol II. Five of the 11 drop-outs were due to family reasons; the other 6 to the onset of other concomitant diseases. None of the drop-outs was reported to be due to the administration of the drug.

Study plan - The study design of both protocols was multicenter, randomized, cross-over, double-blind, controlled *vs* placebo. Randomization was performed separately for each center. The study period lasted 16 weeks: each patient was randomly assigned to one of the two treatment sequences; drug-washout-placebo or placebo-washout-drug. Each treatment period (placebo or drug) lasted 6 weeks. The washout period lasted 4 weeks. The daily ganglioside dose was 20 mg in a single i.m. administration. Clinical and metabolic data as well as symptom scores were recorded at the beginning and at 3, 6, 10, 13 and 16 weeks for protocol I, and at the beginning and at 2, 4, 6, 10, 12, 14, 16 weeks for protocol II.

Electrophysiological data were recorded at the beginning and at 6, 10 and 16 weeks. All patients were included only after formal informed consent. The drug used in the study is a ganglioside mixture *; the excipient solution ** as such was used as placebo.

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* Each ampoule of 10 mg of gangliosides contains: monosialotetrahexosylganglioside (GM_1) 21% equal to 2.1 mg; disialotetrahexosylganglioside (GD_{1a}) 40% equal to 4.0 mg; disialotetrahexosylganglioside (GD_{1b}) 16% equal to 1.6 mg; trisialotetrahexosylganglioside (GT_{1b}) 19% equal to 1.9 mg;

** Excipient solution: dibasic sodium phosphate 12 H_2O 6 mg; monobasic sodium phosphate 2 H_2O 0.5 mg; sodium chloride 16 mg; redistilled pyrogen-free water q.s. for 2 ml.

Statistical analysis - The patients of both protocols were assigned to their treatment sequence (drug-placebo or placebo-drug) by means of a restricted randomization scheme using permutated blocks of four units. Analysis of the behavior of metabolic data over the study period was performed by means of regression coefficients and Bravais-Pearson correlation coefficients were calculated to estimate the degree of correlation between these parameters and electroneurological data. Hypotheses regarding the responses to drug and placebo were tested by covariance analysis, using as covariates: center, observation period, patients, age group, paresthesia group, GHb level, mean of the two basal electroneurological measures. Since the raw data presented some really extreme values and since electroneurological variations were to be evaluated referring either to their low or high basis, instead of using raw measures data were transformed into ranks[53].

Qualitative variables related to symptoms were analyzed by the MacNemar test[17] (matched data being available for each patient), and by Gart's test[20], allowing for the correction of basal differences between the two treatment periods. When only data regarding the first treatment period were taken into account, the proportion test was used to compare drug and placebo.

RESULTS

Clinical and metabolic parameters - During the study no significant variations of mean daily plasma glucose (tab. 3), GHb (tab. 4), glycosuria, urine ketone bodies, heart rate, blood pressure, and daily insulin requirement were observed.

Electrophysiological parameters - The results of the statistical evaluation of main and carry-over ganglioside effects on electrophysiological parameters are reported in tab. 5. A significant main effect was observed in protocol I for DL of ulnar nerve ($p < 0.01$) and for MCV of the peroneal nerve ($p < 0.03$; tab. 6). In protocol II a statistically significant main effect of the drug was observed for SCV of the median (wrist-elbow; $p < 0.06$; tab. 7), and for MCV of the ulnar nerve ($p < 0.002$; tab. 8). As for placebo a positive effect ($p < 0.02$) was present in the sural nerve in protocol I.

Carry-over effects were observed in protocol I for SCV of the median nerve (wrist-elbow; $p < 0.02$) and for MCV of the peroneal nerve ($p < 0.1$), and in

sequence		weeks			
		0	6	washout 10	16
protocol I	G-P	197 ± 72	220 ± 92	205 ± 70	204 ± 81
(n = 97)	P-G	236 ± 67	218 ± 89	198 ± 70	195 ± 76
protocol II	G-P	250 ± 78	212 ± 102	241 ± 110	235 ± 90
(n = 43)	P-G	226 ± 79	218 ± 70	224 ± 72	214 ± 113

Tab. 3 - Mean daily plasma glucose ($\bar{x} \pm SD$) levels (mg/dl) in 140 subjects with diabetic peripheral neuropathy treated with gangliosides (G) and placebo (P) for 16 weeks.

sequence		weeks			
		0	6	washout 10	16
protocol I	G-P	10.9 ± 2.3	10.7 ± 2.2	10.7 ± 2.1	10.6 ± 2.2
(n = 97)	P-G	11.2 ± 2.1	11.1 ± 2.2	10.5 ± 2.1	10.6 ± 1.7
protocol II	G-P	11.1 ± 2.3	10.9 ± 1.6	10.9 ± 2.0	10.5 ± 2.2
(n = 43)	P-G	11.2 ± 2.4	10.8 ± 2.2	11.0 ± 2.2	11.0 ± 1.8

Tab. 4 - Mean (± SD) glycosylated hemoglobin (%) levels in 140 subjects with diabetic peripheral neuropathy treated with gangliosides (G) and placebo (P) for 16 weeks.

nerve	electroneurographic parameters	p value*			
		protocol I		protocol II	
		main	carry-over	main	carry-over
median	SCV (finger-wrist)	n.s.	n.s.	n.s.	n.s.
	SCV (wrist-elbow)	n.s.	0.02	0.06	n.s.
sural	SCV	0.02**	n.s.	n.s.	0.05
ulnar	MCV	n.s.	n.s.	0.002	0.002
	DL	0.01	n.s.	n.s.	0.1
	MAP	n.s.	n.s.	n.s.	n.s.
peroneal	MCV	0.03	0.1	n.s.	0.3
	DL	n.s.	n.s.	n.s.	n.s.
	MAP	n.s.	n.s.	n.s.	0.06

* non-parametric analysis - two sided test; ** placebo

Tab. 5 - Summary of statistical results of electroneurographic findings in 140 subjects with diabetic peripheral neuropathy, in protocol I (97) and protocol II (43).

sequence	weeks			
	0	6	washout 10	16
G-P	A 38.9 ± 4.6 B 38.9 ± 4.6	40.5 ± 4.5	40.7 ± 4.5	40.7 ± 4.4
P-G	A 42.1 ± 4.7 B 41.2 ± 4.4	42.2 ± 3.8	42.3 ± 3.9	43.4 ± 4.3

main: $p < 0.03$; carry-over: $p < 0.1$

Tab. 6 - Motor conduction velocity (m/sec) values ($\bar{x}$ ± SD) of peroneal nerve during ganglioside (G) and placebo (P) treatment in 97 diabetic subjects with peripheral neuropathy (protocol I). A and B represent the two basal values.

protocol II for SCV of the sural nerve ($p < 0.05$), for MCV of the ulnar nerve ($p < 0.02$), for DL of the ulnar nerve ($p < 0.1$), and for MAP amplitude of the peroneal nerve ($p < 0.06$).

Neurological symptoms - Only paresthesias improved significantly after ganglioside treatment. The frequency distribution of paresthesias shows that in the patients with frequent and/or severe paresthesias of protocol II, at the end of the study period only 10 patients still presented this symptom, 17 patients had no paresthesias and 14 reported them rarely (tab. 9); while in two subjects the symptom was not recorded. Moreover, statistical analysis (tab. 9) shows that 22 diabetics revealed a 'drug preference', while 10 preferred placebo and 9 had no preference.

Side effects - No side effects were reported either by the patients or by the physicians during the study period.

DISCUSSION

This multicenter trial shows that in diabetic patients with peripheral neuropathy gangliosides have a direct positive effect improving both some specific neurological symptom and some electrophysiological parameters. In fact, after the two treatment periods, paresthesias improved significantly or disappeared, and both MCV of peroneal (protocol I) and ulnar nerve (protocol II), and SCV of median nerve (protocol II) were significantly improved by the drug. The carry-over effect on some electrophysiological parameters in both protocols could imply a persistent effect of the drug after discontinuing therapy, or a delayed drug effect. The absence of significant variations in metabolic parameters, such as mean daily plasma glucose, glycosuria and GHb, confirms that the improvement is related to drug treatment and not to metabolic control. The results of this multicenter trial confirm the findings of previous studies in animals[21, 24, 38] and man[1, 42]. In fact, i.p. ganglioside administration for 30 days in genetically diabetic mice (C57BL/KS-db/db) significantly improved nerve conduction velocity[38], in association with a normalization of fiber size distribution. In these animals gangliosides also improve Brain Stem Evoked Potentials (BSEP), lowering the thresholds and improving the latencies[21]. Moreover, POZZA et al.[42] reported that the treatment with 20 mg/day i.m. of gangliosides for 40 days in 20 insulin-dependent diabetic patients significantly improved median nerve sensory action potential latency, median nerve mixed conduction velocity, and sural nerve sensory action potential. In a randomized, double-blind study BASSI et al.[1] reported that, in patients with diabetic or alcoholic neuropathy, ganglioside treatment facilitated the reappearance of sensory potentials and significantly increased the MAP amplitude in median, ulnar, and peroneal nerve.

Gangliosides are glycosphingolipids which seem to be involved in the processes of nerve growth, regeneration and tissue reinnervation[16, 39, 45, 46, 51, 52]. Moreover, gangliosides have been demonstrated to be incorporated at the neuronal membrane level in pheripheral nerves[22]; to serve as receptors interacting with some specific toxins, hormones and viruses[9]; increase the physiological process of reinnervation by stimulating 'sprouting' mechanisms[5, 22, 26].

sequence	weeks			
	0	6	washout 10	16
G-P	A 54.2 ± 5.2 B 54.3 ± 6.1	54.4 ± 5.4	53.1 ± 4.6	54.5 ± 6.0
P-G	A 53.9 ± 5.4 B 53.2 ± 4.7	54.1 ± 4.1	53.2 ± 3.6	55.0 ± 5.4

main: $p < 0.06$

Tab. 7 - Sensory conduction velocity values (m/sec) ($\bar{x} \pm SD$) of median nerve (wrist-elbow) during ganglioside (G) and placebo (P) treatment in 43 diabetic subjects with peripheral neuropathy (protocol II). A and B represent the two basal values.

sequence	weeks			
	0	6	washout 10	16
G-P	A 48.1 ± 4.8 B 47.9 ± 3.8	51.2 ± 6.6	50.3 ± 4.9	50.1 ± 6.8
P-G	A 49.9 ± 6.2 B 48.3 ± 5.1	49.1 ± 3.6	49.0 ± 3.5	50.4 ± 5.0

main: $p < 0.002$; carry-over: $p < 0.02$

Tab. 8 - Motor conduction velocity values (m/sec) ($\bar{x} \pm SD$) of ulnar nerve during ganglioside (G) and placebo (P) treatment in 43 diabetic subjects with peripheral neuropathy (protocol II). A and B represent the two basal values.

paresthesias	weeks			
	0	6	10	16
none	–	13	12	17
rare	–	14	17	14
frequent	31	10	9	5
every day	12	6	5	5

	G-P	P-G	total
drug preference	17	5	22
placebo preference	2	8	10
no preference	3	6	9

$p < 0.07$ (Gart's test); $p < 0.02$ (proportion test)

Tab. 9 - Frequency distribution of paresthesia score in 43 subjects with diabetic peripheral neuropathy (protocol II) during treatment with gangliosides (G) and placebo (P) for 16 weeks.

The favorable effect of exogenous gangliosides on experimental peripheral neuropathies, such as the diabetic, has been related to ganglioside incorporation into the cell membrane and to a stimulation of processes occurring as a repair mechanism after nerve damage. This action seems to be related to the presence of growth factors and to the fact that gangliosides, acting as receptors, enhance the internalization of the complex[15, 51]. Recent data show that ganglioside incorporation into synaptic membranes will activate enzymes, such as (Na^+, K^+) adenosine triphosphatase, adenylcyclase, phosphodiesterase[11, 33], causing functional changes in these membrane activities and stimulating sprouting. Activation of (Na^+, K^+) ATPase may lead to a proper ion balance between inside and outside of the axons, improving nerve conduction velocity. Moreover, the evidence that gangliosides have a favorable influence on fiber size distribution in diabetic animals may confirm the hypothesis that they also favor the action of some endogenous factor responsible for axon trophism[24]. The relatively slight mean improvement (1.5-3 m/sec) seen in conduction velocities can be related to some changes in the membrane ionic transport phenomena, as can be expected in such a very short period of observation.

The lack of detectable effects in some of the nerves tested may also be ascribed to the short treatment period and/or to the low dose administered (20 mg/day), for istance the lack of an effect on the peroneal nerve in protocol II may be due to the fact that this nerve was very severely affected, as shown by its MCV values, so as to require a longer period of treatment. In fact, the best results in mice[24] were obtained with higher doses of drug (up to 10 mg/kg) given for a longer period.

Another point of discussion is that patients of protocol II with symptomatic neuropathy and with longer duration of diabetes responded better than those of protocol I. Recent experimental data might explain this: it has been demonstrated that diabetic neuropathy in genetically diabetic mice is characterized by early insulin-dependent metabolic alteration and by later structural modifications, that are no longer influenced by insulin treatment. These structural non-insulin-dependent alterations seem to be improved by exogenous gangliosides[25].

In conclusion, ganglioside treatment seems to have a positive effect on diabetic peripheral neuropathy, leading to improvement both in some of the symptoms and in electrophysiological parameters.

Further studies are now in progress to evaluate the effects of gangliosides, particularly on the clinical aspects of diabetic neuropathy, after a longer period of treatment.

SUMMARY

Ganglioside treatment was evaluated with a multicenter, randomized, double-blind, controlled, cross-over vs placebo trial in 140 insulin-treated diabetic subjects with peripheral neuropathy. The patients entered the study when they showed an impairment in at least two of the electroneurographic parameters, and were assigned to two protocols according to the presence and severity of their neurological symptoms. Ninety-seven diabetic subjects with no or mild symptoms were assigned to protocol I, whereas 43 symptomatic patients were assigned to protocol II. The treatment periods lasted 6 weeks with an intermediate washout period of 4 weeks. The treatment consisted in the daily i.m. administration of 20 mg gangliosides or of placebo. Electroneurographic parameters were recorded at the beginning and at the end of each treatment period, whereas clinical and metabolic data (mean daily plasma glucose,

glycosuria and glycosylated hemoglobin) were evaluated every three weeks in protocol I and every two weeks in protocol II. No change in the metabolic parameters was observed throughout the trial period. However, the treatment induced a statistically significant improvement of paresthesias (protocol II) and of some electrophysiological parameters; in particular, ganglioside treatment improved MCV of peroneal nerve ($p < 0.03$) in patients of protocol I, MCV of ulnar nerve ($p < 0.002$) and SCV of median nerve ($p < 0.06$) in patients of protocol II. Furthermore, 22 subjects of protocol II showed a 'drug preference' while 10 preferred placebo and 9 had no preference. In conclusion, ganglioside treatment seems to have a positive effect on diabetic peripheral neuropathy, improving both some symptoms and some electrophysiological parameters.

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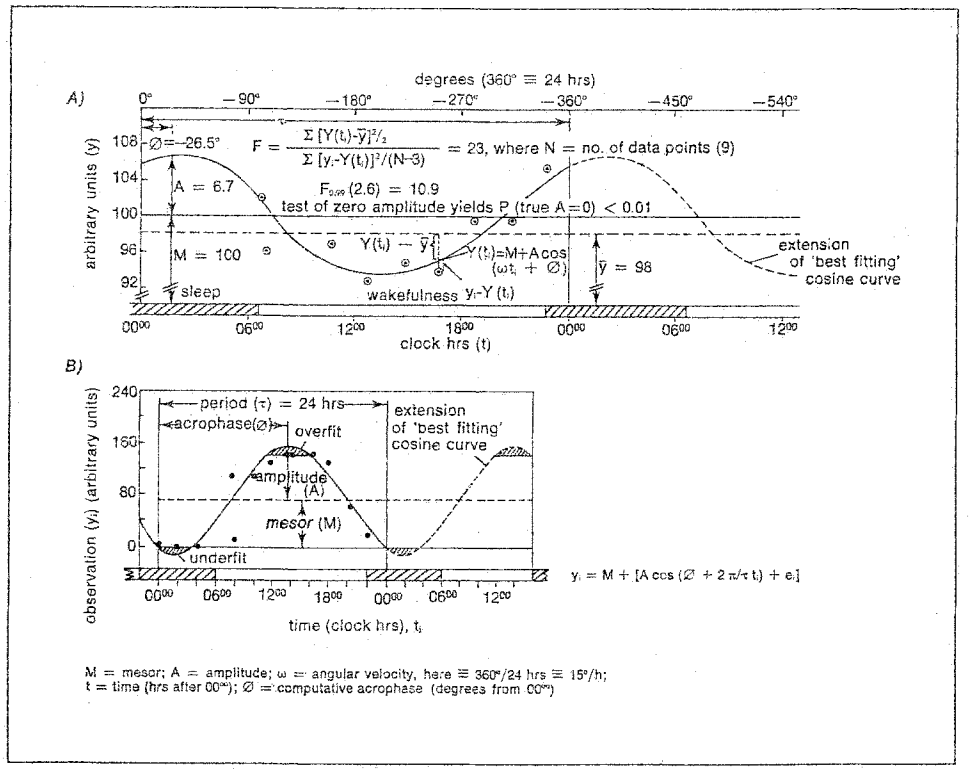
time: the fourth dimension in medicine

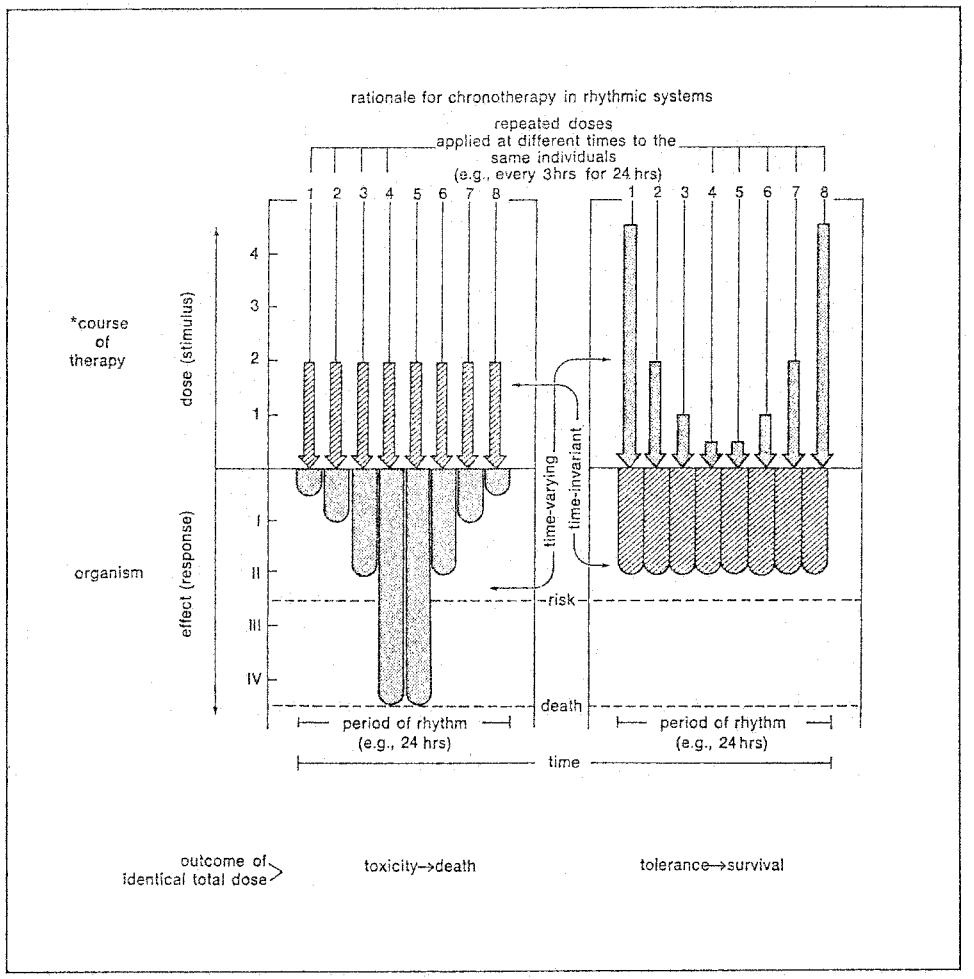


A) Testing rhythm sinusoidality by variance ratio, F;
abstract example with 24-h cosine function y(t), continuous curve, fitted by least squares to data $y_i(o)$ obtained during wakefulness span;
M = mesor; A = amplitude; ω *= angular velocity, here ≡ 360°/24 hrs ≡ 15°/h; t = time (hrs after 00^00); Ø = computative acrophase (degrees from 00^00);* $\omega = 2\pi/\tau$.

B) Parameter M, A and Ø estimation by least squares fit of cosine model with fixed period: $y_i = M + [A \cos(Ø + 2\pi/\tau t_i) + e_i]$;
t_i = time; y_i = observation at t_i; e_i = error at t_i, having an independent normal distribution with mean zero and unknown variance σ^2.

Computer programs facilitate inferential statistical rhythm detection in noisy data such as those usually collected in a clinic (*A*) and the estimation of the rhythm's properties (*B*).

...'the recognition of a spectrum of frequencies characterizing so many functions underlies a dynamic concept of health. This view includes focus upon the individual's predictably changing ability to react properly to both usual and unusual stimuli by adjustments in the extent or timing of rhythms. Indeed, as the chronobiologist resolves predictable, spontaneous changes, he also estimates the nature and the extent of responses to environmental factors. Thus eventually, as a dynamic approach to the problem of health assessment and maintenance, he may be able to optimize the timing of activities, in work and leisure: for best immediate productivity compatible with long-term health'.



The effect of equal (time-invariant) doses results, as a rule, in unequal responses, in rhythmic systems. Accordingly, for a time-invariant response, unequal doses (time-variant treatment) is essential. Chronotherapeutic schemes may involve variations, as a function of time, in kind as well as dose and time (if treatment is to be eventually optimized).

..... 'it is shown that the administration of a potentially harmful yet otherwise useful agent such as a drug has drastically different effects along the 24-h scale' (Halberg, modified).