Relevance for Chronopharmacology in Practical Medicine

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Nearly all functions of the body, including those influencing pharmacokinetic parameters, such as drug absorption and distribution, drug metabolism, and renal elimination display significant daily variations. Also, the onset and symptoms of diseases such as asthma attacks, coronary infarction, angina pectoris, stroke, and ventricular tachycardia are circadian-phase dependent. Asthma attacks predominatly occur around 4 o'clock at night. Blood pressure and heart rate in normotensives and essential (primary) hypertensive patients display highest values during daytime followed by a nightly drop and an early morning rise. In about 70% of forms of secondary hypertension, however, this rhythmic pattern is abolished or even reversed exhibiting nightly peaks in blood pressure. Similar findings were obtained in children. This form of hypertension is accompanied by increased end organ damages. These observations call for a circadian time-specified drug treatment. In nocturnal asthma unequal dosing of antiasthmatic drugs with a higher/single evening dose is recommended. In secondary hypertension not only the elevated blood pressure must be reduced but the disturbed blood pressure profile should be normalized, too, possibly best achieved by evening dosing. Pharmacokinetics may also not be constant within 24 hours of a day as shown for cardiovascular active drugs, antiasthmatics, anticancer drugs, psychotropics, anlagesics and local anesthetics, antibiotics to mention but a few. Far more drugs were shown to display significant daily variations in their effects even after chronic application or constant infusion. Because circadian rhythms undergo maturation with development, drug therapy in children can/may also be modified by circadian time of drug dosing as shown for anticancer drugs. In conclusion, there is clear evidence that the dose/concentration-response relationship of drugs can be significantly dependent on the time of day. Thus, circadian time has to be taken into account as an important variable influencing a drug's pharmacokinetics and/or its effects or side effects.

Circadian rhythms have been documented throughout the plant and animal kingdom at every level of eukariotic organization. Circadian rhythms (the about-24-hour rhythm; circa = about, dies = day,[1]) by definition are endogenous in nature, driven by oscillators or clocks[2] and persist under free-running conditions. In various species (*Drosophila nelanogaster, Neurospora crassa,* Mouse, Golden hamster) even the genes controlling circadian rhythms have been identified (genes: per, frq, clock, tau).[3] In 1971, Konopka and Benzer[4] were then able to identify on the X chromosome of *Drosophila* a region that controlled the period in the eclosion rhythm of 3 mutants (per clock gene). In 1984, Bargiello et al[5] could even show that a fragment of the per gene injected into embryos of an arrhythmic mutant of *Drosophila* could restore rhythmicity in eclosion. This data provided first evidence that the biological clock is not a fiction but is genetically determined and can even be transplanted from one animal into the other thereby inducing the rhythmicity of the donor into the recipient. Recently, the precise feedback regulation of the clock gene in the fruit fly has been unraveled.[6-8]

In general, the endogenous clock in man does not exactly runs at a frequency of 24 hours but somewhat slower. The rhythm in human body temperature which is timed by the biological clock has an about 25-hour period under free-running conditions, ie, without environmental time-cues or Zeitgebers (eg, light, tem-

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perature). Zeitgebers[9] entrain the circadian rhythm to a precise 24-hour period. Zeitgebers are, therefore, necessary to entrain a living subject to a "normal" period of 24 hours! Thus, rhythmicity inherent to all living systems allows them to adept more easily and to better survive under changing environmental conditions during the 24 hours of a day as well as during varying conditions of the changing seasons.

In experimental animal and in man, however, most rhythmic fluctuations were not or even cannot be studied under free-running conditions, leaving the answer open to what a degree they are really "circadian." Purely exogenous rhythms are better termed as "24-hour" or "daily" rhythms. Thus, an overt 24-hour rhythm in a given parameter can be endogenous or predominantly exogenous in nature. Within the literature published, however, the term "circadian" is not always used in the above mentioned restricted sense, the broader term will be used here, too.

Chronobiological Background

It is a common paradigm in clinical pharmacology that pharmacokinetic parameters are considered not to be influenced by the time of day of drug administration. Concerning drug concentrations-versus-time profiles "the flatter the better" is also a common aim in drug targeting. However, there is convincing evidence that this paradigm cannot hold any longer. The reason is that it is now well established that nearly all functions of the body, including those influencing pharmacokinetic parameters, display significant daily variations. Circadian or 24-hour rhythms exist in heart rate, body temperature, blood pressure, blood flow, stroke volume, peripheral resistance, parameters of electrocardiogram (ECG) recordings, in the plasma concentrations of hormones, neurotransmitters and second messengers (eg, cortisol, melatonin, insulin, prolactin, atrial natriuretic hormone, noradrenaline, cAMP [cyclic adenosine monophosphate]), in the renin-angiotensin-aldosterone-system, in blood viscosity, aggregability and fibrinolytic activity, in the plasma concentrations of glucose, electrolytes, plasma proteins, enzymes, in the number of circulating red and white blood cells and blood platelets. Also gastric acid secretion, gastro-intestinal motility, gastric emptying time and GI-tract perfusion exhibit pronounced circadian variation. Moreover, various functions of the lung such as minute volume, peak flow, FEV_1, dynamic compliance, functions of the liver (metabolism, estimated hepatic blood flow, first pass effect) and of the kidneys (glomerular filtration, renal plasma flow, pH, urine volume, electrolyte excretion) vary with the time of day (for review see refs[10-16]). It may be noted that in contrast to adults children of an age between 5 days and 1 year however, did not display significant daily variations in urine volume and urine pH.[17] This can have an impact on the renal elimination of week acidic drugs (see below).

In man the organization in time can also be seen in certain states of disease in which the onset and symptoms do not occur at random within 24 hours of a day: Asthma attacks are more frequent at nightly hours than at other times of day as already observed about 300 years ago by John Floyer: "I have observed the fit always to happen after sleep in the night."[18] Similarly, the occurrences of coronary infarction as well as of angina pectoris attacks and of pathologic ECG-recordings are unevenly distributed over the 24-hour span of a day with a predominant peak in the early morning hours. Moreover, subtypes of a disease entity such as forms of vasospastic and stable angina pectoris or of primary and secondary hypertension may exhibit pronouncedly different 24-hour patterns in their symptoms [for review see ref[19]]).

Chronopharmacology

Having in mind the organization in time of living systems including man it is easy to conceive that not only must the *right* amount of the *right* substance be at the *right* place, but also this must occur at the *right* time. This is the more important when an organism or individual itself has to act or react in favorable biotic or environmental conditions which by themselves are highly periodic. Thus, it is easy to understand that exogenous compounds including drugs may differently challenge the individual depending on the time of exposition.

In the last decade numerous studies in animals as well as clinical studies have provided convincing evidence that the pharmacokinetics[20-22] and/or the drugs' effects/side effects can

be modified by the circadian time and/or the timing of drug application within 24 hours of a day (for review see refs[11-16]).

Chronopharmacotherapy of Asthma Bronchiale

Pulmonary functions such as FEV_1 are circadian phase-dependent with lower values at night. Most important, the amplitude increases with increasing severity of asthma (Fig 1) and this observation has been included in the staging of the disease.[32,33] Moreover, the lungs are also more sensitive to bronchoconstrictor substances such as acetylcholine, histamine, housemite dust, and grass pollens at nightly hours than during daytime.[23-26] This holds true for adults as well as children suffering from asthma. Recently, it has also been shown that the ciliary frequency in the human nouse displays a circadian rhythm with a higher frequency at night, but not in patients with chronic obstructive pulmonary disease.[27]

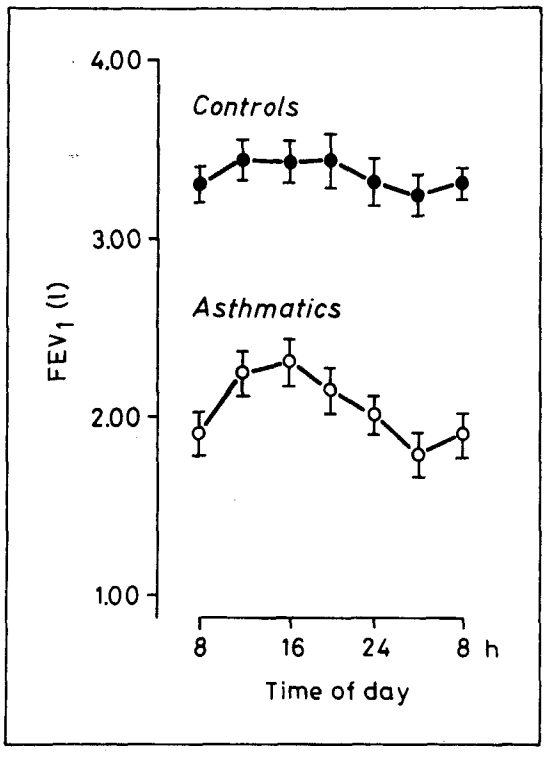

Figure 1. Daily variation in FEV_1 in healthy subjects and in patients suffering from asthma bronchiale. Note that FEV_1 is not only lower but that the amplitude is greater in diseased patients than in healthy subjects.

Because nocturnal asthma is a common event in asthmatic disease it is not surprising that antiasthmatic drugs have also been studied in relation to time of day. Theophylline was one of the first drugs for which daily variations in its pharmacokinetics were reported and up to now more than 50 studies were published[28,29] covering different theophylline preparations in different galenic formulations. Interestingly, the first study on the chronokinetics of theophylline was done in children[30] (Fig 2). This data in general demonstrated that Cmax (peak drug concentration) was lower and/or tmax (time to Cmax) was longer after evening than after morning application of oral theophylline. This data provided evidence that theophylline might be dosed higher during the night than during daytime hours—or even a single evening dose might be used—to overcome the nocturnal decrease in pulmonary function adequately.[31] Consequently, international guidelines[32] and the German Asthma League,[33] therefore, recommend that a single evening dosing or a higher evening dose of theophylline may be advantageous in the treatment of nocturnal asthma.

In contrast to the general belief concerning drug concentrations curves as mentioned above ("the flatter the better") it seems, therefore, to be adventageous to accept greater fluctuations in drug concentrations throughout 24 hours of a day in order to better achieve the therapeutic goal. In the treatment of asthmatic patients $beta_2$-sympathomimetic drugs are still remedies of first choice. Clinical data indicate that not only the pharmacokinetics of the $beta_2$-sympathomimetic terbutaline but also its effects on peak expiratory flow were circadian phase dependent: After a 7 day treatment with oral terbutaline (7.5 mg at 7.30 hours and at 19.30 hours) Cmax was higher after morning than evening drug application with tmax being 3.5 hours and 6.2 hours, resp,[34] thus, resembling the daily variations observed with theophylline. A further study with oral terbutaline indicated that doubling the dose in the evening, ie, an unequal dosing during 24 hours, can better control the nocturnal fall in peak flow.[35] These studies do not only give evidence for daily variations in the pharmacokinetics and the effects of oral terbutaline but also point out that the dose–

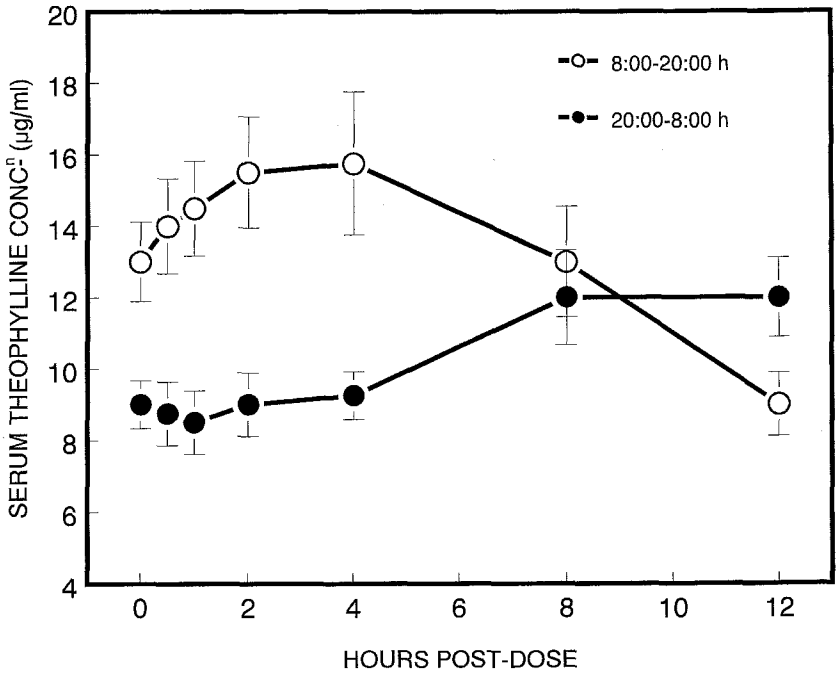

Figure 2. Plasma concentrations of retarded theophylline after chronic administration of twice daily (morning and evening) in 13 asthmatic children.

response relationship of a drug can be circadian phase dependent.

Recent data with the long-acting beta-agonist bambuterol showed that the once in the evening dosing (20 mg at 20 hours for 4 weeks) increased the lung function at night without affecting the 24 hour-value but reducing the amplitude.[36] The evening application of bambuterol lead to pronounced daily variations in the plasma concentrations of bambuterol and its active metabolite terbutaline, chronokinetics ie, morning versus evening dosing, however, were not studied. Inhaled application of formoterol or salmeterol improved the lung functions in patients with mild asthma[37]; equal doses of the drugs inhaled at four different circadian times improved airway function but did not affect the 24-hour profile.

Anticholinergics also seem to improve expiratory flow better after evening dosing.[38] There is no doubt about the importance of inhaled glucocorticoids in the early treatent of asthma.[32,33] In a recent study, inhaled budesonide (0.4 mg at 8 hours and 20 hours for 4 weeks) improved the 24 hour-mean in FEV_1 with a more pronounced effect at night and reduced the amplitude in the rhythm in FEV_1.[39] This is of special interest since worsening of asthma is accompanied by an increase in amplitude of FEV_1. Whether inhaled clucocorticoids display a chronokinetics has not yet been investigated.

In conclusion, the chronopharmacologic data indicate that antiasthmatic drugs such as theophylline and beta-sympathomimetics may/should be dosed higher in the evening than during daytime when asthma is predominantly nocturnal. The best dosing time and the best dosing interval of inhaled glucocorticoids is still an open question.

Chronopharmacotherapy of Peptic Ulcer Disease

In 1970, Moore and Englert[40] first described the circadian rhythm in gastric acid secretion in man. This chronobiologic finding unanimously led to the recommendation that H_2-blockers (ranitidine, cimetidine, famotidine, roxatidine, nizatidine) should be taken once a day in the late afternoon or early night when acid secretion is increasing, independently of whether the compounds have a short or a long half-life.[41]

Recently another paradigm was pulled down: It has been generally assumed that constant drug infusion leads to constant drug concentrations which in turn should lead to constant drug effects. However, it has been shown[42] that a constant infusion of ranitidine over a period of 24

hours does not lead to a constant effect: The increase in gastric pH by ranitidine was less during the nightly than during the daytime hours of drug infusion, indicating that there might be a partial nocturnal resistance to H_2-receptor blockade. This interesting finding calls not only for further investigations on the regulatory mechanisms of gastric acid secretion but could also indicate that drugs with a different mechanism of action may be added to the treatment with H_2-blockers during the nightly hours.

In contrast to H_2-blockers proton pump inhibitors (PPI) should be dosed in the morning[41] since the increase by lansoprazole and omeprazole in intragastric pH is more pronounced after morning than evening administration.[41] At least for lansoprazole a reduced absorption has been reported for the evening (see ref[41]).

Chronopharmacological Implications in Hypertension

The development of easy-to-use devices to continuously monitor blood pressure and heart rate in man (ABPM = ambulatory blood pressure monitoring) clearly opened the eyes of the clinician for chronopharmacology in showing that blood pressure in normotensive and in hypertensive patients are clearly dependent on the time of day. Moreover, different forms of hypertension may exhibit different circadian patterns: In normotension as well as in primary hypertension there is in general a nightly drop in blood pressure ("dippers"), whereas in secondary hypertension caused by eg, renal disease, gestation, Cushing's disease, the rhythm in blood pressure is in about 70% of the cases abolished or even reversed with highest values at night ("non-dippers")[43,44] (for review see ref[45]).

Drug treatment of hypertension includes various types of drugs such as diuretics, β- and α-adrenoceptor blocking drugs, calcium channel blockers, converting enzyme inhibitors, and others that differ in their sites of action. Because the main steps in the mechanisms regulating the blood pressure are circadian phase-dependent [see ref[15]], it is not a surprise that also antihypertensive drugs may display a circadian time-dependency in their effects and/or their pharmacokinetics. Moreover, these drugs differ in their half-life, galenic formulations and, thus, in dosing interval. Despite the great number of studies published in evaluating antihypertensive drug efficacy, the time of day of drug application was only scarcely a specific point of investigation.

In hypertensive patients no cross-over (morning versus evening) study with β-adrenoceptor antagonists has been published. From studies performed without time specified drug dosing it is difficult to draw definite conclusions on the importance of the circadian time of drug dosing for the antihypertensive drug efficacy. A resume of 20 "conventionally" performed studies[46] showed that β-adrenoceptor antagonists do either not affect or reduce or even abolish the rhythmic pattern in blood pressure. In general, however, there is a tendency for β-adrenoceptor antagonists to predominately reduce daytime blood pressure levels and not to greatly affect night-time values, being less/not effective in reducing the early morning rise in blood pressure.[46,47] Consistently, decreases in heart rate by β-adrenoceptor antagonists are more pronounced during daytime hours. In healthy subjects a cross-over study with propranolol similarly showed a more pronounced decrease in heart rate and blood pressure during daytime hours than at night.[48] Interestingly, the agent with partial agonist activity, pindolol, even increase heart rate at night.[49] In conclusion, clinical data indicate that β-adrenoceptor mediated regulation of blood pressure dominates during daytime hours and is of less or minor importance during the night and the early morning hours. This correlates well with the circadian rhythm in sympathetic tone as indicated by the rhythm in plasma noradrenaline and cAMP [see ref[37]].

The effects of calcium channel blockers were also analyzed mainly by visual inspection of the blood pressure profiles. In primary hypertensives, 3 times daily dosing of nonretarded verapamil did not greatly change the blood pressure profile being, however, less effective at night.[50] A single morning dose of a sustained-release verapamil showed a good 24-hour blood pressure control.[51] Dihydropyridine derivatives [DHP], differing in pharmacokinetics, seem to reduce blood pressure to a varying degree during day and night, drug formulation and dosing interval may play an additional role. In eight studies in essential hypertensives using a cross-over design (Table 1) DHP did not differently affect the 24-hour blood pressure profile after once morning or once evening dosing.[53-61] Most interest-

Table 1. Effects of Calcium Channel Blockers on the 24-Hour Pattern in Blood Pressure

				Effect on 24-Hour Blood Pressure			
Drug	*Dose mg/d*	*Duration Dosing time*	*Patients (n) Diagnose*	*Day*	*Night*	*24h-Profile*	*Reference*
Amlodipine	5	4 wks	20, EH				Mengden et al,[53] 1992
		−a.m.		++	++	Preserved	
		−p.m.		++	++	Preserved	
Amlodipine	5	3 wks	12, EH				Nold et al,[81] 1998
		−08.00h		+	+	Preserved	
		−20.00h		+	+	Preserved	
Nifedipine GITS	30	1 or 2 wks	10, EH				Greminger et al,[55] 1994
		−10.00h		++	++	Preserved	
		−22.00h		++	++	Preserved	
Nitrendipine	20	4 wks	41, EH				Meilhac et al,[57] 1992
		−07.00h		+	+	Preserved	
		−19.00h		+	+	Preserved	
Nitrendipine	10	3 days	6, EH				Umeda et al,[58] 1994
		−06.00h		++	++	Preserved	
		−18.00h		+	++	Changed	
Isradipine	5	4 wks	18, EH				Fogari et al,[54] 1993
		−07.00h		++	++	Preserved	
		−19.00h		++	++	Preserved	
Nisoldipine ER	20	4 wks	75, EH				White et al,[61] 1999
		−07-11.00h		++	++	Preserved*	
		−21-23.00h		++	++	Preserved*	
Isradipine	5	4 wks	16, RH				Portaluppi et al,[52] 1995
		−08.00h		++	++	Not normalized	
		−20.00h		+	+++	Normalized	
Nifedipine i.r.	10	single dose	12, NT				Lemmer et al,[56] 1991
		−08.00h		+	++	Preserved	
		−19.00h		+	+	Preserved	
Lacidipine	4	6 w	33, EH				van Montfrans et al,[60] 1998
		−07.00-09.00		++	(+)	Preserved	
		−22.00-24.00		(+)	+(+)	Preserved?	

NOTE. Table includes only data from cross-over studies.
Abbreviations: EH, essential (primary) hypertensives; RH, renal (secondary) hypertensives; NI, Normotensives.
* Small differences in dippers versus nondippers.

ingly, the greatly disturbed blood pressure profile in secondary hypertensives due to renal failure was only normalised after evening but not after morning dosing of isradipine.[52]

Five cross-over studies (morning *v* evening dosing) with converting enzyme inhibitors in essential hypertensive patients were published (Table 2). They show in 3 studies that evening dosing in contrast to morning dosing resulted in a more pronounced nightly drop and the 24-hour blood pressure (BP) profile was distorted. Evening dosing of quinapril also resulted in a more pronounced effect than morning dosing, the BP pattern, however, was not greatly modified.[63] In the light of a reduced cardiac reserve of patients with hypertension at risk,[66,67] a too pronounced nightly drop in BP after evening dosing might be a potential risk factor for the occurrence of ischemic events.[68]

In conclusion, there is some evidence that in primary hypertension antihypertensive drugs should be given at early morning hours, whereas in secondary hypertension it can be necessary to add an evening dose or to apply a single evening dose. In addition, rhythmicity can also be seen in the pharmacokinetics of cardiovascular active drugs. However, the galenic formulation and/or the indiginous half life of a drug has to be considered in order to come to a final recommendation. This clearly calls for additional chronopharmacological studies using a cross-over design.

Our studies have shown that different cardiovascular active compounds such as propranolol,

Table 2. Effects of ACE Inhibitors on the 24-Hour Pattern in Blood Pressure

				Effect on 24-Hour Blood Pressure			
Drug	*Dose mg/d*	*Duration Dosing time*	*Patients (n) Diagnose*	*Day*	*Night*	*24h-Profile*	*Reference*
Benazepril	10	single dose	10, EH				Palatini et al,[59] 1993
		−09.00h		+++	++	Preserved	
		−21.00h		+	++	Changed	
Enalapril	10	single dose	10, EH				Witte et al,[62] 1993
		−07.00h		++	+	Preserved	
		−19.00h		++	+++	Changed	
		3 wks					
		−07.00h		++	+	Preserved	
		−19.00h		+	++	Changed	
Quinapril	20	4 wks	18, EH				Palatini et al,[63] 1992
		−08.00h		++	+	Preserved	
		−22.00h		++	++	Preserved	
Ramipril	2.5	4 wks	33, EH				Myburgh et al,[65] 1995
		−08.00h		+	(+)	Preserved	
		−20.00h		(+)	+	Preserved	
Perindopril	2	4 wks	18, EH				Morgan et al,[64] 1997
		−09.00h		++	+	Preserved	
		−21.00H		+	++	Changed	

NOTE. Table includes only data from cross-over studies.
Abbreviation: EH, essential (primary) hypertensives.

oral nitrates and the calcium channel blocker nifedipine showed higher peak drug concentrations [Cmax] and/or a shorter time-to-peak concentration [tmax] after morning than evening oral drug dosing, at least when non-retarded formulations were used. In the case of retard formulation of IS-5-MN and nifedipine, however, no circadian phase-dependency in their pharmacokinetics were found (for details see references[15,22,45]). Concerning the underlying mechanisms responsible for this chronokinetic behaviour of these lipophilic compounds a faster gastric emptying time in the morning[69] and—more important—a higher gastro-intestinal perfusion in the morning than in the evening are assumed to be involved.[70]

Special Considerations for Children

As mentioned above, the first study on the chronopharmacokinetics of theophylline was performed in asthmatic children[30] a shorter tmax and a higher Cmax after morning than evening dosing even under chronic drug application. Several studies with antiasthmatic drugs did not give evidence for a difference in circadian drug effects when compared to adults.[71] Another chronokinetic study in asthmatic children aged 5 to 13 years[72] confirmed the pattern described by Scott et al[30] for the oral application. Moreover, these authors gave evidence that the lower evening predose concentration of theophylline was due to slower drug absorption because no diurnal variation were found after drug infusion during morning and evening.[72]

As mentioned earlier, children do not display a circadian rhythm in urinary pH.[72] The same authors could demonstrate that not only the elimination rate of sulfonamides is much lower in newborns than in adults but that in children below 1 year the elimination rate of sulfisomidine during the night was about 20% lower than during daytime, indicating a daily variation in non-ionic tubular reabsorption.[72]

In children aged 9 to 12 years[73] or 241 children from 6 to 16 years,[74] the 24 hour blood pressure pattern as evidenced by ambulatory blood pressure measurements was not principally different from that found in adults showing dipping behavior at night. Data from a multicenter study in 1,141 healthy children and adolescents, aged 5 to 21 years, provides well-based limits of normal ABPM pattern in mid-European children,[75] however, a detailed rhythm analysis is still lacking. In accordance with findings in adults, ABPM was shown to provide more reli-

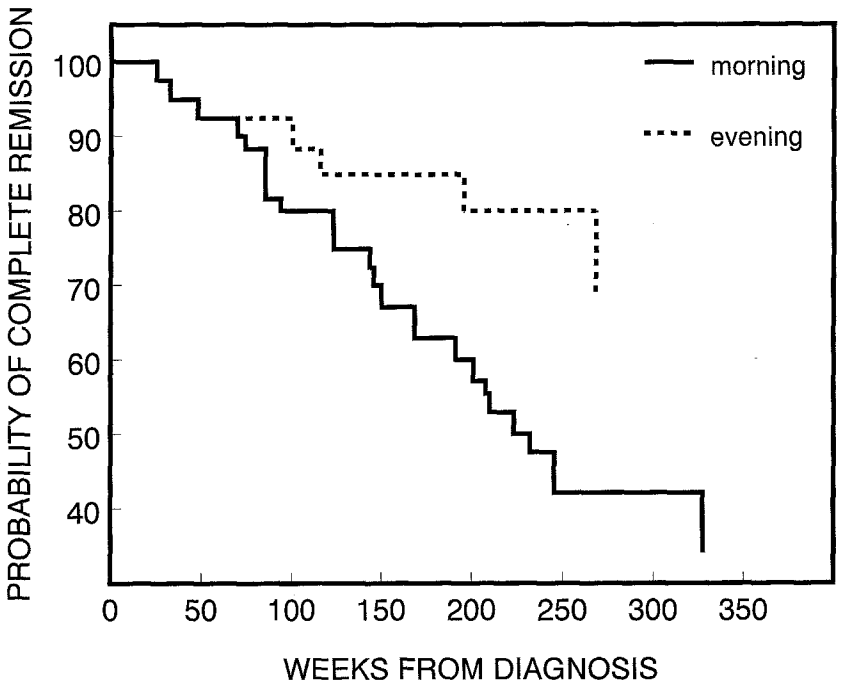

Figure 3. Kaplan-Meier analysis of the disease-free survival in 118 children with lymphoblastic leukemia receiving mercaptopurine either in the morning or in the evening.

able results on the 24 hour blood pressure profile in pediatric patients after renal transplantation with an attenuated or reversed nocturnal dip.[76] In untreated children with kidney disease and conserved renal function a distorted circadian rhythm in blood pressure was found in about 25%, both daytime and nighttime hypertension was observed.[77] There is only limited data in premature infants; a preliminary study with an oscillometric method (every 15 minutes for 24 hours) in premature infants (31 to 42 weeks) showed a mean arterial pressure of 48 mm Hg with a dominant BP but variable rhythm period from 24h to 4h, which was neither synchronized to heart rate rhythm nor to the light-dark cycle.[78]

Most interesting findings were obtained in children suffering from acute lymphocytic leukemia: Koren et al[79] could give evidence that the elimination half-life of mercaptopurine was significantly longer in the evening (7.1 hour) than during day (2.9 hours) during maintenance therapy. Moreover, a sharp fall in white blood cell count was the consequence of switching morning dosing to evening dosing. Thus, they concluded that daily variations in mercaptopurine disposition results in clinically important myelotoxicity of the drug. In accordance with this observation evening dosing of mercaptopurine in 118 children with lymphoblastic leukemia resulted in a significant increase in disease-free survival as shown by Kaplan-Meier analysis[80] (Fig 3).

Conclusion

Experiments in single cells, plants, animals, and man have provided sound evidence that all living creatures are structured not only in space but also in time. Biological clocks time us and help us to entrain our time structure to the changing environmental conditions. Having in mind these fundamental properties of living systems it is not surprising to note that this has also implications for pharmacology, pharmacy and drug therapy in that drug effects and/or pharmacokinetics can be modified by circadian time as well as drugs are able to influence circadian rhythms. Since maturation of circadian rhythms takes place in humans during development circadian time-specified drug application in children has to be studied much more frequently and in more detail. It has to be kept in mind that chronotherapy in children can—but may not—result in different drug efficacy and/or toxicity compared with adults.

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