CHAPTER 11

TREATMENT WITH OPEN EYES: MARKERS-GUIDED CHRONOTHERANOSTICS*

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CONTENTS

* Dedicated to Earl E. Bakken, who started chronotheranostics over half a century ago by restoring a failing heart rhythm and who led the way to closing the loop between diagnosis and treatment by his development of implantable devices.

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11.1 INTRODUCTION

Chronobiological trials have demonstrated the merits of assessing the time structure of marker variables to recognize a heightened risk of disease, to specify the kind and timing of treatment, and to evaluate and optimize the patient's response [1, 2]. By so doing, abnormalities in the pattern of the circadian (or other) rhythm are part of the diagnosis (and prognosis) (chronodiagnosis) [2]. Because such abnormalities are usually observed within the physiological range, before there is overt disease, preventive measures can be initiated in a timely fashion. Correcting an ongoing treatment plan can also be carried out earlier, in order to avoid excessive toxicity and most importantly to improve efficacy [3]. Chronobiological trials have also demonstrated the merit of timing the administration of treatment (chronotherapy), even for 24-hour formulations [4]. Very often, however, the desire to generalize and to seek "the optimal time (or schedule)" to administer a given treatment overlooks the even greater necessity of individualizing the therapeutic plan. Therein lies a major merit of marker-rhythmometry. Adjusting the scheduling of treatment according to the kind of abnormal pattern diagnosed, a procedure referred to as chronotheranostics, addresses this need of individualized temporal and all other therapeutic optimization.

Markers-guided chronotheranostics is best implemented when the following requirements are met:

1. One or several marker variables are available and can easily and cost-effectively be measured repeatedly. Longitudinal around-the-clock measurements of variables directly pertinent to the medical problem on hand are ideal but may not always be feasible or cost-effectively practicable.
2. Protocols can be designed to effectively determine optimal treatment schedules. It is often thought that chronotherapeutic trials are much more costly than usual trials because the cost needs to be multiplied by the number of treatment schedules tested. This misconception will be dispelled. More difficult to achieve but not impossible is the

implementation of *N*-of-1 designs that make the individualization of timed treatment feasible.

3. Outcome measures are available that can shed light on the efficacy of treatment and identify the presence of any undesired effects.
4. Statistical methods can be applied not only for groups of patients but also for individual subjects, to recognize abnormality, to assess whether treatment was efficient, and to determine whether one treatment schedule is superior to another.

While chronotherapy has been used successfully in different medical specialties, applications in relation to blood pressure disorders will be emphasized herein, for the simple reason that, in this case, blood pressure is a most pertinent variable easily monitored around the clock for long spans. It also has the merit that it can be used as a multiple-purpose marker, to assess the efficacy of a given intervention, to guide the optimization of its timing, and to survey its continued efficacy. Before turning to medical applications, a brief, generally relevant methodological overview introduces chronobiological concepts for study designs and for data analysis.

11.2 METHODOLOGICAL OVERVIEW

Currently, no general provisions are made for defining what the optimal circadian (or other) rhythm stages are at which to administer a given pharmacological or nonpharmacological treatment, despite much evidence suggesting that timing is important [5, 6]. For drugs found to be effective preclinically, Phase I clinical trials are designed to determine tolerated doses associated with acceptable side effects, at times usually dictated by convenience rather than pertinence [7]. Timing, the critical ingredient of chronobiological designs, is not just another factor like genetics and nutrition that can be ignored, when under the standardized conditions of the experimental laboratory, it has been repeatedly shown that timing can tip the scale between health and disease and even between death and survival after exposure to a fixed dose of a potentially noxious agent (Figure 11.1) [8]. While timing remains an essential factor in Phase II and Phase III trials focusing on treatment efficacy and a comparison with the current best treatment, respectively, the case has been made to incorporate "Phase 0" chronobiological pilot designs at the outset [9–11]. These would aim at detecting, sooner and with smaller sample sizes [12], desired or undesired effects that may otherwise be missed.

The misconception that chronobiological designs are too expensive, assuming that the needed sample size should be multiplied by the number of rhythm stages to be tested, is easily dispelled by the following considerations:

1. Most biological variables are characterized by an ubiquitous time structure composed of multifrequency rhythms, chaos, and trends.

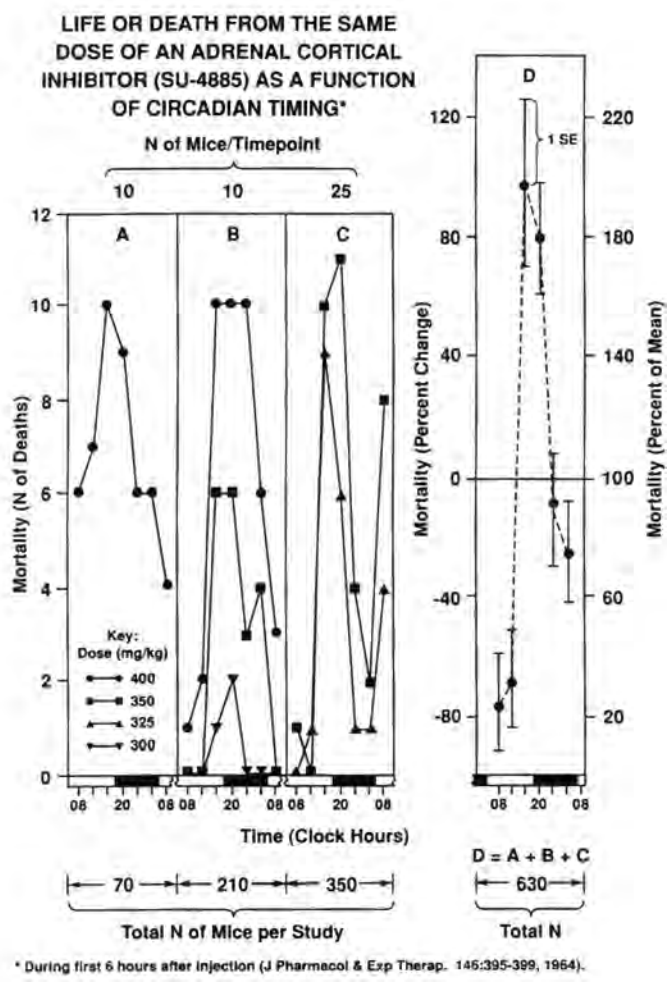


Figure 11.1. Dose affects circadian amplitude of neuron susceptibility resistance cycle to SU-4885, assessed by single component model.

Rather than unnecessarily inflating the error term by ignoring rhythms, their assessment is incorporated into study designs, samples being spread uniformly to cover several stages of the rhythm under consideration rather than collecting samples without time specification. Circadian rhythms are usually prominent. Ignoring such large-amplitude predictable variation by sampling at any time during usual office hours yields estimates of mean values associated with an inflated dispersion index, as illustrated in Figure 11.2.

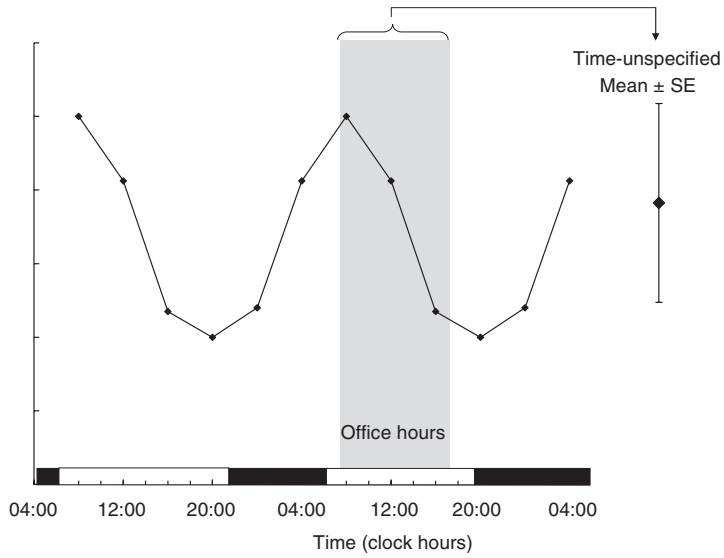


Figure 11.2. Large dispersion when measurements are not time specified. Physiological variables such as cortisol are characterized by large-amplitude circadian rhythms. Ignoring the rhythmic structure of this variable may unduly inflate the dispersion index, notably when measurements are taken during only a portion of the cycle such as regular office hours (e.g., from 0700h to 1700h), a span that covers most of the predictable range of variation assumed by this variable.

2. When the merits of two treatments need to be compared, the lack of time specification is associated with the need for large sample sizes to detect a difference in view of the inflated standard deviation (Figure 11.3, top).
3. With large sample sizes, however, some spurious differences may result from a phase difference when sampling is limited to part of the cycle (e.g., during office hours; Figure 11.3, bottom). This situation may arise when two anticancer drugs that act on a different stage of the cell cycle are compared: their mechanism of action being different, the circadian stage of highest efficacy and/or tolerance is likely to differ accordingly. While the two drugs are administered during the same subspan of the 24-hour day, differences in effect may hence not reflect a true difference in treatment efficacy. Figure 11.4 summarizes results from 35 studies on 5266 rodents investigating the tolerance of seven different anticancer drugs, with corresponding therapeutic gains shown in Figure 11.5 [13].
4. Failure to account for the rhythmic behavior of the variable investigated may further lead to controversial results, large differences in opposite directions being reported by investigators working at two different times (e.g., in the morning or in the late afternoon or evening). As illustrated in

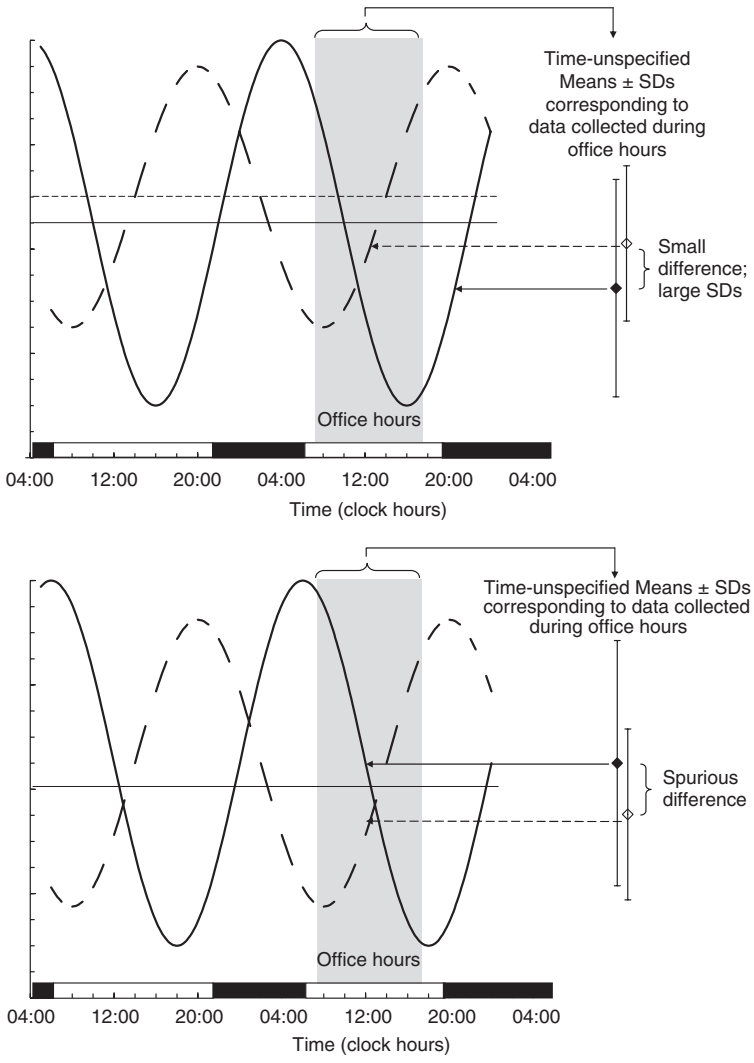


Figure 11.3. A heavy price for ignoring the chronome's rhythms in current Phase III trials. Top: The need for large sample sizes may arise from the following situation: When rhythmic variables are sampled during "regular" hours (e.g., from 0700h to 1700h) and are characterized by large-amplitude circadian rhythms with different phases, the dispersion indices around the mean value of each variable will be very large, much larger than the difference in mean value between the two rhythmic variables. A large sample size is hence needed to detect such a difference, which may be small compared to the dispersion index. Bottom: There is also the danger that in the absence of a real difference, large sample sizes may detect spurious differences. This situation may arise from a phase difference between two rhythmic variables sampled during the same span (e.g., from 0700h to 1700h), corresponding to only a subspan of the full cycle of variation of these variables.

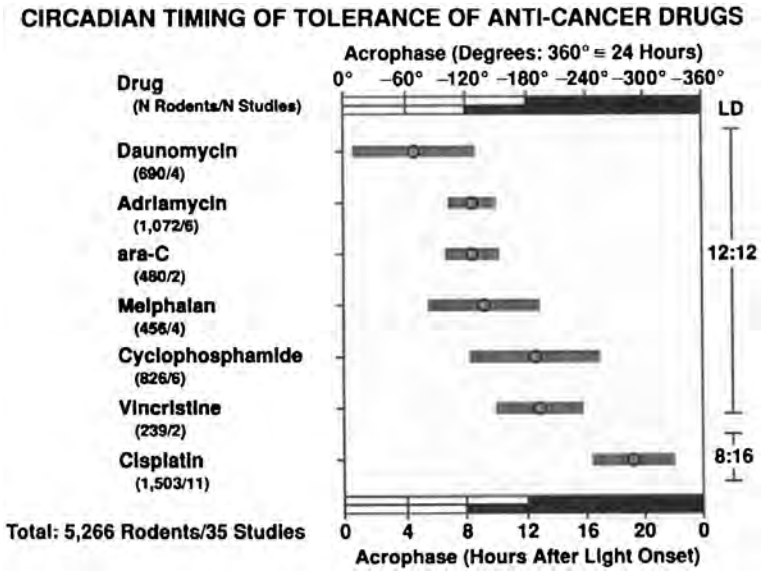


Figure 11.4. Different times of optimal resistance to the toxicity of a few anticancer drugs. Acrophase chart of cosinor results.

Figure 11.6, controversial differences may stem from a difference in amplitude, phase, or period.

5. When treatments are being compared, concern about statistical power usually prompts investigators to limit the comparison to two or as few groups as possible [14–17]. Whereas statistical power is indeed greater with fewer groups when relying on conventional tests such as the one-way analysis of variance, it is not the case when results are assessed in the light of a model such as the cosinor, used in chronobiology to find optimal times of treatment administration [9]. As illustrated in Figure 11.7, limiting the design to two test times may be optimal when the times of highest and smallest responses are known a priori (top left), but if this is not the case, there is the risk of inadvertently selecting two test times corresponding to the midline crossings, thereby failing to detect differences of potential great benefit to the patients (top right). Whereas three timepoints are sufficient to determine a rhythmic pattern if it does not deviate too much from sinusoidality (bottom left), six timepoints are usually sufficient to define a rhythm (bottom right). In this case, estimates of uncertainties for all parameters involved can also be obtained.
6. Critical for the individualized optimization of treatment timing is the availability of statistical procedures applicable to the individual patient providing a longitudinal record of one or several marker variables. As illustrated later for the case of blood pressure, parameter tests [18] can detect not only a change in the mean value but also differences in

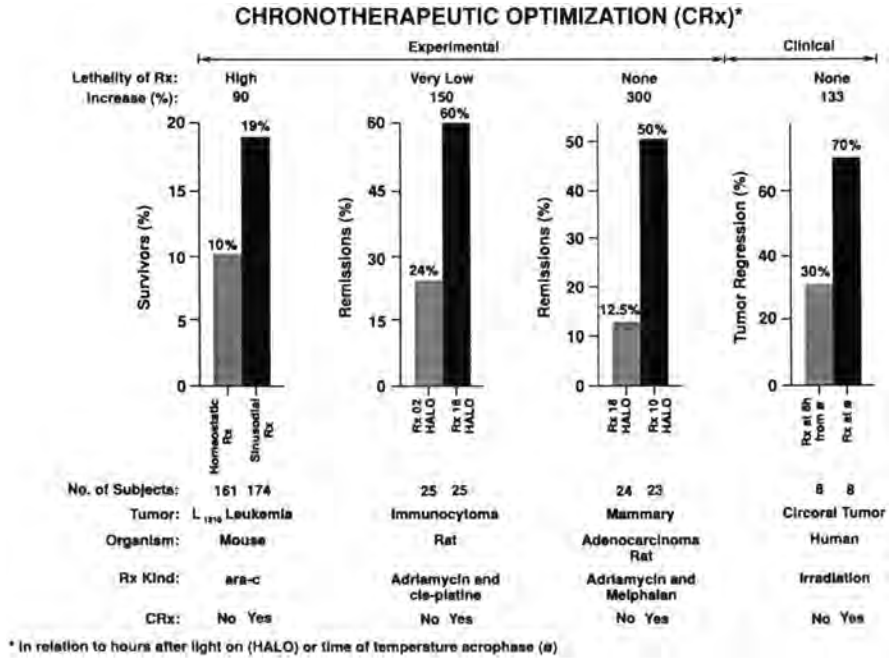


Figure 11.5. Chronotherapeutic optimization of cancer treatment in experimental animals (first 6 columns), and in clinical radiotherapy research (last 2 columns). In each pair, the column on the right shows the gain from timing, with the column on the left in each pair serving as reference standard.

other rhythm characteristics such as the amplitude and acrophase (timing of overall high values recurring in each cycle). Cumulative sum (CUSUM) control charts [19, 20]; see also Ref. [21] can also determine whether a desired therapeutic goal has been achieved, insofar as a given endpoint departs from a decision interval, the latter corresponding to "no statistically significant change." When used to assess a patient's response to treatment, a directional change can be anticipated. Since the time of intervention is known, the CUSUM can further relate the effectiveness of the treatment to the time of its administration, thereby providing an inferential statistical approximation of causality.

11.3 APPLICATIONS IN ONCOLOGY

11.3.1 Chronoradiotherapy of Perioral Tumors

It had been shown in the laboratory that subgroups of mice responded differently to the exposure to 400, 450, or 500 roentgens of total body X-irradiation at one of six different circadian stages, 4 hours apart [22].

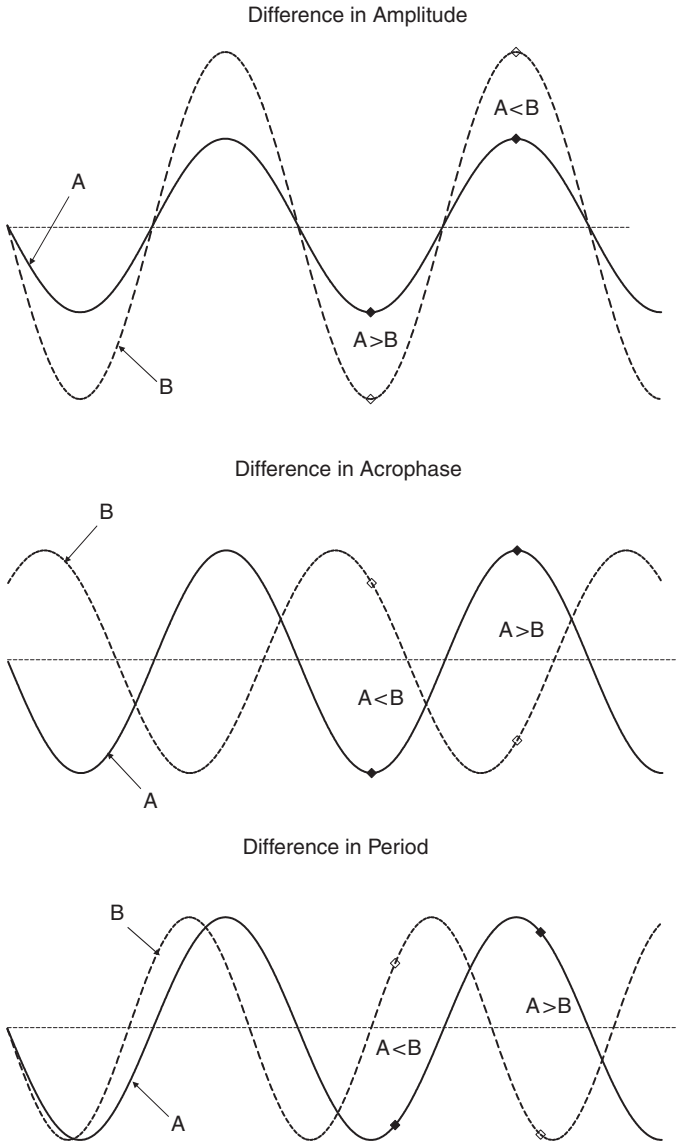


Figure 11.6. Opposite results may be obtained at two different test times when the underlying rhythmic patterns of two groups being compared are characterized by a difference in amplitude, acrophase, or period.

From mortality at each dose and at each test time, a dose was computed that killed 50% of the animals within 30 days. The highest tolerance was found to occur during the second half of the light (rest) span [22]. Against this background, a study in India sought the optimal circadian stage to administer

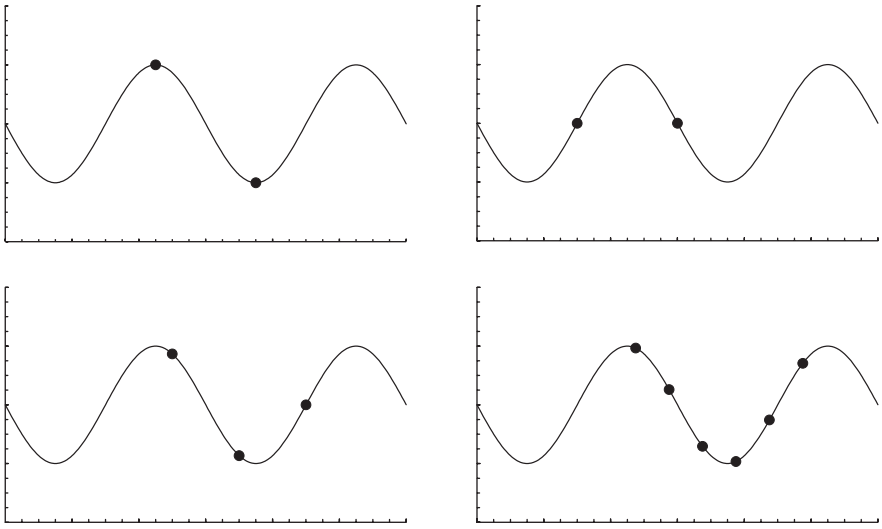


Figure 11.7. A two-test-time approach on a cycle is often carried out and thought to be optimal (top left). This is true only when the times of spontaneous minima and maxima or of the minimal and maximal response are known. The spread of the observations is then maximal. Correspondingly, the power may also be maximal. If, however, the exact timing of the rhythmic function is not known, or is likely to be altered in certain circumstances or may differ among individuals, then one takes the risk of sampling at the midline crossings (top right). A prominent rhythm may hence remain undetected and unexploited. Major gain is obtained by adding single subjects and test times, for example, from three to six equidistant experimental units (bottom) such as patients per cycle investigated. Three test times per cycle allow the estimation of rhythm characteristics (#1) but do not suffice for hypothesis testing (#2) or for the derivation of confidence intervals for the parameters (#3). A design involving six test times per cycle is parsimonious and powerful for achieving all three purposes.

radiotherapy in patients with large cancers of the oral cavity amenable to temperature measurement around the clock for a few days prior to treatment [13, 23]. The response to radiotherapy could also be readily assessed at least semiquantitatively by measuring tumor size. Groups of 8 patients each were scheduled for 5 weeks of radiotherapy, 5 days per week. Patients were randomly assigned to be treated at the time of peak tumor temperature, or 4 or 8 hours before or after that time. Another control group was treated "as usual," with no regard for timing. As seen in Figure 11.8 (left), the average tumor regression rate was much higher for patients treated at the optimal time of peak tumor temperature than for patients treated at the worst time (8 hours after the time of peak tumor temperature). Moreover, the tumor regression rate follows a predictable circadian pattern depending on the circadian stage of treatment administration in relation to the patient's circadian temperature variation used as marker rhythm ($P = 0.042$); see Figure 11.8 (right) [13, 23]. These short-term

results based on tumor regression rate are in keeping with longer-term outcomes, as shown elsewhere (see Chapter 10 in this volume). Patients treated at the time of peak tumor temperature had the highest 2-year disease-free survival rate, which was about twice as high as for patients treated at other times or for patients treated as usual, without regard for timing [13, 23].

11.3.2 Unspecific Markers Preferred to Clock Hour: Successful *N*-of-1 Chronochemotherapy

An earlier attempt focusing on reducing toxicity while relying on results from the experimental laboratory for efficacy was successful, the patient being alive 30 years later [24]. In this patient (CN) diagnosed with a rare and highly malignant ovarian endodermal sinus tumor with spillage into the peritoneal cavity, who is alive and well more than 30 years after subsequent individualized chronochemotherapy, the timing of drug administration was varied from month to month for the first 4 months and autorhythmometry (mood, vigor, nausea, temperature) and complete blood counts were followed to determine the patient's time of highest tolerance. Prior to each course of medication, blood samples were drawn every 4 hours for 24 hours at specific timepoints to determine the circadian rhythm of circulating platelets, white blood cells (WBCs), and differential, hemoglobin, hematocrit, and red blood cell (RBC) indices. Core temperature, blood pressure, pulse, mood, and vigor were also assessed around-the-clock every 4 hours for months. Then, at scheduled times subsequent to medication, WBCs were obtained to assess bone marrow suppression. Oral temperature was measured five or more times a day, on most days, mostly during wakefulness. Regularly, around the clock, mood and vigor were self-rated by admittedly subjective criteria. In March, the medications were given between 0800h and 1100h, in April between 2000h and 2200h, in May at 0400h, and in June again at 2200h. After the fourth course, 0400h was chosen as the preferred time of administration based on criteria for the patient's drug tolerance, as well as by extrapolation of optimal cyclophosphamide timing from mice (correcting for differences in diurnality vs. nocturnality of activity) [25]. CN received 20 courses of treatment covering 19 months.

11.3.3 Chronosensitivity Complements Chemosensitivity Tests: The Erna Test

More recently, several protocols were designed to seek optimal cancer treatment administration schedules to benefit a patient (EH) with advanced ovarian cancer focusing primarily on treatment efficacy [26]. Difficulties related to *N*-of-1 designs relate primarily to confounding effects due to the patient's response to prior treatment, any development of drug resistance, and to any progression of the disease [10, 27, 28]. Optimization of efficacy also requires repeated measurements of pertinent variables such as tumor markers that are

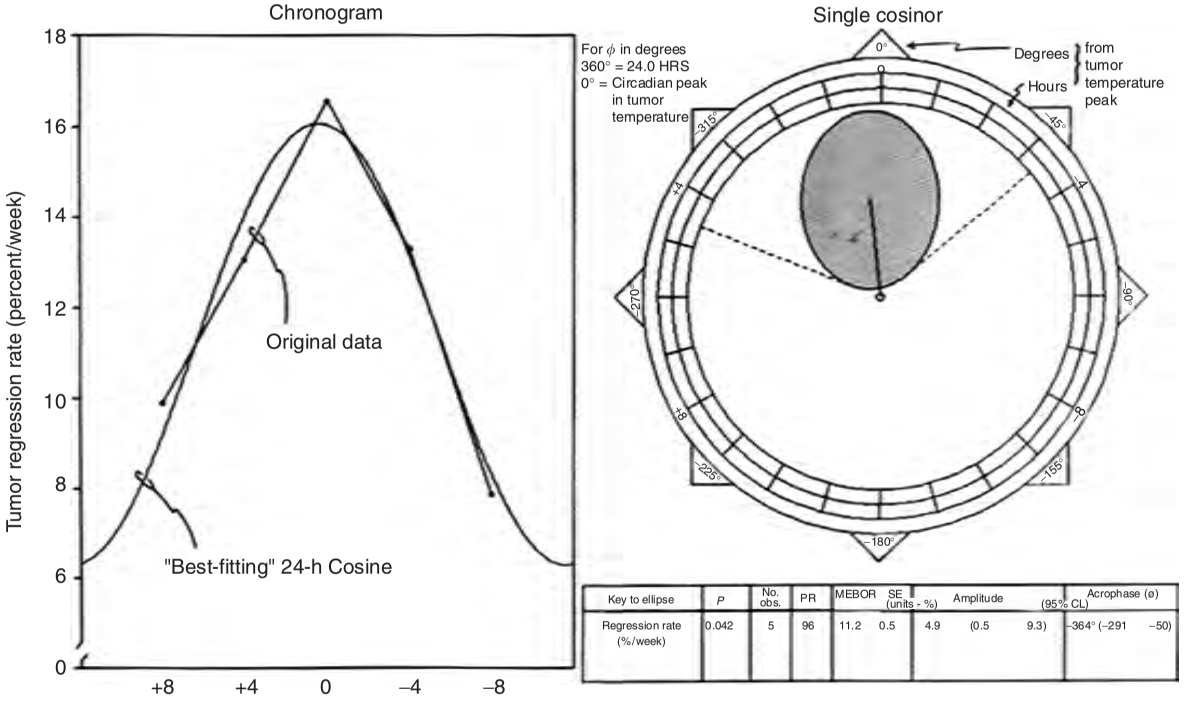
usually invasive, when the validity of a treatment effect is to be ascertained. For treatment timing, however, serial blood samplings for several days can be substituted by determining tumor marker rhythms in urine (such as UGP) or AU2 saliva (such as CA125 or CA130) [29, 30]. The fact that some markers (such as CA125 or CA130) can be determined in saliva, where they are characterized by a large-amplitude circadian rhythm and also by a circaseptan variation of lesser prominence, but where their pertinence may be limited to guiding the timing of treatment (since a determination in saliva is less specific than in blood), made it possible to obtain around-the-clock measurements before, during, and after a given course of chemotherapy in an unusually cooperative patient (EH) [11, 27]. Whereas around-the-clock determinations of tumor markers during treatment, such as taxol [31] or cisplatin [10, 32], administered at a constant rate for AU1 24 hours, or time-specified differences in tumor markers during versus before treatment such as doxorubicin [33] administered differently from one day to the next over several days via a programmable pump may provide information regarding the individual patient's response to treatment, this approach is more problematic for treatment administered as a bolus. In the latter case, an answer may not be obtained until at least three courses have been administered, which may be too late to benefit that particular patient, unless it is done early, as in the case of CN [24]. In this case, treatment timing was guided with unspecific markers insofar as efficacy and resistance other than that to myelotoxicity are concerned.

Circulating CA125 was assayed in about 3-hourly samples during a 24-hour infusion of taxol (135 $mg/m^2/24\,h$) [31]. A statistically significant decrease in the circulating marker concentration could be ascertained ($P = 0.007$). The largest decrease in CA125 in EH occurred at a time similar to that of largest tumor proliferation, which had been independently assessed by flow cytometry from intraperitoneal washings obtained on 30 patients with ovarian cancer [34]. A similar trend was observed for another ovarian marker, M-CSF, during this treatment [35], and for serum CA125 during another 24-hour infusion of taxol administered to the same patient [31]. CA125 was determined in around-the-clock unstimulated saliva samples collected during 7 days before treatment and AU2 during the 24-hour constant infusion of cisplatin (25 $mg/m^2/24\,h$). A decrease in the MESOR of salivary CA125 ($P = 0.046$) suggests that, overall, the

Figure 11.8. Illustration of a five-timepoint chronobiologic design, with results analyzed by single cosinor. The original data (left) are tumor regression rates, expressed as a percentage per week, plotted as a function of the timing of radiotherapy in relation to peak tumor temperature. The five timepoints are the time of peak tumor temperature, 4 or 8 hours before the peak, or 4 or 8 hours after the peak, described by 0, + 4, + 8, −4, and −8, respectively. The 24-hour cosine curve best fitting these results is plotted together with the data (left). The cosinor results are also shown in a polar display (right). The fact that the 95% confidence ellipse for the joint estimation of the circadian amplitude and acrophase does not cover the pole (center of the plot) attests to the statistical significance of the fitted model (rejection of the zero-amplitude or no-rhythm assumption).

Validation of effectiveness of human chronoradiotherapy

Faster peri-oral tumor regression rate with radiotherapy at circadian peak in tumor temperature *



Key to ellipse	P	No. obs.	PR	MEBOR	SE (units - %)	Amplitude		(95% CL)	Acrophase (ø)	
Regression rate (%/week)	0.042	5	96	11.2	0.5	4.9	(0.5	9.3)	–364° (–291	–50)

* Determined in a patients/group (total = 40 patients) receiving radiotherapy 5 days/week for 5 weeks at 1 of 5 treatment times in relation to macroscopic peak of tumor temperature (based on 2-hourly measurements taken around–the–clock for 1–5 days prior to treatment onset) (Helberg, et al., 1977).

treatment was effective. The depression in cancer marker occurred primarily between 0600h and 0900h with a further response of lesser extent lasting until 1600h [32].

A treatment course of doxorubicin administered continuously for 7 days by means of a programmable pump offered the opportunity to vary the infusion rate in a circadian stage-dependent manner and to vary the timing of the high rate infusion span from one day to the next [33]. After an about 2-day infusion of doxorubicin at a constant rate (0.6 mL/h of a 25 mg/100 mL solution), a high 6.6 ml dose was programmed to be administered over 4.8 hours followed by a low dose rate of 0.4 mL/h for the remainder of the 24-hour cycle. The 4.8-hour span of high infusion rate was shifted by 4.8 hours each day. The ovarian cancer marker UGP was assessed in fractionated urine samples collected around the clock before treatment and during the entire treatment span. Optimal timing of treatment could be assessed for efficacy by time-specified differences in UGP during constant infusion of doxorubicin as compared to values before treatment. Results in this case could also be compared with an assessment of treatment efficacy, gauged by UGP on days with highest infusion rate at five different circadian stages, analyzed by cosinor, the UGP response being assigned to the midpoint of the interval during which the high dose was administered. An overall decrease in both UGP and CA125 during treatment was noted, as an indication that it was effective. Conceivably, to the extent to which the marker reflects tumor metabolism, the time of largest marker response serves as an indication of the time of highest drug efficacy [33].

Chemosensitivity assays in vitro on tumor cells removed at surgery can help determine the most appropriate choice of treatment [36], its effectiveness being further checked in vivo by changes in noninvasively sampled marker rhythms in response to treatment [37]. The chemoresponse assay helps determine whether drug resistance has developed and accordingly secure the putatively most useful drug. A chemoresponse assay for nine antineoplastic agents found the patient to be sensitive to 5-FU. A 1-hour infusion of 5-FU (12 mg/kg) was administered at 2200h (given at the end of a 700-mg/m^2/day leucovorin infusion for 24 hours). During the first 2 days after treatment, UGP dropped markedly, this response being replicated after another similar treatment also administered at 2200h, but not when it was administered at 0600h or at 1400h [33]. The optimal timing determined in this *N*-of-1 study (in the Erna test) corresponds closely to the recommended infusion schedule for 5-FU (with maximal dosing at night) based on a subsequent large clinical trial [38].

11.3.4 Triangulation

Many anticancer drugs and radiation disrupt cell reproduction and have their greatest effect on tissues that are growing most rapidly. Drugs or radiation can destroy some cancer cells whenever they are administered. But, depending on the timing of treatment, a variable amount of healthy tissue is also destroyed. The healthy cells most sensitive to such treatment are the fast-growing cells in

hair follicles, the lining of the intestine, and bone marrow. Hence the usual side effects of anticancer treatment are loss of hair, nausea, and a reduction in the red and white blood cells formed in bone marrow. By charting rhythms of cell division in healthy and cancerous tissue, it should be possible to find the time when chemotherapy or irradiation is least harmful to healthy cells. Such an approach has already been extensively used in the experimental laboratory [39, 40]. Extension of the findings to the clinic can thus shield healthy tissues of cancer patients by timing treatment [24], albeit without losing sight of the primary objective of optimizing treatment efficacy for the given patient. A rhythm in the concentration of a drug in blood (in bioavailability, the false gold standard), brought about by interactions within the body among effects of metabolic and excretatory mechanisms, may well be timed quite differently as compared to that desired for an optimal treatment effect. In the case of a carcinostatic drug, one strives to give a potentially toxic drug at a time that represents the most desirable compromise between the times when the drug is best tolerated by the host, insofar as this is compatible with a time when it is most effective against the tumor [28].

In a clinical trial of advanced ovarian and bladder cancer at the University of Minnesota [41, 42], combination chemotherapy consisted of about nine courses of doxorubicin followed 12 hours later by cisplatin. Patients were randomly assigned to one of two treatment times, doxorubicin being administered either about 1 hour before awakening or about 11 hours after awakening. Heart rate was monitored around the clock before each treatment course. The difference in heart rate MESOR between the last and first profiles served to assess the cardiotoxicity of doxorubicin for each patient. These differences in heart rate MESOR were then assigned to a time code representing the time of doxorubicin treatment in hours and minutes from the pretreatment heart rate acrophase. A cosinor analysis of these data (consisting of differences in heart rate MESOR assigned to the time of doxorubicin administration relative to the pretreatment heart rate acrophase, pooled over all patients and all treatment courses) indicated a statistically significant circadian rhythm in the cardiotoxicity of doxorubicin ($P = 0.043$), being highest about 10 hours after the heart rate acrophase. Accepting the possible increase in heart rate MESOR as unfavorable leads to the inference that treatment at the cardiosensitivity chronorisk (corresponding to the time of anticipated largest increase in heart rate MESOR) should be avoided [43].

Triangulation is defined as the location of a point in circadian (or other) time which is associated with the best compromise between desired and undesired effects [43]. First, optimal circadian stages may be evaluated by reference to several marker rhythms, each used for the double purpose of gauging the patient's response to treatment and the timing of treatment administration relative to that marker's acrophase. Each marker rhythm assesses the different toxicities of the drug(s) (such as cardiotoxicity, myelotoxicity, and nephrotoxicity) as well as any benefit derived therefrom (e.g., by tumor markers or tumor size and survival time). As such, they provide information concerning

the merits and/or demerits of the treatment. Second, the marker rhythms can provide information concerning the timing of treatment by means of their acrophase. To investigate optimization by timing, treatment time is not specified only in terms of clock hours but also relative to the acrophases of the various marker rhythms. Thus the patient's responses to treatment (evaluated as an increase in heart rate MESOR for cardiotoxicity, as the area under the curve of weekly WBCs, neutrophils, and platelets for myelotoxicity, and by creatinine clearance for nephrotoxicity) can be referred as outcomes to the given patient's prospectively determined internal time structure. This approach allows the individualization of timed treatment. Triangulation is then based on a collateral hierarchy of estimates relating to the anticipated benefits from and risks of the treatment [43]. It has been suggested by the founder of the specialty of oncology in the United States, B. J. Kennedy, that this approach added a few years to the life of a patient with a very advanced ovarian cancer at the time of its diagnosis [44].

When relying on more than one marker rhythm, several indications of desired treatment time are obtained. These may or may not converge; that is, they may point toward a similar rhythm stage overall or toward conflicting rhythm stages. When there is convergence of desired times, an average optimal time can be derived from all estimates by "triangulation." In the case of divergence, however, optimization may be carried out by assigning different weights to the different marker variables in view of the given patient's strengths and weaknesses, more weight being given to the estimate thought to describe the risk to which the patient is thought to be most susceptible [43].

Studies by Blank et al. [45] of mitotic activity of bone marrow and tumor of sarcoma-bearing rats and of bone marrow of healthy controls indicate the possibility to achieve concomitantly near-maximal efficacy and near-minimal myelotoxicity, with similar results already observed in humans with different kinds of malignancies (Blank et al., unpublished). Indeed, whereas the circadian rhythm in mitotic activity of bone marrow was similar for intact and tumor-bearing rats, with a peak occurring shortly after the onset of the dark (activity) span, the circadian rhythm in mitotic activity of tumor had a much smaller amplitude and a different acrophase occurring late in the dark span (Figures 11.9 and 11.10). Also to be considered is a set of usually cyclic environmental conditions that may affect healthy cell division. For instance, corneal mitotic activity was found to be affected by magnetic storms [46]. Geomagnetic activity was also reported to influence hematotoxicity in cancer patients [47, 48].

11.4 APPLICATIONS IN INFECTIOUS DISEASE: IMPORTANCE OF CHRONOPHARMACOKINETICS

As an illustrative example of the chronoavailability of drugs (e.g., see Ref. [49]), three erythromycin test preparations were tested [50]. Twenty-four adult men were given 250 mg of erythromycin as one of three different preparations in a

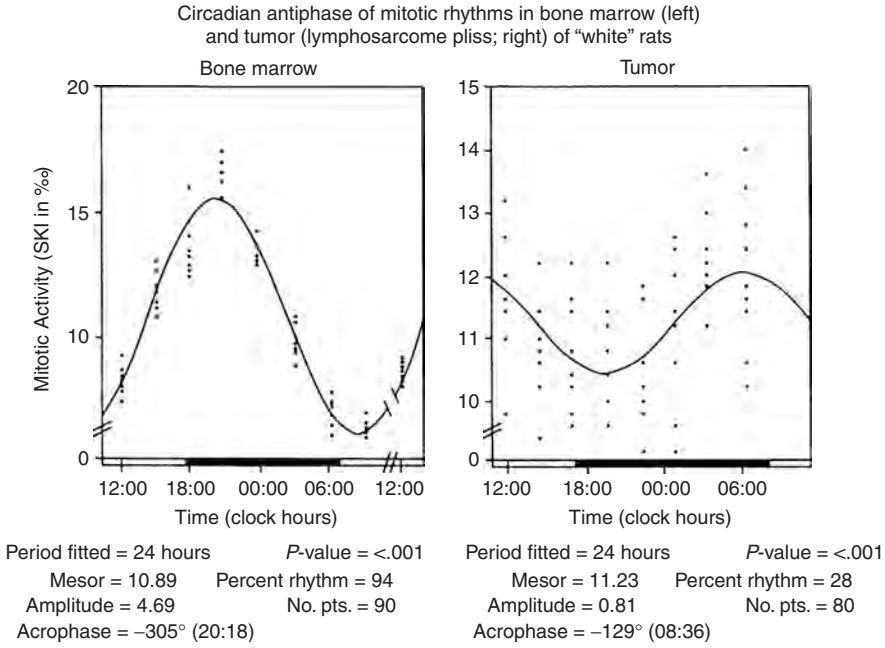


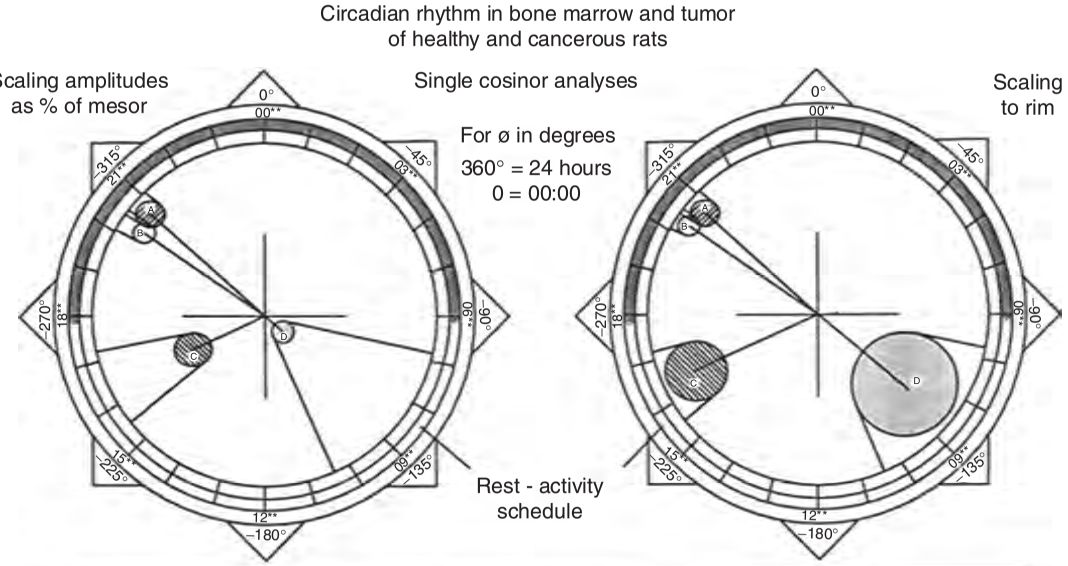
Figure 11.9. Comparison of circadian mitotic rhythm in bone marrow and tumor of rats bearing a lymphosarcoma. Note large difference in acrophase between the two circadian mitotic rhythms, suggesting the possibility of optimizing treatment efficacy without excessive toxicity. Data from M Blank.

crossover study with 1-week intertreatment intervals. All subjects were diurnally active and nocturnally resting, and fasted for 2 hours before each dose and for at least 2 hours after each medication. Dosing started at 0800h of one day and continued every 6 hours for 3 days. Blood concentrations of erythromycin were determined at 0, 1.5, 3, 4.5, and 6 hours in relation to each dose for 24 hours, and again with the same sampling schedule beginning 48 hours after the first dose. On a group basis, a circadian rhythm was invariably demonstrated with statistical significance for all endpoints considered, namely, the time to peak, the peak concentration, the area under the curve, and the nadir. The largest area, and hence the greatest coverage in terms of antimicrobial activity, was found with drug administration around noon [50].

11.5 APPLICATIONS IN TRANSPLANTATION

11.5.1 Chronopharmacodynamics Override Chronopharmacokinetics

Cyclosporine is a powerful immunosuppressive drug able to prevent or greatly delay the onset of acute allograft rejection both in experimental [51] and in



Variable (animal model)	Units	*P*	N	PR	Mesor ± SE	Amplitude*	Acrophase (Ø)*
A Bone marrow mitosis (health)	‰	< 0.001	91	90	11.47 0.13	5.18 (4.74 5.63)	–311° (–307 –316)
B Bone marrow mitosis (cancer)	‰	< 0.001	89	94	10.89 0.09	4.69 (4.37 5.01)	–305° (–301 –308)
C Bone marrow prolif.pool (cancer)	%	< 0.001	89	57	14.90 0.23	3.50 (2.68 4.33)	–245° (–232 –258)
D Tumor mitosis (cancer)	‰	< 0.001	80	28	11.23 0.11	0.81 (0.44 1.18)	–129° (–102 –156)

P = Probability of hypothesis: amplitude = 0; N = number of observations
PR = Percent rhythm (percentage of variability accounted for by cosine curve)
* Conservative 95% confidence limits (parentheses) derived from cosinor ellipse

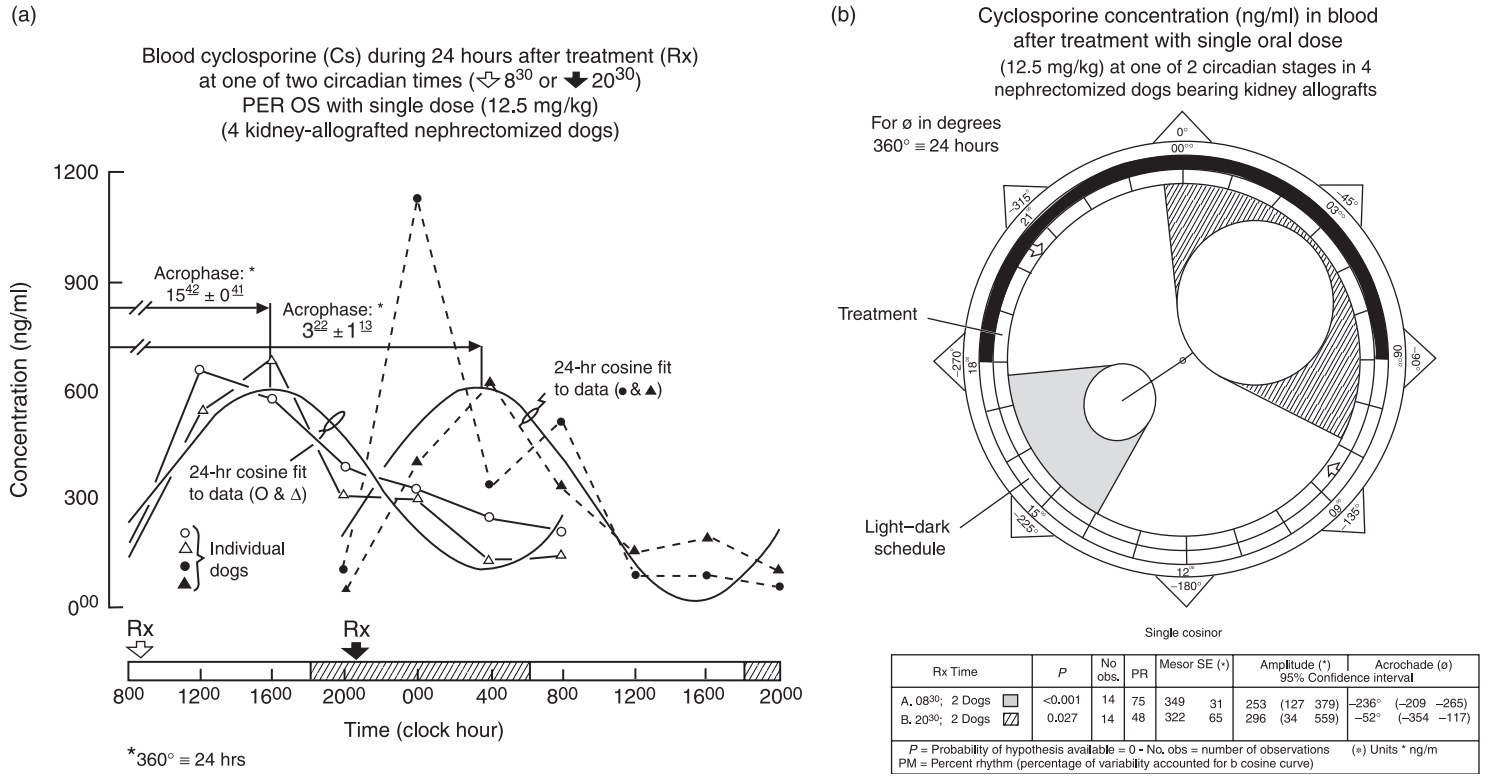
Figure 11.10. Polar representation of circadian mitotic rhythms in bone marrow and tumor of rats bearing a lymphosarcoma as compared to circadian mitotic rhythm in bone marrow of healthy control rats. Whereas mitotic activity of bone marrow peaks shortly after the onset of the dark (activity) span in both healthy and cancerous rats, the circadian rhythm in mitotic activity of tumor has a smaller amplitude and a different acrophase occurring late in the dark span. This difference has important implications for scheduling the administration of oncotherapy. Data from M. Blank.

clinical [52] transplantation. Cyclosporine's nephrotoxicity being dose-related, the optimization of this drug's immunosuppressive properties could lower the dosage needed to prevent acute graft rejection and thus reduce nephrotoxicity [53]. Studies on rats had shown that the toxicology of cyclosporine was circadian stage dependent [54]. Moreover, rejection of a heart allografted across a major histocompatibility barrier was delayed with low single IP daily doses of cyclosporine given during the daily dark (active) span. A circannual variation in the immunosuppressive effect of cyclosporine was also noted, manifested by a prolongation of graft function [55]. Studies with segmental pancreas allografts from ACI rats into diabetic Lewis inbred rats confirmed that circadian timing determined the extent of prolongation of graft function [53].

The potential benefit from the circadian timing of cyclosporine was further tested in the dog with a kidney allograft. Treatment was administered IV via an implanted, externally programmable pump (Medtronic Inc., Minneapolis, MN). A circadian sinusoidal schedule of cyclosporine peaking in the middle of the dark span was associated with nearly a doubling in graft survival as compared to a schedule peaking in the middle of the light span [56]. In this experimental model of kidney-allografted dogs, the possibility was then examined that the results obtained with a pump could be exploited for the more practical oral administration of the drug [53].

Kidneys were exchanged between 10 pairs of mongrel dogs, the remaining kidney of each dog being discarded. Quasirandomization was used to assign dogs to one of two treatment groups, receiving single daily doses of 12.5 mg/kg BW cyclosporine orally, for up to 60 days, either at 0830h or 2030h. Dogs in a pair were assigned to different circadian stages, one at 0830h, the other at 2030h. A control group of 6 dogs was kept untreated. Dogs were kept in a regimen of light from 0600 to 1800h alternating with darkness from 1800h to 0600h. At death, rejection was confirmed by laparotomy and graft histology. The chronopharmaokinetics were determined by measuring blood concentrations of cyclosporine around-the-clock (7 samples at 4-hour intervals) in four dogs (two dogs for each treatment time) on the 5th postoperative day. Graft survival was used to assess the chronopharmacodynamics [53].

A statistically significant circadian rhythm was found to characterize cyclosporine blood concentrations from the two dogs treated at 0830h ($P < 0.001$) as well as those from the two dogs treated at 2030h ($P = 0.027$) (Figure 11.11). As anticipated, acrophases at 1542 ± 0041h and at 0322 ± 0113h, respectively, are in near antiphase. From a chronopharmacokinetics point of view, treatment was equally effective at the two timepoints tested, as evidenced by the lack of a statistically significant difference in the areas under the curve (8719 vs. 7740 ng/mL $\times$ h, respectively) and in the cyclosporine trough concentrations assessed from 18 dogs (2 dogs were excluded from analysis due to technical failures) (191 ± 60 vs. 141 ± 49 ng/mL, respectively). By contrast, in terms of the chronopharmacodynamics, 8 of the 10 dogs treated at 0830h rejected their graft after 6, 6, 7, 7, 8, 10, 17, and 20 days, whereas only 2 of the



Rx Time	P	No obs.	PR	Mesor SE (•)	Amplitude (*) 95% Confidence interval	Acrochade (ø)
A. 08^30; 2 Dogs	<0.001	14	75	349 31	253 (127 379)	–236° (–209 –265)
B. 20^30; 2 Dogs	0.027	14	48	322 65	296 (34 559)	–52° (–354 –117)

P = Probability of hypothesis available = 0 - No. obs = number of observations (•) Units * ng/m
PM = Percent rhythm (percentage of variability accounted for b cosine curve)

Figure 11.11. Circadian rhythm in cyclosporine blood concentrations from two dogs treated at 0830h (open symbols) and from two dogs treated at 2030h (dark symbols). All dogs were kept in light from 0600h to 1800h daily. Note difference in acrophase (measure of timing of overall high cyclosporine concentrations in blood) in the absence of any difference in chronopharmacologic endpoints, apparent from plot of data as a function of time with best-fitting 24-hour cosine curves (a) and from the polar cosinor display also showing the statistical significance of the circadian rhythms by the rejection of the zero-amplitude (no-rhythm) test (b). Data from M. Cavallini.

8 dogs treated at 2030h rejected their graft after 12 and 15 days ($P < 0.010$ by life table analysis; all 6 control animals acutely rejected their graft within 7 days), See Figure 11.12 [53].

11.5.2 Beyond Circadian Optimization

A circaseptan (about-weekly) periodicity in rejection was found for human kidney transplants in Minnesota, Paris, and Milan [57]. The phase of this circaseptan component was determined by the timing of transplantation rather than by the day of the week when surgery was performed, suggesting that we are dealing with a response rhythm that is amplified after exposure to a single stimulus such as transplantation surgery. The question thus arose as to whether circaseptan rhythms could be exploited to optimize the prolongation of graft function by treatment with an immunosuppressant agent such as cyclosporine [58].

Segmental pancreatic transplantation was performed on Ma Lewis (RT-1^{l}) recipients with ACI (RT-1^{a}) donor rats. Animals were kept on staggered regimens of 12 hours of light alternating with 12 hours of darkness. At 7–14 days prior to surgery, all recipients were made diabetic by a single IV injection of 50 mg/kg BW streptozotocin. All rats with a serum glucose over 400 mg% received a segmental pancreas transplant from an ACI rat standardized on the same lighting regimen. Cyclosporine was administered daily, starting on the day of surgery, at one of six different circadian stages, 4 hours apart (either at 2, 6, 10, 14, 18, or 22 hours after light onset, HALO). A given animal was always treated at the same circadian stage each day. One ''homeostatic'' group (H) received equal daily doses of 3.5 mg/kg cyclosporine. Seven experimental groups (S) received varying daily doses of cyclosporine according to a 7-day sinusoidal pattern, the first largest dose being administered either on the day of surgery or on the second, third, fourth, fifth, sixth, or seventh day after surgery. The average daily dose equaled that of the control group (3.5 mg/kg), with maximal departure of ± 1 mg/kg. Untreated rats and rats receiving the vehicle only served as controls (C).

Serum glucose was measured every 1 or 2 days. The day of graft rejection was defined as the first of at least 3 consecutive days of hyperglycemia (>200 mg%) and confirmed in most cases by laparotomy and graft histology. The circadian stage-dependent effect of cyclosporine treatment was confirmed in rats of group H, the longest times to rejection occurring at about 20 HALO, in keeping with other studies in rats. But the longest graft function was found to occur for rats receiving varying daily doses of cyclosporine, according to a circaseptan sinusoidal pattern with largest daily doses administered either on the third or fifth day after surgery and at 7-day intervals thereafter [58]. The mean number of days (and standard deviation) elapsed until pancreas rejection was 6.0 ± 1.4 days in the 11 control animals. By comparison, in the 19 rats receiving equal daily doses of cyclosporine, it was 8.8 ± 4.0 days, and in the 49 rats receiving cyclosporine according to a circaseptan schedule, it was 11.5 ± 4.2

days. As shown in Figure 11.13, as compared to the average rejection time of group H, the untreated control rats had a 31% shorter duration of graft function (Student $t = 6.50$, $P < 0.01$), whereas rats in group S had a 30% prolongation of graft function (Student $t = 4.41$, $P < 0.01$) [58]. A 30% increase in treatment efficacy associated with circaseptan optimization is not negligible, for two reasons: first, it can be achieved in addition to benefit from circadian optimization; and second, from a practical viewpoint, chronotherapeutic optimization may be easier to achieve along the scale of the week than along the circadian scale, notably when the best circadian stage happens to fall outside clinic hours and treatment cannot be administered automatically (e.g., via a programmable pump) or by controlled release [59].

Circaseptan optimization was also achieved in studies on the immunomodulation of malignant growth in LOU rats bearing an immunocytoma [60]. The effect of a 7-day pretreatment with the immunomodulator lentinan was compared to that of pretreatment with saline. The growth of the malignant tumor was inhibited and survival time lengthened when lentinan was administered daily during the light (rest) span in doses varying sinusoidally from day to day as a circadian–circaseptan chronotherapy. By contrast, when lentinan was conveniently given during the active span in the usual equal daily doses, tumor growth was accelerated and survival shortened.

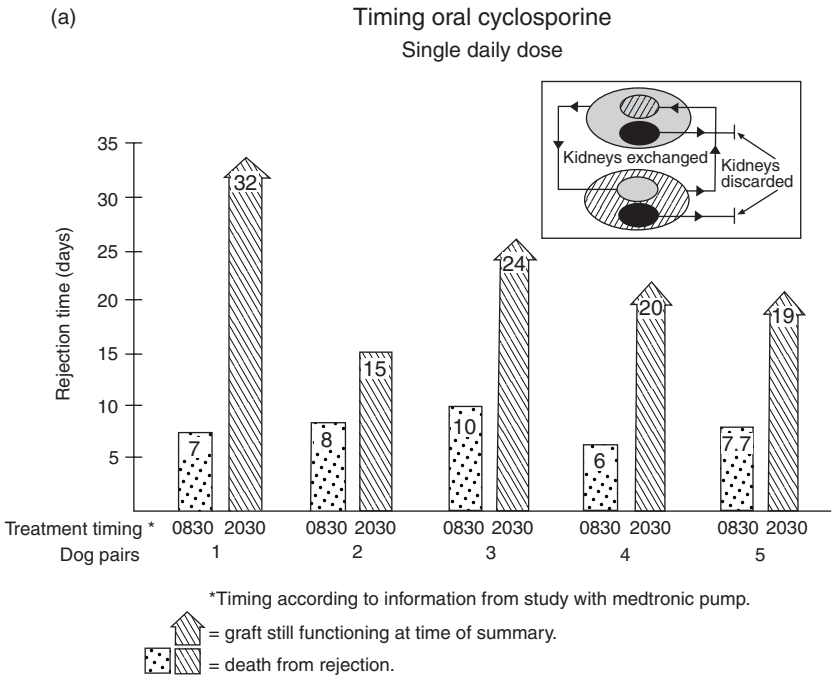
For both cyclosporine and lentinan, circannual differences were also documented [58, 60].

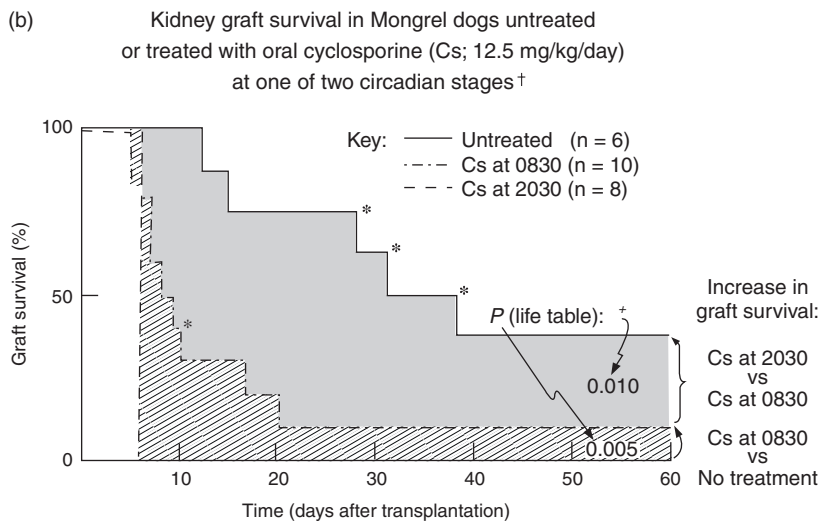
11.5.3 Early Detection of Cyclosporine Side Effects in Heart Transplant Patients

Nephrotoxicity represents a frequent and severe complication associated with cyclosporine treatment, reported in heart transplant recipients [61]. Cyclosporine therapy has also been associated with a persistent elevation of blood pressure developing within the first weeks posttransplantation in 60–90% of heart allograft recipients, requiring intensive and combined antihypertensive regimens [61].

Twenty-four-hour ambulatory blood pressure monitoring (ABPM) of 14 patients, 43–61 years of age, 1–33 months after heart transplantation, revealed

Figure 11.12. Despite lack of differences in terms of chronopharmacokinetics (Figure 11.11), there is a large difference in pharmacodynamics between treatment at 0830h and 2030h. (a) Intermediate results show that in pairs of dogs with an exchanged kidney, one receiving 12.5 mg/kg cyclosporine at 0830 h and the other at 2030h, the evening dose is consistently associated with a longer time to organ rejection, being over twice as effective as the morning dose in prolonging kidney allograft function. Arrows correspond to functioning graft at time of summary; flat bars correspond to death from rejection. (b) Summary of results at end of study: kidney graft survival in untreated dogs and in dogs receiving oral cyclosporine at 0830h or at 2030h. Dogs were kept in light from 0600h to 1800h daily. Data from M. Cavallini.





* Died or killed with functioning graft

† 0830 or 2030 (dogs kept in daily light from 0600 to 1800)

+ E.T. Lee: statistical methods for survival date analysis. Life-time learning publ. Belmont, Ca., 1980 (552pp.)

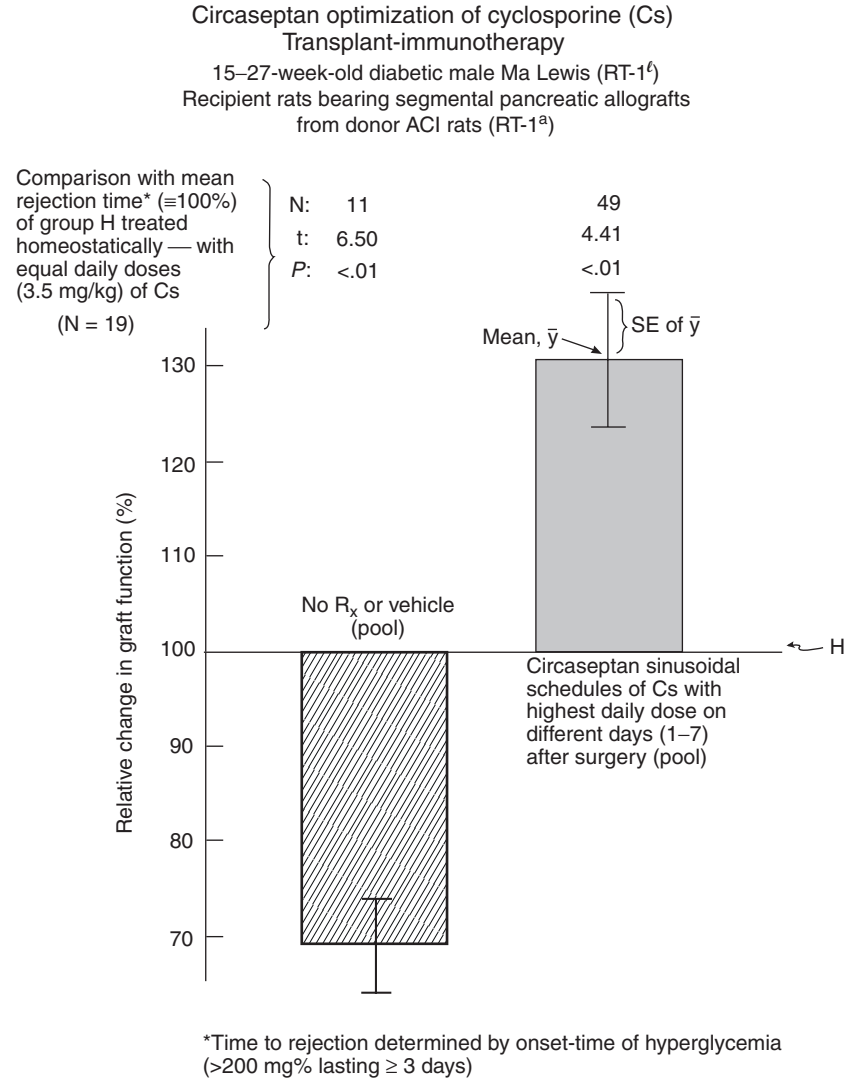


Figure 11.13. Cyclosporine chronotherapy of pancreas-allotransplanted rats suggests further gain in graft function from doses varying from day to day according to a weekly periodicity, beyond the circadian stage dependence of equal daily doses. Data from T Liu.

a nocturnal increase in blood pressure [62]. All patients received standard immunosuppressive therapy. ''Hypertension'' was diagnosed clinically by the repeated recording of cuff blood pressure readings above 145/95 mm Hg. Antihypertensive treatment, initiated on the basis of isolated readings in the

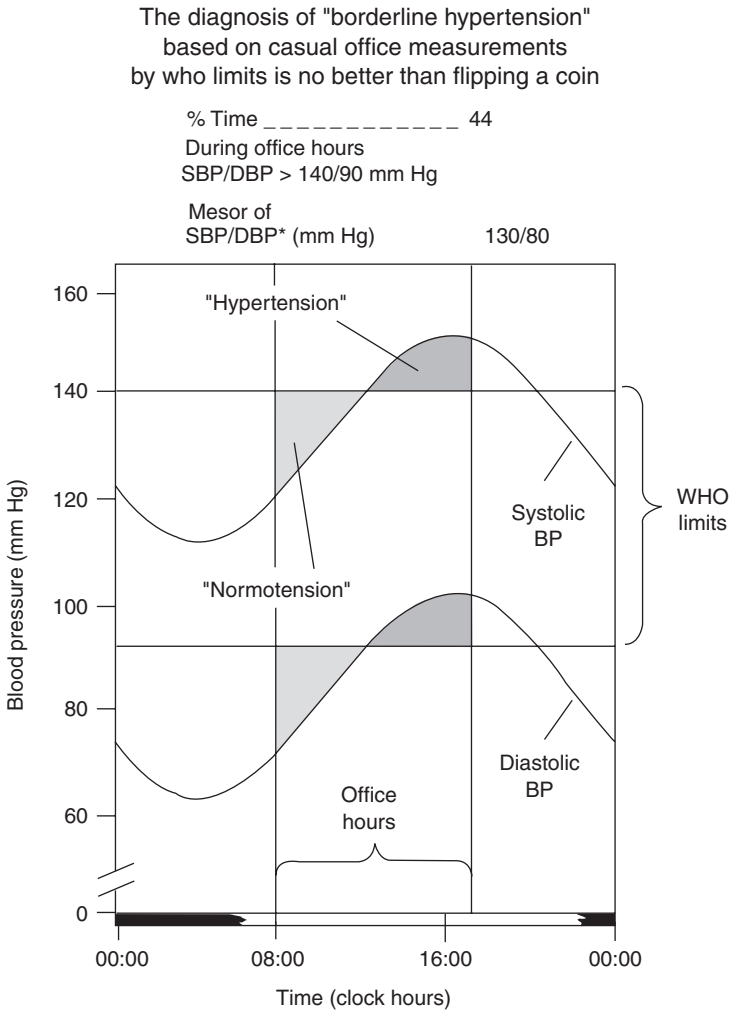
clinic, did not start until after the third month posttransplantation. ABPM revealed nocturnal blood pressure excess already at 2 months after heart transplantation, however. Blood pressure monitoring, as a marker for potential side effects, could prompt the institution of antihypertensive treatment when needed, earlier than on the basis of spotchecks in the clinic. The earlier diagnostic stems both from the fact that abnormality occurred at times when blood pressure is not likely to be checked in the clinic and from the use of time-specified reference values accounting for the usually prominent circadian variation in blood pressure instead of imaginary fixed limits used conventionally [62].

11.6 APPLICATIONS IN CARDIOVASCULAR MEDICINE

11.6.1 Diagnosis of Blood Pressure Disorders

The merit of treating elevated blood pressure is no longer disputed and is widely viewed as a critical way of reducing morbidity and mortality associated with cardiovascular disease [63]. It is also accepted that a diagnosis based on 24-hour ABPM is superior to that based on isolated measurements in the clinic, despite the fact that the latter are taken by trained professionals with an accurate mercury sphygmomanometer under strictly standardized conditions. There are several reasons accounting for the superiority of ABPM over clinic measurements and for the large estimated percentage of misdiagnoses (over 40%) based on single casual readings [64]:

1. The measurements are taken repeatedly, yielding an average value associated with a reduced standard error.
2. They are obtained during usual daily activities rather than at times when the patient's blood pressure may either be spuriously elevated in association with anxiety about the physical examination (''white-coat hypertension'') or spuriously lowered in association with the quieter environment away from loads of usual daily life (''masked hypertension'').
3. Measurements being obtained around-the-clock, the diagnosis does not depend on when during the day the patient happens to be examined, the current situation ignoring the usually large-amplitude circadian rhythm in blood pressure by neglecting to specify when the measurement is taken and by using fixed rather than time-varying limits for its interpretation; Figure 11.14 [65].
4. Around-the-clock measurements convey not just an average value but patterns of change during both the rest and active span, so that if abnormality is present only during times when the patient is not likely to be examined by a health professional, it will be missed. This was the case of a 78-year-old man treated once a day in the morning with a 24-hour formulation of 10 mg Vasotec (ACE inhibitor) [4]. ABPM revealed



* Circadian amplitude of 20 mm Hg and acrophase of –240° (16h from 00:00).

Figure 11.14. Limitations in dealing with blood pressure (BP) interpreted by fixed limits (and casual measurements or automatic office-hour profiles). Theoretic evidence points to the need to replace fixed thresholds by time-varying reference standards (chronodesms) and to rely on more than one or a few casual BP measurements. Assuming that BP is measured precisely and accurately (without error), ignoring the circadian rhythm in BP results in contradictory diagnoses whether BP is taken in the morning or in the afternoon.

nightly elevated values of diastolic blood pressure during 11 consecutive nights, reaching near 120 mm Hg on the average between midnight and 0300 h, whereas during midday when he saw his treating physician, it was below 90 mm Hg.

Just as there is usually large blood pressure variability from moment to moment, accounted for in part by the circadian variation, there can be large day-to-day, week-to-week, and other variability in the circadian blood pressure pattern as well in some patients, to the point that a decision to treat or not to treat may depend on the day a 24-hour ABPM happened to be obtained [2]. For instance, a 33-year-old untreated man who monitored his blood pressure around the clock for over 30 days was found to have 24-hour average values of systolic blood pressure below 120 mm Hg on some days but above 135 mm Hg on other days, with 77% acceptable values during clinic hours on one day but with all readings above 140/90 mm Hg on another day [1]. The question may be raised as to what constitutes optimal affordable blood pressure monitoring, short of continuous longitudinal records with an inexpensive cuffless device, a goal pursued by the Phoenix Project (www.phoenix.tc-ieee.org), a group of volunteering members of the Institute of Electrical and Electronics Engineers. Table 11.1 lists some advantages and limitations of options available today.

11.6.2 Toward a Chronobiologic Blood Pressure Prevention Clinic

With the possibility to monitor blood pressure automatically around the clock, the conventional question of whether a blood pressure measurement is acceptable or too high (or too low) needs to be replaced by a meaningful interpretation of serial measurements obtained during both the rest and activity spans under usual rather than standardized conditions [66]. Keeping these differences in mind, by 1984 we proposed the use of time-specified reference limits (chronodesms) [67] further qualified by gender and age as replacement for arbitrary time-invariant limits relying on actuarial statistics of morbidity and mortality data from previous generations. By 1984, we also had advocated the use of a set of new endpoints in addition to the actual readings and the circadian rhythm characteristics derived therefrom [68]. The latter are obtained by cosinor, using a two-component model consisting of cosine curves with periods of 24 and 12 hours to account for the usual non sinusoidal circadian waveform of blood pressure and heart rate [69–71]. The new endpoints include the following:

- The percentage time an individual's profile lies above the upper time-specified reference limit during 24 hours (or below the lower limit)
- The extent of excess (or deficit) defined as the area delineated by the reference limit and the individual's profile when it lies outside the reference range
- The timing when most of the excess (or deficit) occurs within 24 hours

These model-independent (nonparametric) endpoints are computer derived by numerical integration. Cosinor-derived parametric and nonparametric

Table 11.1. Advantages and Limitations of Different kinds of Blood Pressure Monitoring

Procedure Number	Procedure	Usage	Advantages	Limitations
1	A few measurements on a few occasions under standardized conditions (rest for 5 min).	Current clinical practice for detecting an elevated blood pressure (BP).	Treatment of thus detected high BP reportedly associated with decline in incidence of heart attacks and strokes.	Does not account for large variability in BP. Potentially associated with misdiagnoses (e.g., ''white-coat hypertension,'' ''masked hypertension'').
2	24-hour ABPM (ambulatory BP monitoring).	In current practice, reserved for ''special cases'' (when diagnosis of hypertension is difficult to make).	The 24-hour BP mean has better prognostic value than (1). Allows assessment of BP under usual conditions during both the rest and active daily spans and hence can detect BP abnormality at times when it would usually not be measured. Also allows a rough assessment of the circadian variation.	Does not account for the day-to-day changes in BP and in circadian BP pattern that can be very large in some subjects. Hence it may lead to unwarranted decision to treat or not to treat depending on results on a given day that may or may not be representative. Circadian parameters can only be estimated with a relatively large error margin.

(*Continued*)

3	48-hour ABPM	Only done in some clinical settings abroad, notably in Japan, reserved for ''special cases.''	Extending ABPM from 24 to 48 hours is associated with large improvement in estimating circadian characteristics (error term reduced by about 35%). Allows subject to become habituated to monitor.	Still too short to fully account for day-to-day changes in BP characteristics, notably differences often observed between workdays and weekends. Does not allow assessment of any weekly variation in BP that may also contain valuable information in its own right.
4	7-day/24-hour ABPM	Practiced only within BIOCOS.	Yields a more reliable assessment of the circadian characteristics and allows an assessment of the weekly pattern as well. Outcome studies have found associations with results from 7-day record but not with those of first 24 hours.	In some cases, 7-day/24-hour ABPM may not be enough due to large day-to-day changes in BP. When abnormality is detected, a second weeklong profile is advocated. For treated patients, 7-day/24-hour ABPM does not detect any change in patient's response to treatment.

(*Continued*)

Table 11.1. Continued

Procedure Number	Procedure	Usage	Advantages	Limitations
5	Home monitoring (daily measurement upon awakening and/or at bedtime)	Used in some settings for patients treated for hypertension, notably in Germany and in Japan.	Can detect any change in patient's need for treatment adjustment. Amenable to longitudinal scrutiny, with applications in research.	Does not assess circadian variation or risk associated with abnormalities in BP variability.
6	Home monitoring (daily measurements several times daily, e.g., every 3 hours during waking, with occasional nightly measurement).	Used by some chronobiologists interested in autorhythmometry.	Same advantages as (5) with additional rough assessment of circadian rhythm.	Circadian assessment is often insufficient, notably when nightly values are lacking.
7	Longitudinal ABPM.	Used in some patients in Japan and by a few chronobiologists.	Can detect changes in BP status in a timely fashion. Data also useful for research purposes.	More labor intensive.

endpoints are summarized on a form known as the sphygmochron [1, 2] (www.phoenix.tc-ieee.org/011_Data_Analysis_Methods/Phoenix_Data_Analysis_Methods.htm). In addition to a usually more accurate and more precise estimate of location in the derivation of a MESOR (midline estimating statistic of rhythm) instead of the arithmetic mean, circadian characteristics are also estimated that may reveal abnormalities in their own right.

In 1988, the Mayo Clinic suggested the use of a blood pressure load [72], an index similar to the percentage time elevation, except that it is computed by comparison to fixed limits, rather than chronodesms. Only the latter account for the circadian variation and for differences by gender and age [73]. Clinical issues discussed conventionally by others within the context of ABPM use are the amount of excess, albeit computed versus either fixed limits of 140/90 mm Hg or with respect to daytime and nighttime averages [74]. The use of the hyperbaric index, as the extent of excess has been called, was also advocated by the (U.S.) National High Blood Pressure Education Program Coordinating Committee [75]. As a measure of blood pressure excess, the hyperbaric index helps refine the treatment modality and prognosis that may vary greatly among patients who might have a similar percentage time elevation (or blood pressure load) but different hyperbaric indices, as shown, for instance, in Figure 11.15 [66]. The timing of overall excess is also useful to specify treatment timing.

For a preventive as well as curative medicine, it is critical to map the partly genetically anchored, socioecologically synchronized chronomes (time structures), as their characteristics vary with age, gender, and ethnicity, among others. Chronome maps obtained so far stem from linked cross-sectional (hybrid) studies, in the sense that each individual is sampled around the clock for at least 24 hours, preferably for 7 days or longer. An added longitudinal element along the age scale derived from low-risk, long-lived individuals, validated by outcome, will be indispensable for deriving trustworthy reference standards capable of identifying the presence of a heightened risk in a timely fashion. It will then also become possible to distinguish between rhythm alterations indicative of an elevated risk and naturally occurring changes with age [73]. A website dedicated to this task is being planned by Larry Beaty, a member of the Phoenix Project (www.phoenix.tc-ieee.org/), as a step toward this goal. The merit of such a chronobiologic approach became apparent from a study of pregnant women who were presumably healthy at the start of monitoring, some of them eventually developing complications at a later gestational age [76–78]. Differences on the order of 10 mm Hg were found well within the range of acceptable values between uncomplicated pregnancies and pregnancies that were subsequently complicated by gestational hypertension and/or preeclampsia (Figure 11.16). These MESOR differences detected retrospectively by chronobiological methods could have been acted upon prospectively, already during the first trimester of pregnancy, well before the appearance of any complication, insofar as the systolic and diastolic blood pressure MESORs were below 125/75 mm Hg. This task is a challenge for the future.

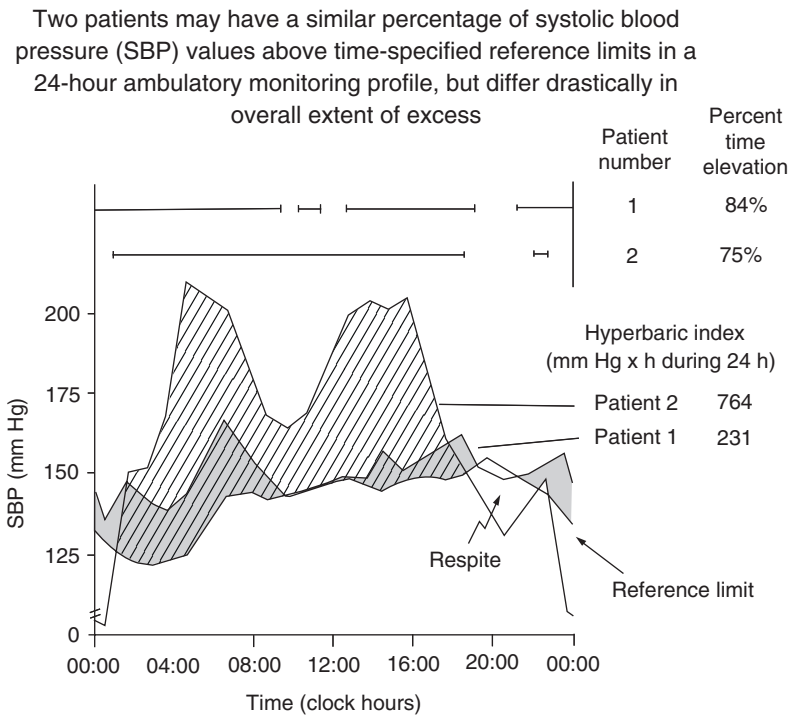


Figure 11.15. The amount of blood pressure excess should be taken into consideration in making decisions regarding treatment since two patients with a similar percentage time elevation (above peer-group chronodesmic limits rather than arbitrary fixed thresholds used to derive blood pressure load) may show drastic differences in extent of excess.

11.6.3 Blood Pressure Variability Disorders

Current guidelines do not specify in the United States the number of measurements to be taken, which in some government-reported studies is less than or equal to 3 [79], whereas the German League Against High Blood Pressure specifies the need for repeated measurements and the Austrian guidelines are even more advanced, specifying a minimum of 30 measurements before a diagnosis is made: hypertension versus normotension if there is more than 26.7% versus less than 23.3% values above fixed limits of 135/85 mm Hg [80]. Indeed, guidelines still focus primarily on the average blood pressure value, with target thresholds of acceptability defined for all adults 18 years or older [81]. The status quo ignores ethnic [82] and gender [83] differences and trends as a function of age both in terms of average value (Figure 11.17), and of the circadian pattern itself (Figure 11.18). Also ignored are results from prospective as well as retrospective studies by others as well as by us indicating that abnormal patterns of blood pressure and/or heart rate are associated with an

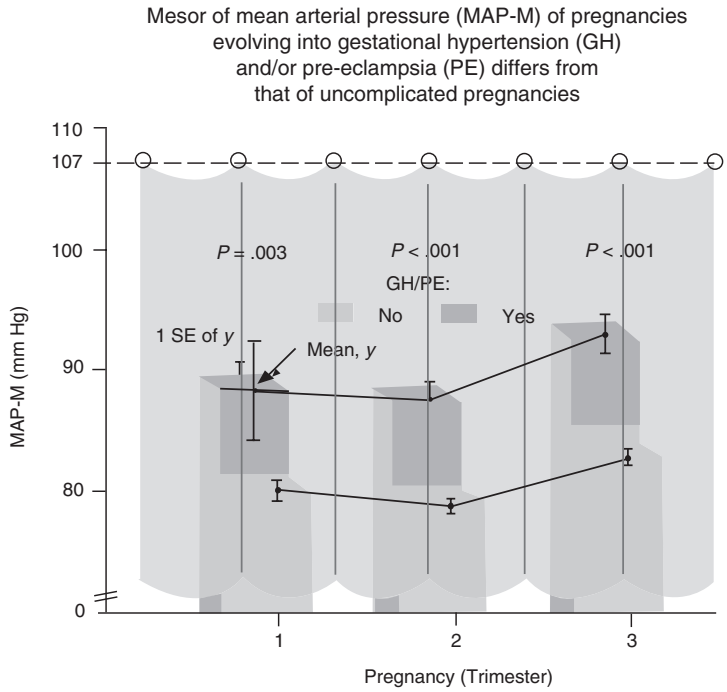


Figure 11.16. Separation on a group basis of the MESOR of mean arterial pressure (MAP) between clinically healthy women at the outset who will develop gestational hypertension and/or preeclampsia ($N = 9$, 21, and 26 in the first, second, and third trimesters, respectively) from those whose pregnancy will remain uncomplicated ($N = 60$, 123, and 88 in the first, second, and third trimesters, respectively). The separation in MAP MESOR between the two groups occurs below a cut off of acceptability at 107 mm Hg. The difference in MAP MESOR exceeds 8 mm Hg on the average between the two groups in all three trimesters. This difference can be detected with statistical significance already during the first trimester of pregnancy, when casual measurements usually fail to recognize a problem (not shown).

increase in vascular disease risk independently of the risk associated with an elevated blood pressure. Risks associated with an excessive pulse pressure [84] and those associated with a decreased heart rate variability [85, 86] have long been recognized and have been confirmed in a 6-year prospective study [87–92] (Table 11.2). An excessive circadian amplitude of blood pressure, a condition known as CHAT (brief for circadian hyperamplitude tension), has also been associated with a large increase in vascular disease risk, notably cerebral ischemic events and nephropathy, even in otherwise MESOR-normotensive patients [1, 2, 87, 91]. An odd timing of the circadian blood pressure rhythm but not of the concomitantly assessed circadian heart rate rhythm, a condition known as ecphasia, sometimes found in patients with non-insulin-dependent diabetes mellitus with autonomic nervous dysfunction [93, 94], has also been

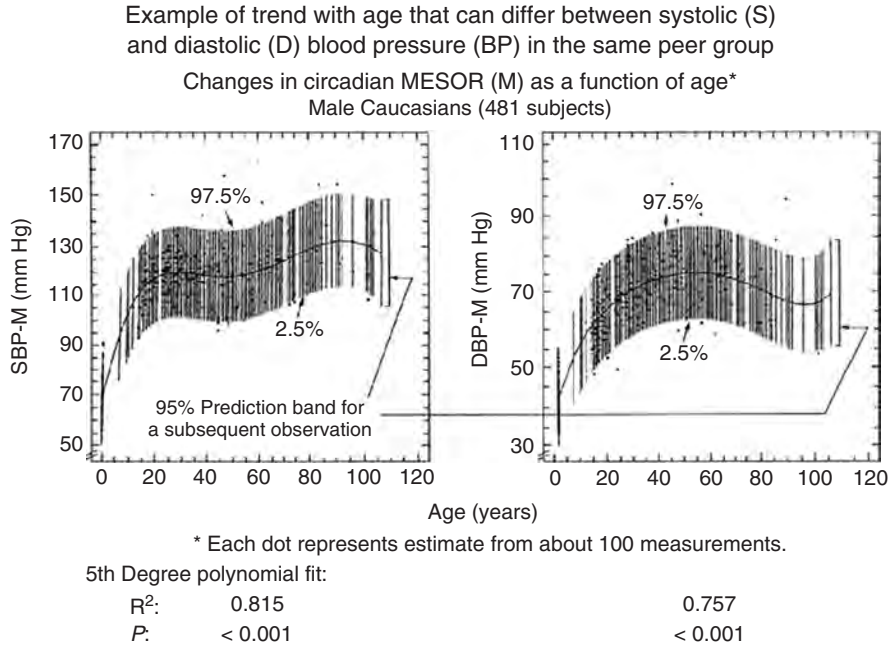


Figure 11.17. The diagnosis of blood pressure disorders could be improved by considering differences by gender (not shown here) and changes as a function of age mapped in presumably clinically healthy people, shown for the MESOR of systolic (left) and diastolic (right) blood pressure of healthy Caucasian males. Note that diastolic blood pressure reaches maximal values around 50 years of age but systolic blood pressure increases until about 80 years of age.

associated with a large increase in vascular disease risk [95–97]. Table 11.3 summarizes outcomes of chronobiological screens.

The increased vascular disease risk associated with an excessive blood pressure variability has been corroborated by others, relying on the standard deviation rather than the circadian amplitude [98, 99]. Much literature dealing with risk associated with ''nondipping'' [100], that is, an insufficient drop in blood pressure by night, another alteration of the circadian pattern, is accounted for in part by ecphasia, a reversal of the circadian blood pressure acrophase [101, 102], rather than on a reduced excursion of the circadian variation in blood pressure, notably in patients with untreated hypertension or in healthy individuals. A comparison of the risk associated with altered circadian characteristics versus a day–night ratio outside acceptable limits indicates the superiority of the chronobiological approach, whether risk is assessed prospectively in terms of actual outcomes [103]—Figure 11.19—or by the left ventricular mass index, used as a surrogate outcome measure available for all study participants [96]—Figure 11.20. Perhaps one reason why the increase in risk associated with an excessive circadian amplitude of blood

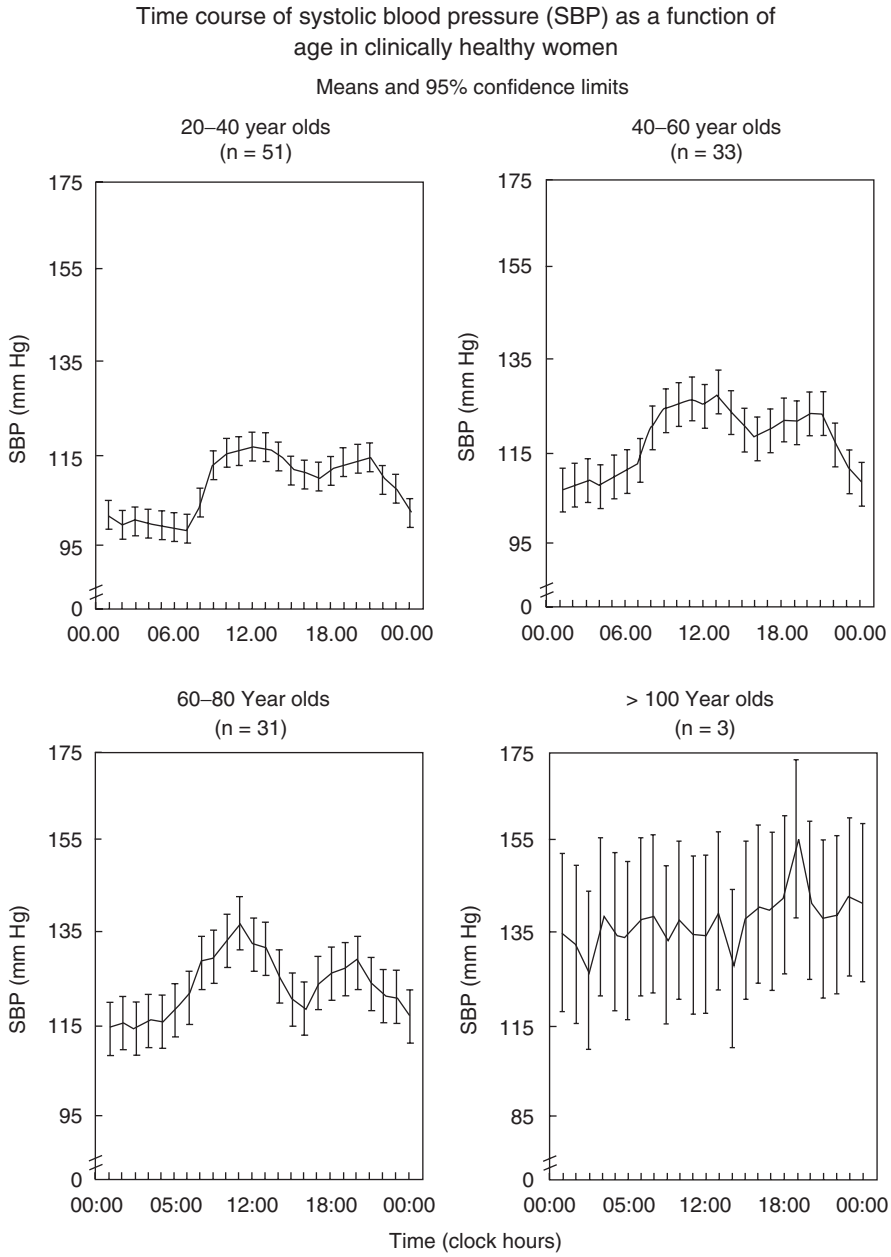


Figure 11.18. Changes in the circadian pattern of systolic blood pressure as a function of age. With increasing age, nightly values tend to increase and the postprandial dip in early afternoon becomes accentuated, at least until 80 years of age. These changes as a function of age seen in presumably clinically healthy people directly affect the day–night ratio. Using fixed values such as 10% and 20% for a classification among nondippers, dippers, and extreme dippers fails to recognize such natural changes with age.

Table 11.2. Relative Risk (RR) and 95% Confidence Interval (CI) of Diastolic Circadian Hyperamplitude Tension (D-CHAT), Decreased Heart Rate Variability (DHRV), and Excessive Pulse Pressure (EPP), Alone or in Combination[a]

Group 1: Reference (N_1) Risk?	Group 2: Test (N_2) Risk?	RR	[95%; CI]
None (214)	D-CHAT (17)	6.294	[2.108; 18.794]
None (214)	DHRV (13)	8.231	[2.847; 23.797]
None (214)	EPP (39)	8.231	[3.600; 18.819]
None (214)	D-CHAT & DHRV (2)	13.375	[2.857; 62.621]
None (214)	D-CHAT & EPP (3)	26.750	[13.554; 52.795]
None (214)	DHRV & EPP (6)	17.833	[7.364; 43.189]
None (214)	D-CHAT & DHRV & EPP (3)	26.750	[13.554; 52.795]
D-CHAT (17)	D-CHAT & DHRV (2)	*2.125*	[*0.417; 10.840*]
D-CHAT (17)	D-CHAT & EPP (3)	4.250	[1.804; 10.013]
D-CHAT (17)	D-CHAT & DHRV & EPP (3)	4.250	[1.804; 10.013]
DHRV (13)	DHRV & D-CHAT (2)	*1.625*	[*0.325; 8.113*]
DHRV (13)	DHRV & EPP (6)	*2.167*	[*0.803; 5.846*]
DHRV (13)	D-CHAT & DHRV & EPP (3)	3.250	[1.438; 7.345]
EPP (39)	EPP & D-CHAT (3)	3.250	[2.030; 5.204]
EPP (39)	EPP & DHRV (6)	2.167	[1.038; 4.523]
EPP (39)	D-CHAT & DHRV & EPP (3)	3.250	[2.030; 5.204]

D-CHAT is defined as circadian amplitude of diastolic blood pressure (BP) above the upper 95% prediction limit of clinically healthy peers matched by gender and age; DHRV is defined as 48-hour standard deviation of heart rate in the lowest 7th percentile of distribution; and EPP is defined as a pulse pressure (MESOR of systolic BP − MESOR of diastolic BP) above 60 mm Hg, where the MESOR is a chronome-adjusted mean value.

[a] Assessed in population of 297 patients, among whom 39 had a morbid event during the following 6 years. RR is computed as ratio of incidence of morbid event in Group 2 versus that of Group 1. A 95% CI not overlapping 1 indicates statistically significant increase in risk in Group 2 versus Group 1.

pressure has not been recognized earlier is that the relationship between risk and blood pressure variability is nonlinear, by contrast to the linear relationship between risk and the MESOR of blood pressure—Figures 11.21 and 11.22 [71, 103]. In most statistical analyses relying on multivariate linear regression, nonlinear associations such as those shown in Figures 11.21 and 11.22 are likely to be missed. Moreover, scholarly papers introducing spectral methods for the study of blood pressure variability [104] or following-up on the same topic include at best variability along the 24-hour scale, while components with periods covering a broad infradian range, including not only circaseptans and circannuals but also transyears and decadal variations, have already been mapped and shown to have congruent counterparts in the near or far environment [105].

11.6.4 Chronotherapy: Experimental Designs

Güllner et al. [106] documented the circadian stage-dependent action of a short-acting anti-hypertensive drug, prazosin, against the background of studies

Table 11.3. Outcomes of Chronobiological Screens of Blood Pressure and Heart Rate[a]

N of Patients (Reference)	*N* at Follow-up	Sampling	*N* Measurements: Total (Outcomes)	Finding
10 (1)	10 (up to 5 years)	5/day daily	Up to 9,125 (only partially analyzed)	Among P. Scarpelli's patients, the 4 who died with malignant hypertension had a larger circadian BP amplitude than the 6 who were still alive (SBP: $t = 1.84$; $P = 0.103$; DBP: $t = 2.99$; $P = 0.017$)
63 (2, 3)	21 after 28 years	~q4 h for 2 days	756 (252)	9 of 10 Subjects without CHAT are alive while 7 of 11 subjects with CHAT are dead 28 years later ($\chi^2 = 6.390$; $P < 0.01$).
56 (4)	56: Concomitant LVMI	q15 min for 24 h	5,376 (5,376)	Classification by Y. Kumagai of patients by LVMI (<100; 100–130; >130 g/m^2) reveals elevation of circadian amplitude at LVMI in 100–130 range whereas MESOR elevation occurs only at LVMI >130.
221 (5, 6)	221 (time of delivery)	q1 h/48 h in each trimester of pregnancy (336 profiles)	16,128 (16,128)	In addition to an 8-mm Hg difference in mean value between women who will or will not develop complications (gestational hypertension, preeclampsia) already observed during the first trimester of pregnancy, the occurrence of complications is also associated with BP profiles characterized by an elevated circadian BP amplitude. In particular, one case (JK) of CHAT where warning was not heeded, was followed 8 weeks later by severe preeclampsia, premature delivery

(*Continued*)

Table 11.3. Continued

N of Patients (Reference)	*N* at Follow-up	Sampling	*N* Measurements: Total (Outcomes)	Finding
				and 26 months of hospitalization of offspring at a cost of about $1 million.
297 (7–12)	297 after 6 years	q15 min for 48 h	57,024 (57,024)	CHAT or a reduced circadian standard deviation of heart rate, or an excessive pulse pressure (>60 mm Hg) are large risk factors (larger than hypertension) for cerebral ischemic events, nephropathy, and coronary artery disease, even when the blood pressure is within acceptable limits.
2039 (13–15)	2039 Concomitant LVMI	Hourly averages for 24 h	48,936 (48,936)	LVMI is increased in patients with CHAT, a reduced circadian standard deviation of heart rate, or an elevated pulse pressure. The relation between LVMI and the circadian endpoints is nonlinear.
23 (16)	12 after 7 years	q15 min for 9 days	19,872 (10,368)	10 of 20 Patients with no consistent BP abnormality are alive and well; 2 of 3 patients with consistent BP abnormality reported an adverse vascular event ($P = 0.015$ by Fisher's exact test).
80 (17, 18)	80: Response to treatment administered 2 h before daily BP peak	q4 h for 24 h before and on treatment	960 (960)	With smaller doses of medications, BP was lowered by R. Zaslavskaya to a larger extent and treatment was accompanied by fewer complications.

(*Continued*)

	vs. control group treated 3 times a day			Treatment: propranolol, clonidine, or α-methyldopa ($P<0.05$ for each effect)
18 (19)	18 (12 weeks)	q30 min (⩾24 h) on 3 regimens	⩾2592 (⩾2592)	Treating CHAT may prevent adverse vascular events: As compared to placebo, nifedipine (1 mg b.i.d. at 0800 h and 2000 h) increases and benidipine (4 mg/day at 0800 h) decreases the circadian amplitude of blood pressure. The resulting increase versus decrease in the incidence of CHAT on nifedipine versus benidipine may account for the corresponding difference between the number of stroke events of 7.6 versus 3.5 and the total number of cardiovascular events of 20.4 versus 8.8 per 1000 person-years.
Totals:				
2,807	2,754		160,769 (>141,636)	

[a] SBP and DBP, systolic and diastolic blood pressure; HR, heart rate; CHAT, circadian hyper amplitude tension, a condition defined by a circadian amplitude exceeding the upper 95% prediction limit of acceptability (in healthy peers matched by gender and age); LVMI, left ventricular mass index. By comparison with several classical studies, the number of measurements in chronobiological work completed thus far is likely to be larger, and confounding by intersubject variability smaller (Ref. 20).

[a]

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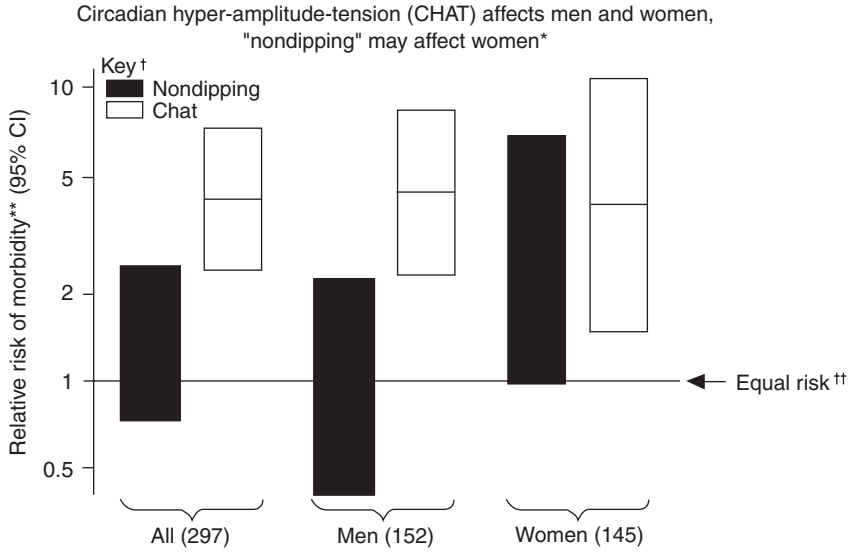
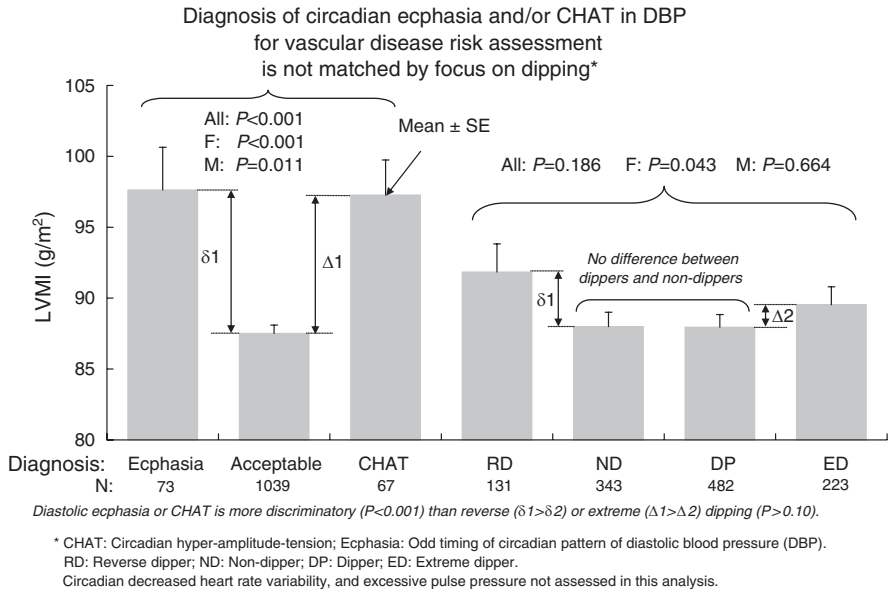


* Data from 6-year prospective study by K. Otsuka. ** Coronary artery disease, cerebral ischemic event, nephropathy and/or retinopathy.
† Diagnosis based on diastolic blood pressure (DBP); nondipping: DNR < 10%, where DHR = day / night ratio = 100% (average daytime [10:00–20:00] DBP-average nightlime [00:00-06:00] DBP) / average 24-hour DBP); CHAT: circadian amplitude (A) of DBP > 90th percentile of DBP-A of clinically healthy peers of same gender and ethnicity and similar age.
†† Incidence of morbid events equal in tested and reference populations (e.g., among dippers and non-dippers).

Figure 11.19. The relative risk (RR) of morbidity occurring within 6 years of 297 patients in Tokyo, Japan, associated with an excessive circadian amplitude of diastolic blood pressure (diastolic CHAT) is statistically significant for both men and women, as well as overall, as evidenced by the 95% confidence intervals of RR values not overlapping one (representing equal risk). By contrast, the relative risk associated with "nondipping" (day–night ratio less than 10%) is only marginally elevated, and only so for women and not for men. Data from K. Otsuka.

testing the timing of hydrochlorothiazide [107]. A follow-up approach, successful on a population basis, relied on just two groups, consisting of comparing the merits of treatment administered at the presumed optimal circadian stage, accounting for the pharmacokinetics of the chosen antihypertensive agent and for the individual's own blood pressure profile obtained prior to the start of treatment, with those of traditional treatment. As shown in Figure 11.23, in studies by Zaslavskaya, treating 1.5–2 hours prior to the time of largest blood pressure excess with propranolol, clonidine, or α-methyldopa was associated with a hypotensive effect of greater extent, achieved with a lesser dose, as compared to treatment with equal doses three times a day [108, 109]. Not shown in Figure 11.23 are additional benefits from chronotherapy, namely, an earlier response to treatment and fewer side effects such as overdosages and treatment-related complications.

Another approach attempting to determine an optimal circadian stage of treatment administration consists of following the Phase 0 design [9, 10, 33]. As shown elsewhere in this volume, this approach was successful in finding that

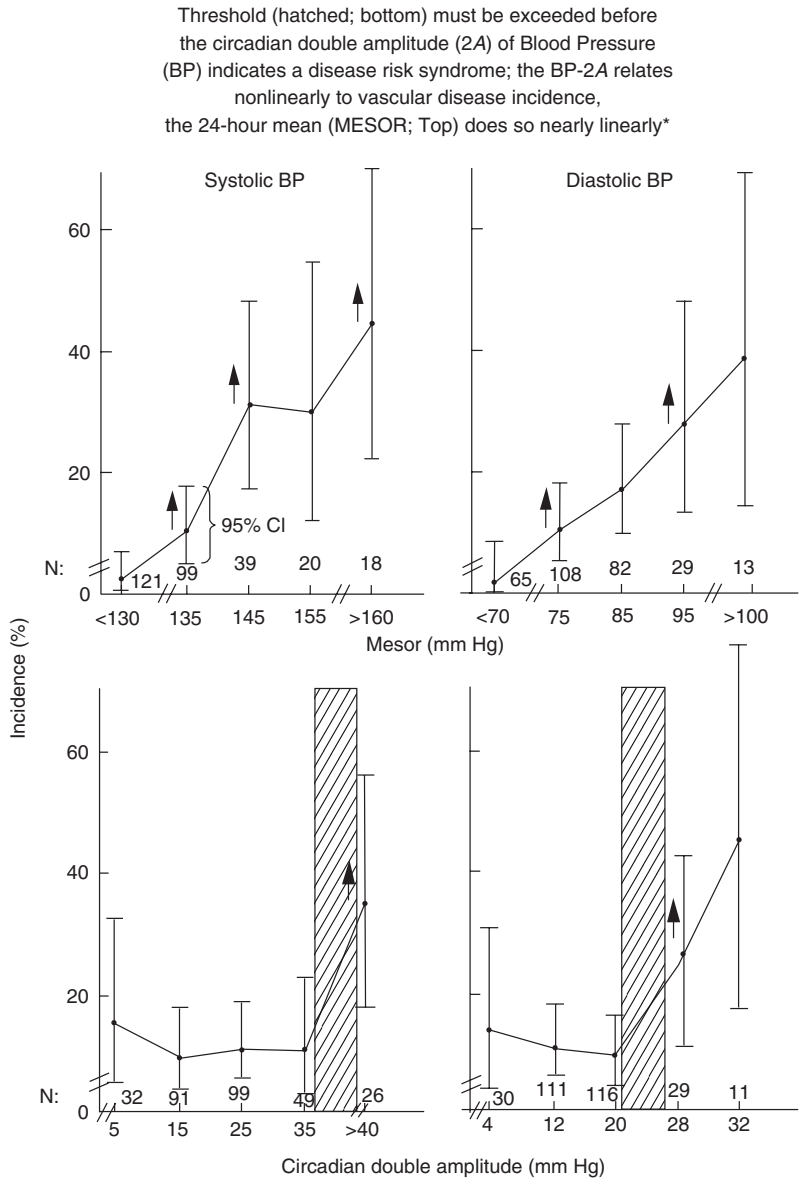


Diastolic ecphasia or CHAT is more discriminatory (P<0.001) than reverse (δ1>δ2) or extreme (Δ1>Δ2) dipping (P>0.10).

* CHAT: Circadian hyper-amplitude-tension; Ecphasia: Odd timing of circadian pattern of diastolic blood pressure (DBP).
RD: Reverse dipper; ND: Non-dipper; DP: Dipper; ED: Extreme dipper.
Circadian decreased heart rate variability, and excessive pulse pressure not assessed in this analysis.

Figure 11.20. The left ventricular mass index (LVMI), used as a surrogate outcome measure, was available from all 1179 participants. It is compared overall and separately for men (M) and women (F) by one-way analysis of variance (ANOVA) among patients classified in terms of circadian characteristics assessed by cosinor (3 columns on left) or in terms of the day–night ratio (DNR) of diastolic blood pressure (''dipping,'' 4 columns on right). LVMI values are greatly elevated when diastolic ecphasia or CHAT is diagnosed, corresponding to circadian patterns of diastolic blood pressure abnormal in terms of timing or extent of predictable change within a day. This is not the case when patients are classified as reverse dippers (DNR $<0\%$), nondippers ($0\% <$ DNR $< 10\%$), or extreme dippers (DNR $> 20\%$). Ambulatory blood pressure monitoring may serve the broader derivation of normative values in health for circadian parameters. Data from C.H. Chen.

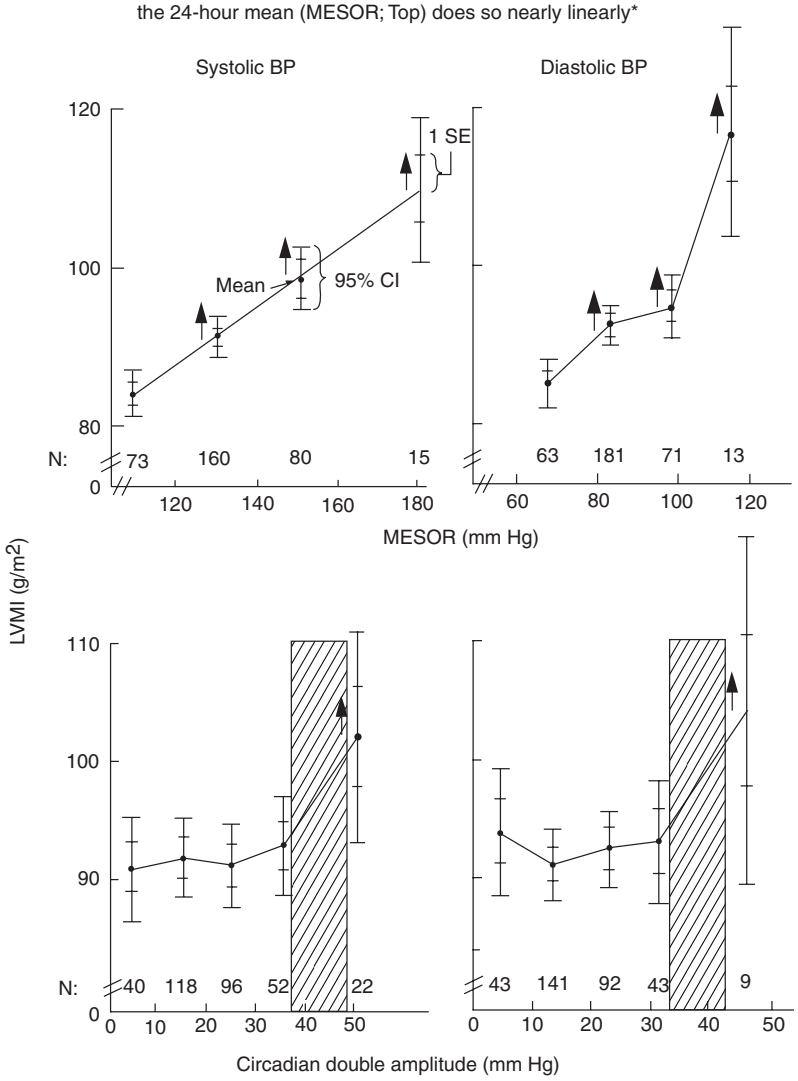
low-dose aspirin is effective as an anticlotting agent when administered after awakening, but not when taken 12 hours later [110]. In the same six subjects, low-dose aspirin was found to be more effective as a hypotensive agent when given in the middle of the activity span [111], a result corroborated independently on a larger sample [112]. This is illustrated in Figures 11.24 and 11.25. Blood pressure was measured around the clock for a total of 16 days by 6 clinically healthy young women. During the first 7 days, aspirin was taken once a day, the timing of its administration differing among the 6 subjects: one took it upon awakening, four others either 3, 6, 9, or 12 hours after awakening, and the last one at bedtime. During the next 2 days, treatment was interrupted (washout), and for the last 7 days, a placebo was administered daily at the same time aspirin was taken during the first week. Each blood pressure profile was analyzed by a cumulative sum (CUSUM) control chart to determine whether



* Statistically significant ($P < 0.05$) increase (▲) found between some consecutive MESORs (top) but only at the transition to the highest (or second highest) 2*A* from the preceding one(s) (bottom); ▨ threshold, N = number of subjects per group; incidence of coronary artery disease, ischemic stroke, nephropathy and/or retinopathy, within 6 years of a 48-hour ambulatory profile of BP in 297 patients in Tokyo, Japan. Data of K. Otsuka.

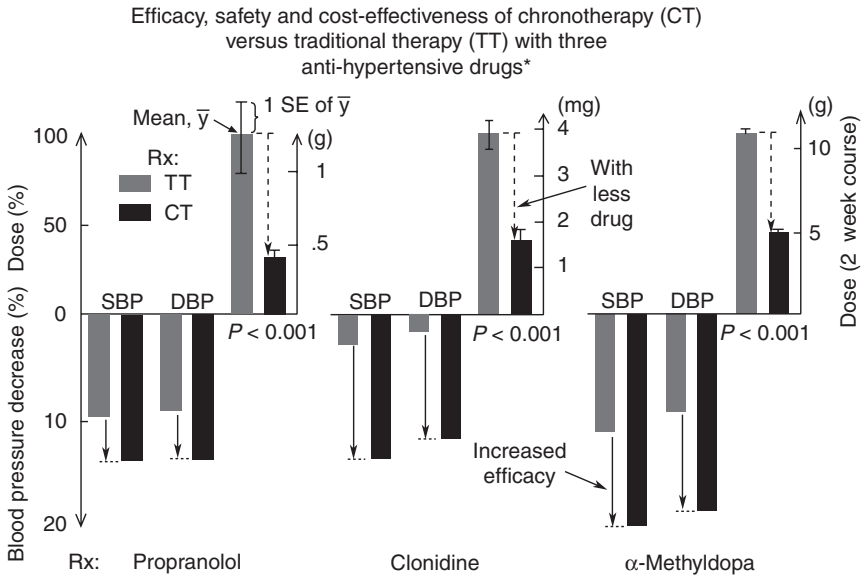
Figure 11.21. The circadian double amplitude (2*A*) of blood pressure must exceed a threshold (hatched; bottom) before cardiovascular disease risk increases. The circadian blood pressure amplitude relates nonlinearly to vascular disease incidence. By contrast, the blood pressure MESOR relates linearly to the incidence of morbid events (top). Data from K. Otsuka.

Threshold (Hatched; Bottom) must be exceeded before the circadian double amplitude (2*A*) of blood pressure (BP) indicates a disease risk syndrome; the BP-2*A* relates nonlinearly to the left ventricular mass index (LVMI), the 24-hour mean (MESOR; Top) does so nearly linearly*



* Statistically significant (P <0.01 corrected for multiple testing) increase (⇑) found between consecutive MESORs (top) but only at the transition to the highest 2A from the preceding one(s) (bottom); ▨ threshold; N = number of subjects per group; 328 untreated men and women, 30–88 years of age, in Taiwan, each providing a 24-hour ambulatory profile. Data of C-H. Chen et al.

Figure 11.22. The circadian double amplitude (2*A*) of blood pressure must exceed a threshold (hatched; bottom) before cardiovascular disease risk increases. The circadian blood pressure amplitude relates nonlinearly to the left ventricular mass index (LVMI), used as a surrogate outcome measure. By contrast, the blood pressure MESOR relates linearly to LVMI (top). Data from C. H. Chen.



* 20 patients per group; hypotensive effect more pronounced on CT than TT ($P < 0.05$)
SBP = systolic blood pressure; DBP = diastolic blood pressure.

Figure 11.23. As compared to treatment with equal doses 3 times a day, treating 1.5–2 hours prior to the time of largest blood pressure excess with propranolol, clonidine, or α-methyldopa is associated with a hypotensive effect of greater extent from smaller doses of the drug. Not shown is the finding that the effect occurs sooner and leads to fewer overdosages and to fewer complications. Data from R. Zaslavskaya.

aspirin was associated with a statistically significant change in blood pressure [19]. A CUSUM consists of two lines, one indicating an increase in mean, the other a decrease in mean. As long as the two curves remain within a given decision interval, any change in blood pressure remains "in control" (no statistically significant change). But once one of the two curves breaks outside the decision interval, it indicates a statistically significant increase (if the positive CUSUM curve breaks above the upper limit of the decision interval) or decrease (if the negative CUSUM curve breaks below the lower limit of the decision interval) in blood pressure.

Figure 11.24 indicates an increase in blood pressure after switching from aspirin to placebo 6 or 9 hours after awakening, but not at other circadian stages. When the response is assigned to the circadian stage of treatment administration, pooling results from all 6 subjects, the circadian stagedependence of the effect becomes apparent (Figure 11.25), as subjects were randomly assigned to a circadian stage of treatment. Parenthetically, it has been reported that aspirin, albeit at a higher dosage, may be better tolerated in the evening [113], yet this report has been later disputed [114, 115]. The different optimal circadian stage of aspirin administration, after awakening for its anticlotting

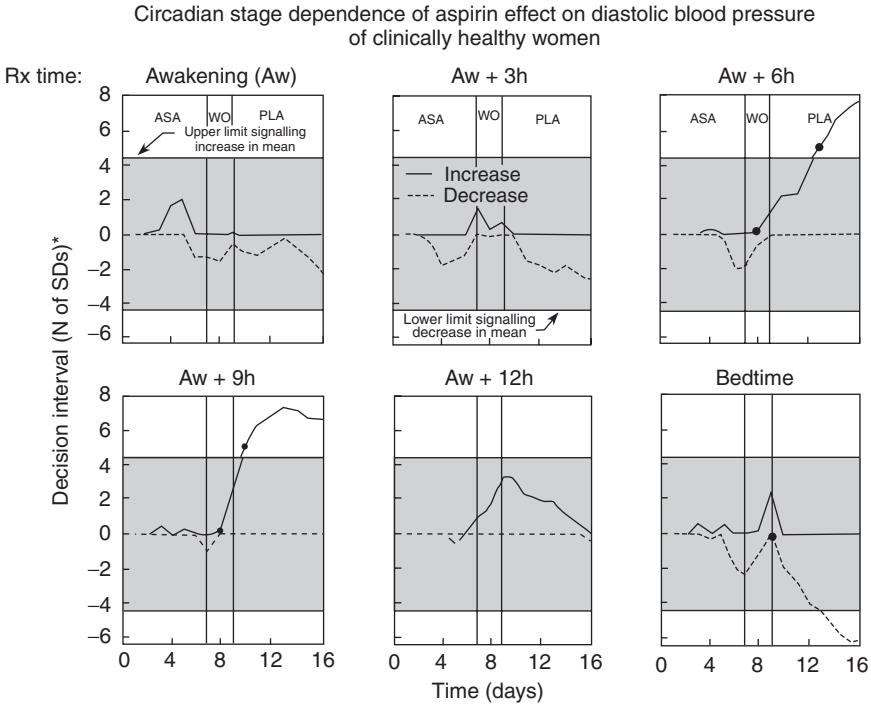


Figure 11.24. In an *N*-of-6 pilot study, 6 clinically healthy women took low-dose aspirin (100 mg per day) for 1 week, and after a 2-day wash-out were switched to another week of placebo. Each woman was treated at a different circadian stage, either upon awakening, 3, 6, 9, 12 hours after awakening, or at bedtime. The treatment time was kept the same for a given subject. Blood pressure was measured around the clock throughout the study. Changes in the MESOR of blood pressure were assessed by means of a self-starting cumulative sum control chart. While the series of daily MESORs of diastolic blood pressure is proceeding ''in control'' (i.e., before the switch from aspirin to placebo), the cumulative sum (CUSUM) comprises two line graphs that generally stay within the limits of the ''decision interval'' (shaded area). The two curves signal increase and decrease in mean (in this case daily diastolic blood pressure MESORs), respectively. When one curve breaks out of the (shaded) decision interval boundary, it provides the rigorous validation of the change (increase or decrease) in DBP MESOR. The time at which the MESOR changed is estimated by tracking the line segment leading to the breakout back to the last occasion on which it lay on the horizontal axis. An increase in DBP MESOR is observed only when aspirin was replaced by placebo 6 or 9 hours after awakening, and treatment at bedtime has the opposite effect. Treatment at other times was not associated with any statistically significant change in diastolic blood pressure. Data from P. Prikryl.

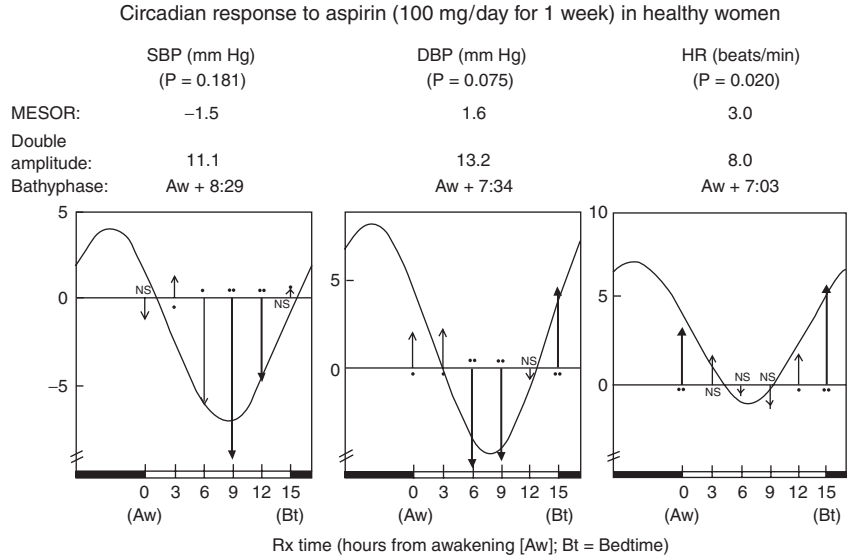


Figure 11.25. Because women in Figure 11.24 were randomly assigned to one of six different treatment times, the response to treatment can be allocated to the timing of its administration. The least squares fit of a 24-hour cosine curve then tests whether the response to treatment was circadian stage dependent. For diastolic blood pressure, the circadian stage dependence of low-dose aspirin treatment reaches borderline statistical significance ($P = 0.075$), the largest effect being observed for aspirin taken in the afternoon, in keeping with independent results on a larger population. Data from P. Prikryl.

properties, midactivity span for its hypotensive effect, or possibly, still to be clarified, before bedtime to reduce its side effects, are a reminder of the need to triangulate among different effects, with consideration for the given patient's condition and susceptibility.

A similar protocol was followed to determine the optimal circadian stage of administration of Micardis (Telmisartan) [116], except that in this study patients served as their own longitudinal control, the time of treatment administration being changed at intervals.

One problem related to this design when it is applied longitudinally rather than in a group is the choice of an interval during which treatment is taken at a given time. In the Micardis study, the time of treatment administration was rotated by 3 hours every day. This may be too rapid, the first treatment time tending to be associated with a larger response, as it also corresponds to the patient's first response to the drug. One way to circumvent this problem is to repeat the rotation around all test times several times. Another consists of starting with different treatment times for different patients and of assessing effects on a group basis rather than for the individual patient. Considering a

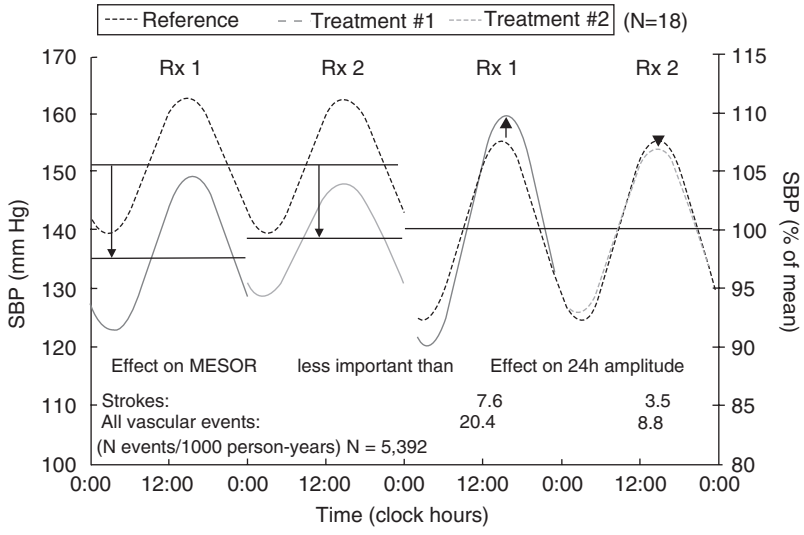
longer interval before changing the treatment time also presents some problem as it lengthens the duration of study, during which trends related to infradian components such as the circannual rhythm may complicate the interpretation of the results that can then no longer be attributed primarily to the response to treatment. Changing treatment times at intervals of 1 to 2 weeks seems preferable, and has been done for an antihypertensive agent [105] and for a nutriceutical with an effect on blood pressure [117].

11.6.5 Chronotherapy: Restoration of an Acceptable Blood Pressure Pattern

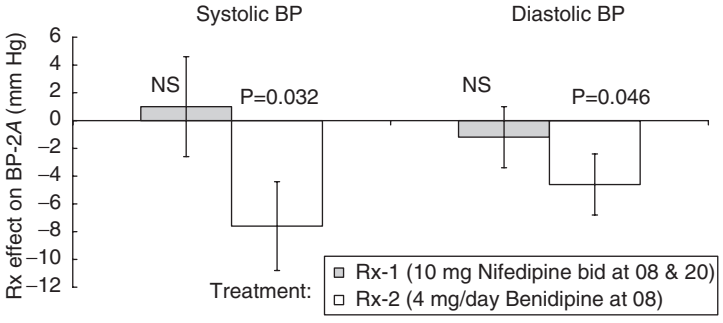
Much emphasis is placed today on lowering an elevated blood pressure, but not much attention is given to restoring an altered circadian variation in blood pressure (and/or heart rate). There is evidence, however, that the reduction of an excessive circadian amplitude of blood pressure to eliminate CHAT is associated with a reduction in adverse cardiovascular outcomes. Large clinical Asian trials [118, 119] indicated that treatment with benidipine in the morning was more beneficial than treatment twice a day (in the morning and evening) with nifedipine. A crossover randomized study comparing both treatments on patients followed by ABPM indicated that treatment with nifedipine was associated with a slight increase in the circadian amplitude of blood pressure. By contrast, benidipine treatment was associated with a reduction of the circadian amplitude of blood pressure (Figure 11.26), and hence with a reduction in the incidence of CHAT [120]. Of further interest is the observation that even though nifedipine lowered the average value of blood pressure more than benidipine, the latter halved undesirable events, in keeping with its reducing the circadian amplitude of blood pressure.

That the timing of treatment administration was relevant rather than the choice of the anti hypertensive agent was further demonstrated in *N*-of-1 studies, that repeatedly showed the presence of a larger circadian amplitude of blood pressure when the same dose of the same drug was taken in the evening as compared to the morning [120]. This is illustrated, for instance, in

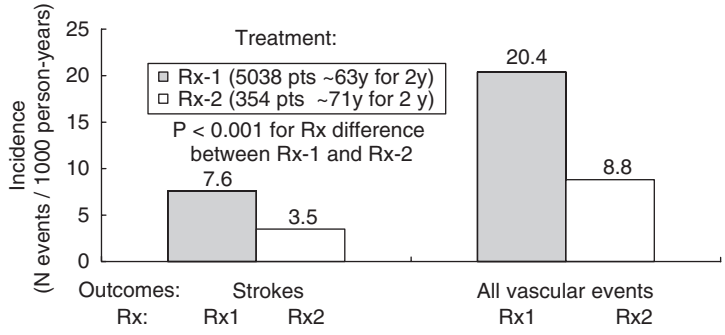
Figure 11.26. (a) A crossover study investigates the effect of two different treatments on the circadian pattern of blood pressure. Whereas nifedipine (taken twice a day, in the morning and in the evening, Rx1) is associated with a slightly more pronounced decrease in the MESOR of systolic blood pressure as compared to benidipine (taken once a day upon awakening, Rx2), it is also associated with a numerical increase rather than with a decrease in the circadian amplitude of systolic blood pressure. For patients with a circadian blood pressure amplitude close to the upper limit of acceptability, taking Rx1 or Rx2 may make the difference between iatrogenic CHAT or reducing the amplitude well within the range of acceptable values. Data from K. Otsuka. (b) Rx2 was found in large Asian clinical trials to be associated with better outcomes than Rx1. Reducing the incidence of CHAT may be the reason accounting for the difference (almost by a factor of 2) in outcomes, whether strokes or all cardiovascular events are considered.



Does treating CHAT reduce morbidity?
(18 Patients in double blind placebo controlled study)
(M Shinagawa et al. Biomed pharmacother 2002; 55: 125–132)



Outcomes of long-acting calcium antagonists trials in Japan



CHAT: Circadian hyper-amplitude-tension, condition defined by circadian double amplitude (2*A*) of blood pressure (BP) above 95% prediction limit of healthy peers matched by gender and age.
Outcomes of 2-year calcium antagonist trials on 5392 patients.
Over 50% reduction of strokes (left) and of all severe vascular events (right) by treatment (Rx) that reduces (Rx2, white bars) vs. one that does not reduce (Rx1, black bars) BP-2*A*. Rx1 vs Rx2 comparison: $P < 0.001$.

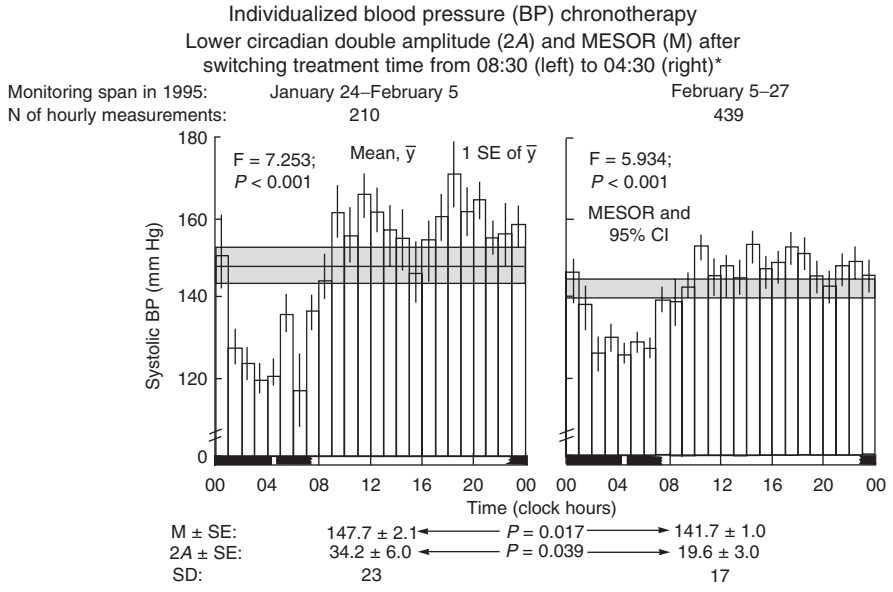


Figure 11.27. Techniques are available to test on an individualized basis the efficacy of treatment. One such method consists of testing the equality of rhythm parameters before and after the start of treatment. In the case of this 75-year-old man, the same dose (240 mg) of the same drug (Diltiazem HCl) was taken either upon awakening (left) or during an interruption of sleep (right). The change in timing of medication was associated with both a further decrease in the MESOR of systolic blood pressure and with a decrease in the circadian amplitude of this variable. Timing treatment (chronotherapy) can hence be useful to treat CHAT as well as MESOR-hypertension.

Figure 11.27. Not only is the MESOR of systolic blood pressure lowered more when the same dose (240 mg) of the same drug (Diltiazem HCl) is taken around 0430h than around 0830h, treatment at 0430h is also associated with a statistically significant decrease in the circadian amplitude of blood pressure [4], as ascertained by parameter tests, another approach applicable to assessing the response to treatment for the individual patient.

Clinical studies have also shown that not all antihypertensive agents act similarly on the circadian amplitude of blood pressure. For instance, long-acting carteolol is capable of lowering the circadian amplitude of blood pressure in most subjects, but captopril Retard is not (Figure 11.28) [121]. This differential effect of different antihypertensive agents in their action on the circadian amplitude of blood pressure was already noted in 1991 [122].

11.6.6 Treating an Elevated Risk: Primary Prevention

Blood pressure variability disorders such as CHAT can occur in otherwise MESOR-normotensive individuals who may not need antihypertensive

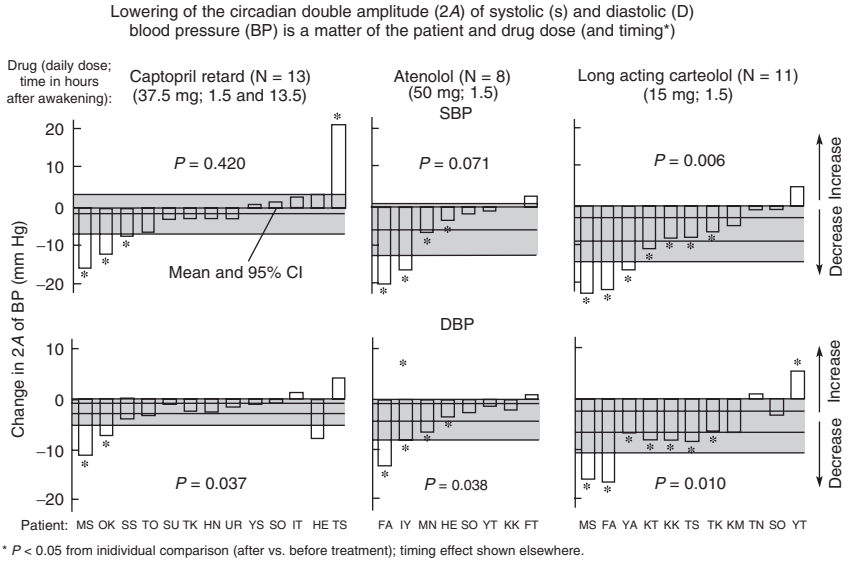


Figure 11.28. Whereas anti-hypertensive drugs are administered to decrease an elevated blood pressure mean, their effect on the circadian pattern of blood pressure, often ignored, can be very different from one drug to another. For instance, on the average, long-acting carteolol but not captopril can reduce the circadian amplitude of blood pressure of most patients participating in the study. This difference should be taken into consideration when CHAT is diagnosed. Data from Y. Watanabe.

medication. Even in MESOR-normotension, CHAT is associated with a large increase in vascular disease risk, however [1, 2]. The diagnosis of CHAT should thus prompt the institution of primary prevention before there is target organ damage.

When there is no need for a pharmacologic intervention, several options are available for the treatment of CHAT. One consists of using relaxation techniques such as autogenic training [121, 123, 124]. Another promising avenue of research relates to the use of ubiquinone (CoQ10), a powerful antioxidant and an integral component of the mitochondrial respiratory chain for energy production [125]. It is found in all tissues and organs of the body, with highest concentrations in the heart. Blood and tissue concentrations of CoQ10 are reportedly reduced with advancing age and in the presence of cardiovascular disease [126]. A recent meta-analysis of 12 clinical trials (362 patients) assessing the efficacy of CoQ10 in reducing an elevated blood pressure concluded that this nutriceutical has the potential of lowering systolic blood pressure by up to 17 mm Hg and diastolic blood pressure by up to 10 mm Hg without marked side effects [127].

In an *N*-of-1 study, a clinically healthy woman, 55 years of age, monitored her blood pressure around the clock for several months prior to the start

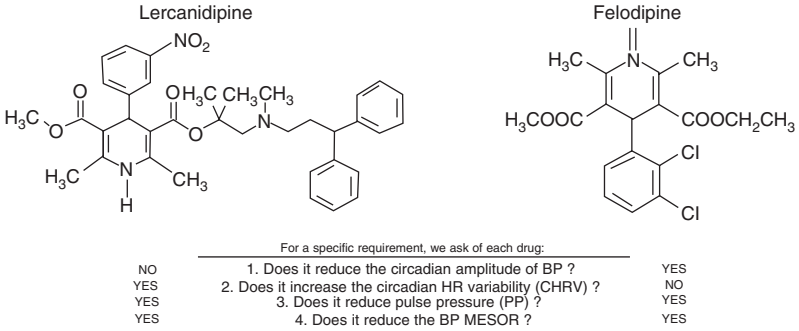
of CoQ10 softgels supplementation (Tishcon Corporation) in daily doses of 100 mg. During the first week, CoQ10 was taken upon awakening, during weeks 2–5, it was taken 3.5, 7, 10.5, and 14 hours after awakening, and during week 6, it was taken 17.5 hours after awakening (corresponding to bedtime). The last 6 weeks prior to the start of treatment were used as reference. A circadian rhythm was invariably demonstrated for systolic and diastolic blood pressure during each of these 12 weeks ($P<0.001$). As compared to the reference span, CoQ10 was associated with a reduction of the circadian amplitude of both systolic ($P<0.001$) and diastolic ($P<0.001$) blood pressure, the effect being circadian stagedependent (SBP: $P=0.043$; DBP: $P=0.012$). The largest reduction in circadian amplitude was associated with CoQ10 supplementation in the evening (around 14 hours after awakening) [117]. Notably in the absence of MESOR-hypertension, CoQ10 supplementation may be preferred to antihypertensive medication for the treatment of CHAT.

11.6.7 Chronotheranostics

Rhythms need to be assessed individually. The most opportune time to administer treatment may differ drastically from one patient to another. A patient diagnosed with CHAT is likely to have blood pressure excess primarily in the afternoon, whereas a ''nondipper,'' or rather a patient with ecphasia, is likely to have blood pressure excess primarily by night, even when their blood pressure MESOR, percentage time elevation, and hyperbaric indices are similar. Model fitting to fractionated indices of excess, that is, hyperbaric indices computed not for the entire 24-hour span but for consecutive intervals of 1 to 3 hours, can determine the time of highest excess [66], an approach underlying the results illustrated in Figure 11.23 [108, 109].

Individualization of treatment includes the consideration of a given drug's effects on the variability of blood pressure and heart rate, in addition to its blood pressure lowering effect. Any differential effects of antihypertensive agents should be targeted to the patient's chronodiagnosis. Figure 11.29 summarizes results from a study comparing the effect of lercanidipine and felodipine on Chinese patients with primary hypertension [128]. Both drugs lower the blood pressure MESOR and reduce the pulse pressure, but only lercanidipine increases the standard deviation of heart rate, while felodipine but not lercanidipine may decrease the circadian amplitude of blood pressure. Lercanidipine may thus be the preferred treatment for patients with a decreased heart rate variability, whereas felodipine may be the preferred treatment for patients with CHAT [2].

For the treatment of risk elevation (prehabilitation) [129], drugs available to restore acceptable patterns of blood pressure and heart rate, thereby eliminating variability disorders, should be chosen as a function of the chronodiagnosis specifying the kind of abnormality encountered. As a first step, Figure 11.29 shows the kinds of questions to be raised and how they may be answered with specific molecules (as no more than illustrative examples). The individualized



*Individualized by 1. kind of chronome alteration detected by a chronodiagnosis that recognizes risk elevation as well as disease (against a gender- and age-qualified standard), 2. kind of drug, i.a., with respect to the desired effect on variabilities of BP and/or HR, and 3. multiple considerations also of timing. For instance, decisions regarding drug choice can be based on a chronodiagnosis using felodipine in the presence of CHAT (circadian hyper-amplitude-tension) (1), with MESOR-hypertension (2) and/or an elevated PP, but not with a decreased CHRV; or using lercanidipine in the presence of a deficit in CHRV, with MESOR-hypertension and/or an elevated PP, but not with CHAT. In each case, individualization is further desirable in relation to the timing of any BP or HR alteration, including the timing of blood pressure excess (1,2). Evidence thus far for the above drugs is available only in MESOR-hypertension. In MESOR-normotension, non-drug, approaches are indicated first, and the action in MESOR-normotension remains to be explored.

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2. Cornélissen G, Delmore P, Halberg F. Healthwatch 3. Why 7-day blood pressure monitoring: What everyone should know about blood pressure. Minneapoits: Halberg Chronobiology Center, University of Minnesota; 2003. 31 pp.

Figure 11.29. Differential effects of lercanidipine and felodipine on the variabilities of blood pressure and heart rate may be used for the individualization of treatment in the light of a chronodiagnosis established on the basis of around-the-clock monitoring of blood pressure and heart rate, analyzed chronobiologically, with results interpreted in the light of time-specified reference values of gender- and age-matched healthy peers. Data from B. Tomlinson and B. Fok.

therapy of risk elevation will eventually have to become an as-one-goes bootstrap operation. It is facilitated by the longitudinal monitoring of blood pressure and heart rate as marker variables useful both for the chronodiagnosis and as a guide for chronotherapy also aimed at restoring altered time structures [97, 105]. A dividend from longitudinal monitoring for self surveillance is the continued checking of the patient's response to treatment, so that any needed adjustment is immediately identified and acted upon, notably since blood pressure disorders are mostly asymptomatic. The aim is to prevent silent incipient target organ damage, such as an elevation of the left ventricular mass index, which was shown to be associated with an increase in the circadian amplitude of blood pressure possibly leading to CHAT, before there is a clear elevation of the blood pressure MESOR [130] (Figure 11.30). In keeping with this result are studies showing the higher prevalence of CHAT among patients with borderline hypertension by comparison with MESOR-hypertensive or MESOR-normotensive individuals [131].

With accumulating databases documenting the great day-to-day variability in blood pressure not just in isolated cases but in most individuals who have

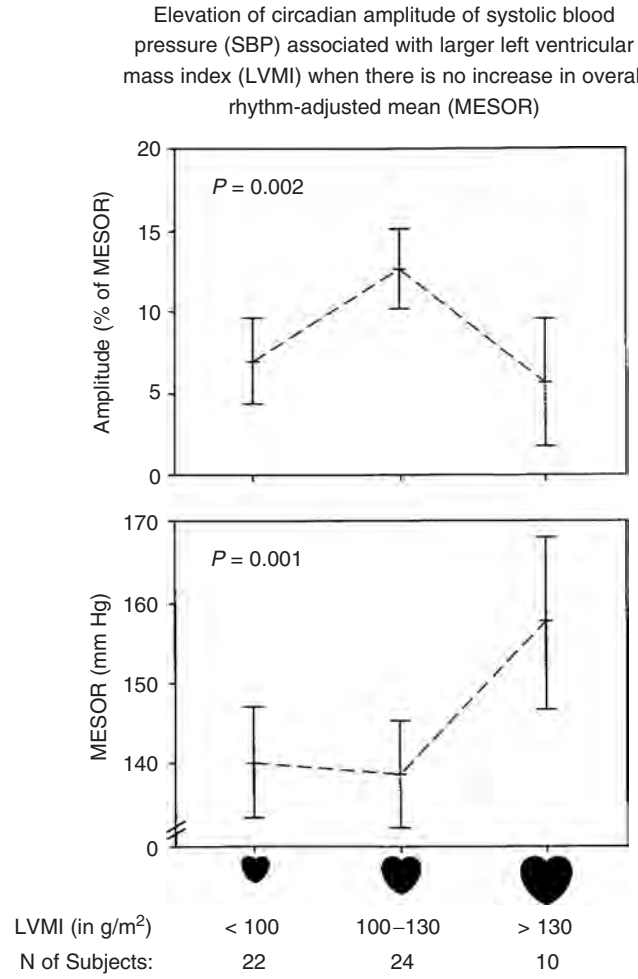

Figure 11.30. When 56 untreated subjects newly diagnosed conventionally as hypertensive are classified in terms of their left ventricular mass index (LVMI), differences are observed in terms of the MESOR and circadian amplitude of blood pressure automatically measured around-the-clock every 15 minutes for 24 hours with an ambulatory monitor. Results shown here for systolic blood pressure are similar for mean arterial and diastolic blood pressure. A transient elevation of the circadian blood pressure amplitude is observed for subjects with intermediate LVMI values, while an elevated blood pressure MESOR is only observed for subjects with the highest LVMI values. Corroborating this finding are results from subsequent studies indicating that CHAT was more frequently observed among patients with borderline hypertension than among MESOR-hypertensive patients or among MESOR-normotensive subjects. Data from Y. Kumagai.

monitored for months, years, and even decades, the wisdom of current treatment modalities that most of the time "fly blind" [132] may also come under new scrutiny. Since blood pressure is so highly variable, should the treatment remain the same every day, in keeping with emphasis now placed on the development of 24-hour formulations? Or should the pharmaceutical industry turn to biosensors that would enable them to close the loop? An implanted blood pressure sensor linked to a drug delivery device capable of programmed patterned delivery of fast-acting antihypertensive medication by means of telemetry relaying the information from the sensor to the pump may be within reach, at least for high-risk patients already requiring the use of an implanted device such as a pacemaker or a defibrillator.

11.7 CONCLUSION

The idea of adjusting the treatment to individual needs emerged a few years ago from advances in molecular biology [133]. The profile of metabolites present in urine before drugs are administered may also help identify whether a patient is a good candidate for a drug. Based on studies in the experimental laboratory, Clayton et al. [134] report that predose metabolic profiles can predict how an individual might respond to a particular drug, a technique dubbed "pharmacometabonomics." Unlike pharmacogenomics [135], pharmacometabonomics includes environmental as well as genetic factors. It has also been suggested that the use of biomarkers for personalized medicine can help reduce drug risks [136], Others have pointed to the danger of relying on averages that can hide individual differences in clinical trials [137]. As an example, these authors cite results from the ATLANTIS B trial that looked at the outcome of stroke patients treated 3 – 5 hours after the onset of symptoms. Whereas collective results showed no difference between t-PA treatment or placebo, benefit from t-PA was found among the one-third of patients who had the least risk of hemorrhage.

Notwithstanding the merits of such recent advances, one critical element remains missing from the aforementioned approaches, namely, the ubiquitous, broad time structures that have repeatedly been shown to make the difference between life and death in the experimental laboratory [8] or between the success or failure of a given treatment in the clinic [23], The transition from conventional treatment, if not from theranostics, to chronotheranostics is facilitated by the development of several technologies for monitoring health status, screening not only for disease conditions but also for risk elevation, for timely treatment scheduled according to bodily rhythms, and for the continued surveillance of the patient's response to treatment:

- Availability of portable, personal, long-term ambulatory monitors of biological variables, such as blood pressure, heart rate, the ECG and EEG, gastric acidity, core temperature and motor activity
- Availability of database systems to acquire and analyze volumes of data

- Availability of statistical procedures to analyze and model the data to devise dosage time patterns optimized for each individual patient
- Availability of portable, programmed devices to administer treatment (e.g., pacemakers, defibrillators, drug pumps)
- A chronobiologic understanding of the health effects of photic and nonphotic cycles

All these technologies could lead to marker rhythms-guided chronotherapy adjusted for the chronodiagnosis of each individual patient.

Table 11.4. Beginnings of Chronopharmacology and Chronotherapy

Year	Description	Author(s)
1952, 1953	2800-fold increase in sensitivity of a corticosteroid assay by accounting for circadian stage	Halberg (1, 2)
1955	Circadian susceptibility rhythm to noise	Halberg, Bittner, Gully, Albrecht, Brackney (3)
1955	Circadian susceptibility rhythm to an endotoxin	Halberg, Spink, Albrecht, and Gully (4)
1958	Detection of (growth) hormone effect on mitoses depends on circadian stage	Litman, Halberg, Ellis, and Bittner (5)
1959	Effect of ethanol depends on circadian stage	Haus, Hanton, and Halberg (6)
1959	Circadian susceptibility rhythm to a drug (ouabain)	Halberg and Stephens (7)
1960	LD_{50} to whole body X-ray irradiation depends on circadian stage	Halberg (8, discussion)
1961	Circadian susceptibility rhythm to Librium	Marte and Halberg (9)
1963	Circadian susceptibility rhythm to acetylcholine	Jones, Haus, and Halberg (10)
1964	Circadian susceptibility rhythm to fluothane	Matthews, Marte, and Halberg (11)
1969	Circadian susceptibility rhythm to penicillin	Reinberg Zagula-Mally, Ghata, and Halberg (12)
1970, 1972	Circadian susceptibility rhythm to arabinosyl cytosine	Cardoso Scheving, and Halberg (13), Haus et al. (14)
1973	Chronotherapy with hydrochlorothiazide and adriamycin	Halberg et al. (15)
1973	Marker rhythmometry introduced for hydrochlorothiazide chronotherapy	Levine Thampson, Shiotsuka, Krzanowski and Halberg (16)

(*Continued*)

Table 11.4. Continued

Year	Description	Author(s)
1974	Formulation of rules of chronopharmacology and chronotherapy	Halberg (17)
1977	Doubling of 2-year survival by timing radiotherapy	Halberg (18)
1979	Ara-C chronotherapy-related cancer cures	Halberg, Nelson, Cornélissen, Haus, Scheving, and Good (19)

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Some milestones in the development of chronopharmacology and chronotherapy are listed in Table 11.4. Already in 1952, it became apparent that the use of timing in a bioassay accounting for rhythms allowed a 2800-fold increase in sensitivity [138], a finding pertinent to drug development. Also, over half a century ago, a small electrical device, cobbled together with spare parts according to a diagram for an electronic metronome borrowed from a popular magazine, kept an infant heart patient alive, by pacing the basic rhythm of the heart. This chronotheranostic ("chrono" since it restored a rhythm) intervention (Bakken, 1999; [139–141] led to current implantable devices, useful in disease. The challenge of the next generation of devices is not only to close the loop between diagnosis and treatment, but to do so by prophylactic intervention in the presence of a heightened risk, before the onset of overt disease.

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