

**BIOLOGICAL RHYTHM -- CHRONOTHERAPEUTICS:
RELEVANCE**

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Summary

A biological clock provides the possibility of anticipating, and therefore preparing for events repetitively associated with daily light-dark alternations, and is at the basis of the rhythmic patterns of biological variables. The reliance on the clock is so entrenched in life that forced disruptions of the natural synchrony between the environment and the internal clock is a risk factor for a number of diseases. Chronotherapeutics refers to a treatment method in which in vivo drug availability is timed to match rhythms of disease. The corollary is to achieve high therapeutic effects with least / minimal adverse effects. There is an interdependent relationship between peak-to-trough rhythmic activity in disease symptoms and risk factors, pharmacologic sensitivity, and pharmacokinetics of many drugs. The specific time that patients take their medication is very important as it has significant impact on treatment success. Biological rhythms not only impact the pathophysiology of diseases, but the pharmacokinetics and pharmacodynamics of medications. Chronotherapeutics is the investigative science that elucidates the biological rhythm dependencies of medications. This review focuses on chronopharmacotherapy of various diseases related to different human body systems, and also provides a molecular biological explanation for the influence of medications on the clock genes. Novel Drug delivery systems and technologies such as time-controlled, pulsed, triggered and programmed drug delivery devices have been developed and extensively investigated in recent years for chronopharmaceutical drug delivery.

Introduction

The term “CHRONO” refers to an observation that every metabolic event undergoes rhythmic changes in time. The study of rhythmic, predictable-in-time differences in the effects and/or pharmacokinetics of drugs both in experimental animals and in men¹⁻³. It investigates the effects/side effects of drugs upon temporal changes in biological functions/ symptoms of a disease as well as drug effects as a function of biologic timing. Two concepts must be considered when dealing with day-related changes of drug efficacy:

- (a) circadian changes in drug bioavailability (chronokinetics);
- (b) circadian changes in the susceptibility to the drug (chronesthesia)

Clinical chronopharmacology (or chronotherapeutics) is the purposeful alteration of drug level to match rhythms to optimize therapeutic outcomes and minimize side effects. Daily rhythms in plants and animals have been observed since ancient times. All physiological, biochemical and molecular functions of living organisms are tightly and reproducibly organized within circadian time, including those responsible for drug distribution, anabolism, receptor binding, bioactivity, catabolism and excretion. A biological clock provides the possibility of anticipating, and therefore preparing for events repetitively associated with daily light-dark alternations, and is at the basis of the rhythmic patterns of biological variables.

The reliance on the clock is so entrenched in life that forced disruptions of the natural synchrony between the environment and the internal clock is a risk factor for a number of diseases.

The molecular mechanisms that underlie the function of the clock are universally present in all cells and consist of gene-protein-gene feedback loops, in which proteins can down-regulate their own transcription and stimulate the transcription of other clock proteins. Although, anchored genetically circadian rhythms are synchronized by (entrained) and maintained by certain exogenous factors- phenomena of Zeitgeber. Therefore, a rhythmic variation under the influence of the Zeitgeber as a resetting factor is involved in adjusting the daily activity pattern to the appropriate time of day.

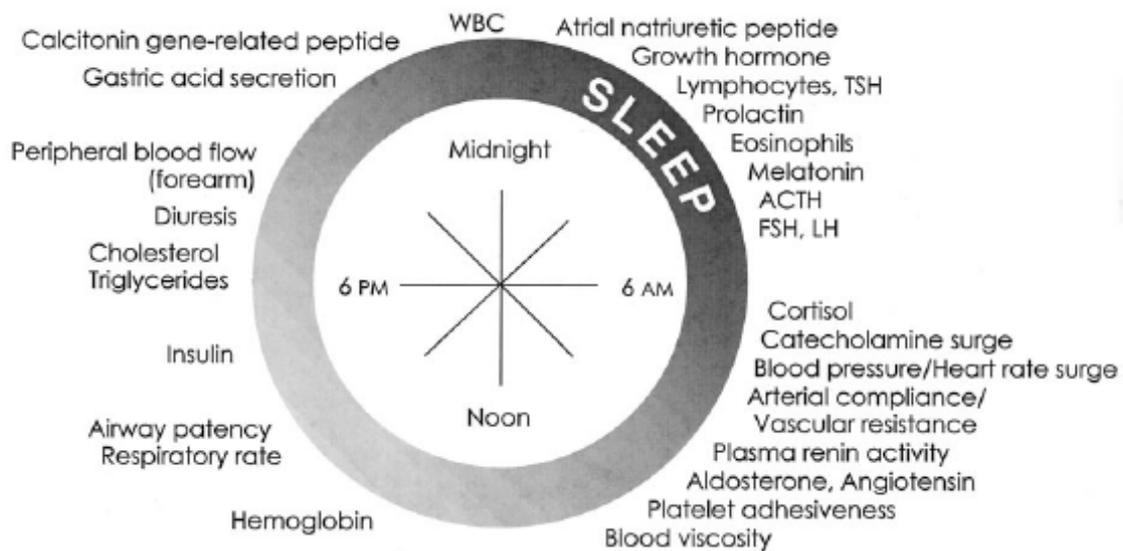
Major circadian oscillator is located in the suprachiasmatic nuclei (SCN) of the hypothalamus. This circadian master clock acts like a multifunctional timer to adjust the homeostatic system, including sleep and wakefulness, hormonal secretions and various other bodily functions, to the 24-h cycle. The sinusoidal output signal produced by the SCN can be described by its period (cycle length), phase (position in the cycle), and amplitude (range between highest and lowest signal). Disruption of amplitude or phase of circadian rhythms can be produced endogenously, like that seen in many psychiatric disorders, blindness, circadian sleep disorders or most chronic diseases. On the other hand, phase maps may undergo transitory disruptions when humans are compelled to make a rapid phase adjustment as, for example, after a rapid move to a new geographic longitude or as a consequence of shift work . Under such circumstances the various individual 24-h components comprising the circadian phase map do not reset their phases to the new environmental times at the same rate, and become somewhat displaced in their relations to one another. Liver is a biological clock capable of generating its own diurnal rhythms. As the body's primary defense against metabolic poisoning, and the target of many toxic substances, the liver is continuously exposed to relatively high amounts of ingested drugs or toxins. Being a major organ of metabolism and detoxification of drugs, knowledge of circadian effects on transcriptional activities that govern daily biochemical and physiological processes in the liver is key for pharmacological and toxicological studies. The major advantage of chronopharmacotherapy is it prevents an overdosing of any class of drug⁴.

GOAL:

1. Match the timing of the treatment with the intrinsic timing of illness.
2. Optimum therapy is more likely to result when the right amount of drug is delivered to the correct organ at most appropriate time.
3. To understand the knowledge of 24 hour rhythm in the risk of disease plus evidence of 24 hour rhythm dependencies of drug pharmacokinetics, effects, and safety.
4. To study and observe the role of novel drug delivery systems and their variations with time.

Table 1. Spectrum of biologic rhythms

Category of Rhythms	Period of Rhythms	Term Used to Describe Rhythm	Illustrative Examples
Short periods	≤Sec	High frequency Oscillations	Electroencephalogram Electrocardiogram
Medium periods	30 min to 20 h	Ultradian	Sleep staging Pulsatile hormone secretion
	20 h to 28 h	Circadian	Most biologic functions (see text and Fig. 1)
Long periods	28 h to 6 days	Infradian	Little studied thus far
	~Week	Circaseptan	Work-rest routine
	~Month	Circamensual	Menstruation, fertility Neuroendocrine functions
	~Year	Circannual	Many biochemical, endocrine, and physiological parameters



Conditions which show a circadian pattern and advantage could be taken by timing and adjusting the administration of drugs according to circadian rhythm of the disease:

1. Myocardial infarction.
2. Hypertension
3. Bronchial Asthma
4. Cerebrovascular accidents
5. Hypercholesterolemia
6. Arthritis
7. Peptic Ulcer

Circadian changes in effects of various chemical agents:

- | | |
|----------------------|--|
| 1. Histamine | 9. PG F ₂ alpha |
| 2. Acetylcholine | 10. Cyproheptadine |
| 3. Ethanol | 11. Lignocaine |
| 4. Chlorthiazide | 12. Orcinprenaline |
| 5. Reserpine | 13. ACTH |
| 6. Oxymetholone | 14. Cortisol |
| 7. Sodium salicylate | 15. Indomethacin |
| 8. Halothane | 16. Various synthetic corticosteroids. |

CAUSES FOR CHRONOPHARMACOLOGY :

There are different reasons for this which may be summarized as:

- 1. Autoinduction:** A repetitive dose of a drug induces or increases enzymes responsible for its elimination, thereby increasing its clearance. This is called as autoinduction. It is dependent on dose and concentration of the drug. It has a number of therapeutic consequences. It affects the time to achieve steady state and limits one's ability to use information from a single dose to predict kinetics. after repeated dose or continuous administration. Carbamazepine shows time dependence in its disposition. The decrease in its peak concentration on repetitive oral administration that either oral bioavailability decreases or clearance increases with time.
- 2. Autoinhibition:** it may occur during the course of metabolism of certain drugs. In this case, the metabolites formed increase in concentration and further inhibit metabolism of the parent drug. In biochemistry, this phenomenon is called as product inhibition or allosteric inhibition or feedback inhibition.
- 3. Food effects:** food is the major cause of diurnal variations. Gastric emptying is slowed or delayed by food, often resulting in a decrease in the peak concentration and an increase in the time of its occurrence following a single dose. It is a major cause of circadian variations in patients tending to eat more in the evening than at breakfast. When absorption is slowed by food, the rate of input into liver and concentration of drug entering liver are lowered and prolonged and thus metabolism is lowered. Hence, a concurrent intake of heavy food in evening and some drugs reduces bioavailability of the drug.

Some of the biological clocks:

- **Cancer Drug Administration** —treatment timing can significantly reduce side effects.
 - Eg: 1. Patients with untreated metastasis from colorectal cancer, the chronomodulated infusion of oxiplatin, 5FU and folinic acid reduced the adverse effects such as stomatitis and peripheral sensory neuropathy.
 2. In patients with lung cancer, chronopharmacology of prolonged etoposide administration by intravenous infusion showed the percentage plasma etoposide concentration at 9 am was significantly higher than 9 pm.

- **Intraocular Pressure (IOP)** —in glaucoma patients IOP peaks at 4 AM and has a trough in the afternoon, opposite that of people with normal IOP;
- **Hormone Secretion** — growth hormone and melatonin are produced at night; testosterone and cortisol in the early morning hours;
- **Allergic Response** — skin tests produce a 3times greater result when given at night;
- **Gastric Motility** — slower at night, which can impact controlled-release product design;
- **Seasonal Affective Disorder (SAD)** — affects 1% to 3% of adults; increased sleep and appetite are well-known phenomena in winter;
- **Atrial Fibrillation** — hospital admissions peak in April with a trough in August;
- **Blood Coagulation** — even with constant heparin infusion rate, thromboplastin time and risk of bleeding vary significantly during the day;
- **Cholesterol Production** — statins dosed in evening have been shown to be more effective;
- **Asthma Treatment** — evening dosing can improve lung function during sleep.

STEPS INVOLVED IN AN EVALUATION OF CHRONOPHARMACOLOGY :

1. Identification of its occurrence—Cause? , Variation? , Does it affect Biological clock?
2. Determination of the parameter affected.
3. Mechanism of nonlinearity—Identify→ Know the mechanism → Solve and fix it.

CHRONOKINETICS:

- Time of administration is a possible factor of variation in pharmacokinetics of a drug.
- Time dependent changes in pharmacokinetics may proceed from 24hrs rhythms in each process⁵. Eg: ADME

Thus 24hr rhythms in

- i) Gastric acid secretion & Ph
- ii) Motility
- iii) Gastric empty time
- iv) GI blood flow
- v) Drug protein binding
- vi) Liver enzyme activity
- vii) Hepatic blood flow

- viii) Glomerular filtration
- ix) Renal blood flow
- x) Urinary pH
- xi) Tubular resorption

All these factors play important role in pharmacokinetic variations.

- Clock genes are expressed in SCN, other brain regions & some peripheral tissues.
- Liver is a biological clock capable of generating its own circadian rhythms & therefore liver is a major organ of metabolism and detoxification, knowledge of circadian elects on transcriptional activities that govern daily biochemical & physiological processes in liver may play a key in toxicology⁶.
- A circadian rhythm is demonstrated for 6 genes involved in the regulation of gene transcription.
- Retinoic acid receptor α & retinoid X receptor (α & γ) play an important role in regulation of gene expression by forming transcriptional active complexes on DNA⁷.
- $P_{1t \times 2}$ & $P_{1t \times 3}$ demonstrate circadian expression⁸.
- Cyp4a3 & putative N- acetyltransferasecamello4 of phase I & phase II of drug metabolism express circadian
- Other members of phase II drug metabolism such as GST & carboxyl esterase demonstrate circadian rhythms A significant circadian rhythm is demonstrated for genes involved in ion transport. They include genes encoding proteins of SLC transporter such as SLC 34a1 & SLC2a8.
- Others like SLC 12a2, SLC 16a1, SLC 19a1, SLC 25a11 & Abcc2 & Aqp9 → show circadian rhythm.
- A significant circadian rhythm is demonstrated for genes involved in ion transport.⁹
Ion transporters → Hcn4, Trpc4, Scn2b, Scn4a, Atp9a, Atp7b & NRITP¹⁰.
- DBP is a major factor controlling circadian rhythm of steroid 15 α hydroxylase & coumarin hydroxylase genes in mouse liver.
- PARnZ1transcription factors DBF, TEF & HLF accumulate in a highly circadian manner in several peripheral tissues such as liver & kidney.

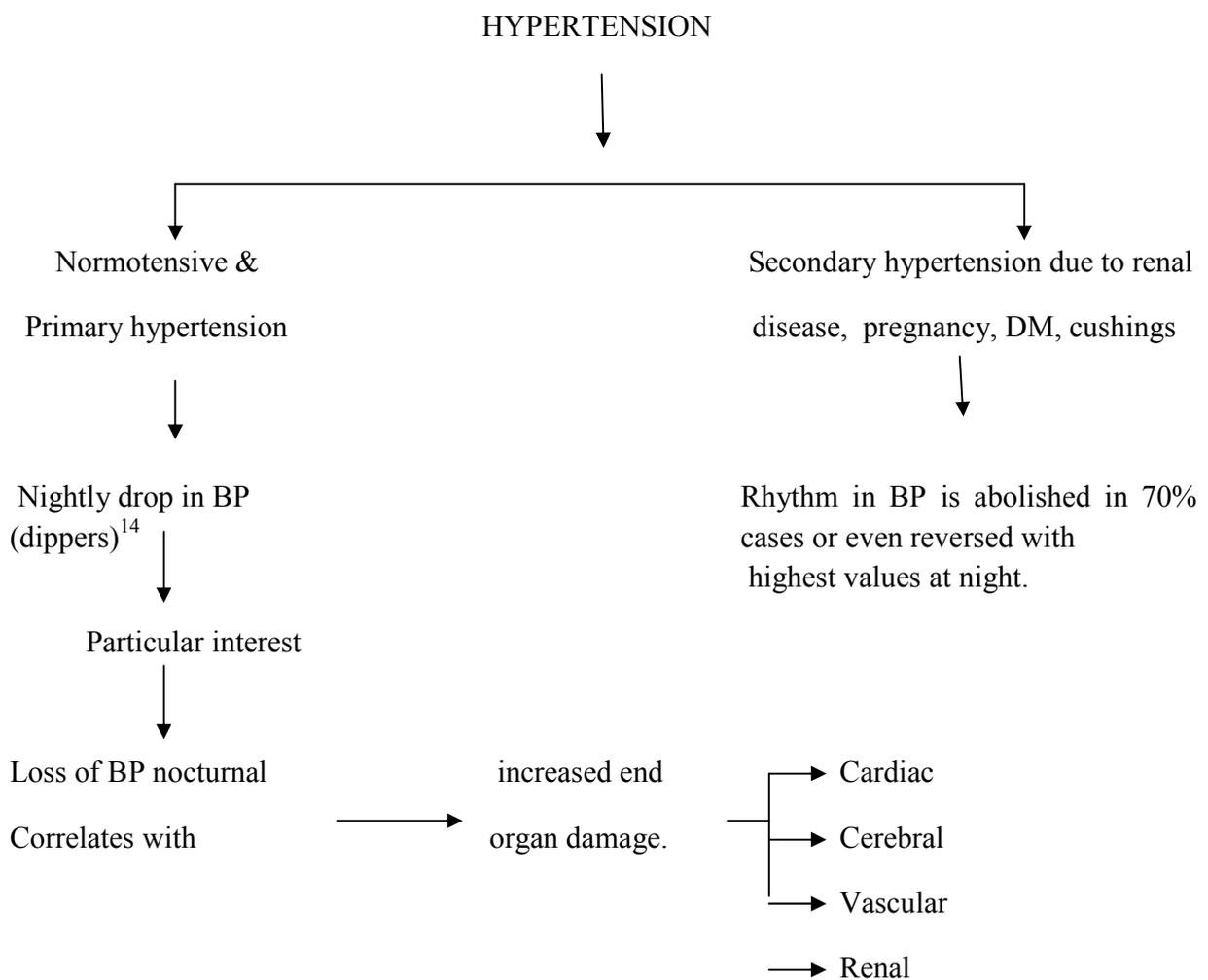
BIOLOGICAL RHYTHMS OBSERVED IN VARIOUS BIOLOGICAL SYSTEMS :

The basic physiological process governing the drug action the absorption the distribution the metabolism and the excretion are controlled by the following systems of the body. Hence it is important to know the circadian rhythms in these systems and their effect on drug action.¹¹

1. **CVS:** Most cardiovascular activities show a circadian rhythm, as do several electrophysiological phenomenon.¹² Cardiovascular functions such as heart rate and blood pressure show 24h variation.¹³

HR & BP are increased in early morning hours.

Different forms of hypertension may exhibit different circadian patterns



- Antihypertensive → taken at early morning hours whereas in secondary hypertension an additional evening dose may be necessary.

- Propranolol, oral nitrates, Ca^{2+} channel x nifedipine & enalapril → show higher C_{max} values & or shorter I_{max} values after morning (oral) administration at least when immediate formulations were used.
- A faster gastric emptying time in the morning and a higher gi perfusion are involved & are responsible for the chronokinetic behaviour of these lipophilic compounds.
- It appears that the circadian phase dependency of the components of the CVS- due to variations in regulatory mechanisms seem to be of greater importance for drug efficacy than daily variations in pharmacokinetics.
- The onset of nonfatal or fatal MI predominantly occur around 6am to 12pm → cause is attributed to rel of catecholamine's, cortisol & increase in platelet aggregation & vascular tone.

Symptoms in CHD



- Myocardial Ischemia
 - Angina attacks
 - Silent Ischemia
- } More frequent during day

Onset of angina attacks in variant angina peaks at 4am.

2. **Asthma:** Numerous investigations have demonstrated the usefulness of chronotherapy for asthma, especially for patients with nocturnal asthma.¹⁵
Development of asthma symptoms & many types of bronchospastic attacks is more clearly common from 2am to 6am.

Aim: Is to get maximal effect from bronchodilator medications during early morning hours.

The general belief about drug concentrations versus time profiles- the flatter the better- it seems therefore that allowing greater fluctuations in drug concentration over the 24hour of the day will lead to better treatment of asthmatic patients.

- a) Theophylline → C_{max} was lower
 T_{max} was higher } → after evening than after morning application of Theophylline.

- b) Terbutaline

- The pharmacokinetics of this β_2 sympathomimetic drug & its effects on PEF are circadian, phase dependent.

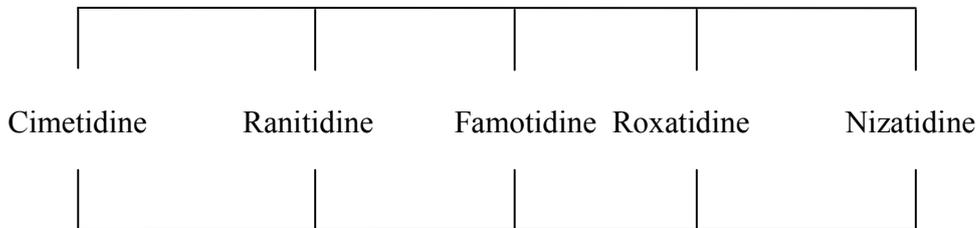
- Administering 2/3 of the daily oral dose of Terbutaline in the evening, improves control of nocturnal fall in peak flow in asthmatics.
- c) Inhaled glucocorticosteroid ciclesonide available → once daily dosing improves patients compliance

Conclusion: Higher doses of theophylline & β-sympathomimetics should be administered in the evening than during day time.

1. PEPTIC ULCER:

- Maximal acid secretion
 - Pain
 - Perforation
- } more common at night

H₂ blockers



Should be taken once daily after the last meal in the Evening regardless of the $t_{1/2}$



Reduces acid secretion
Promotes ulcer healing
Reduces ulcer recurrence

2. CVA:

- Common to occur on the 1st hours of morning between 10 am to 12 noon with Incidence declining in the evening and midnight.
- **Objective** of chronotherapy for CVA is to deliver the drug in higher concentration during time of greatest need & in lesser concentrations when need is less.

Eg: ACE inhibitors more effective when administered at night

→ Atenolol, nifedipine, amlodipine when administered at night

- Antihypertensive & antianginal therapy can be designed to mimic circadian rhythms.

3. ARTHITIS:

- Symptoms of RA → worse in the morning.

Therefore long acting NSAIDS like flubiprofen ketoprofen & indomethacin at bed time achieves Therapeutic effect less A/E.

- Osteoarthritis → less pain in the morning, more at night → Give NSAIDS, like Ibuprofen around noon.
- Ankylosing spondylitis is characterized by swelling and discomfort of the joints of the back¹⁶.

4. Hypercholesterolemia:

Higher rates of cholesterol intake } occur during evening hours even during fasting state & hepatic cholesterogenesis

Therefore evening administration of HMG-COA reductase Θ



Showed significant reduction in serum cholesterol levels.

- CV end points such as MI, unstable angina & stroke rates reduced.

Therefore lovastatin, simvastatin, rasuvastatin, pravastatin, fluvastatin.



Administration between evening meal and bed time.

Exception → atorvastatin → has long elimination $t_{1/2}$.

5. Cancer :

The tumour cells and the normal cells differ in their Chronobiological cycles. This fact is the basis for the chronopharmacotherapy of cancers¹⁷

DNA synthesis in normal human bone marrow cells has a peak around noon while the peak of DNA synthesis in lymphoma cells is nearly midnight.

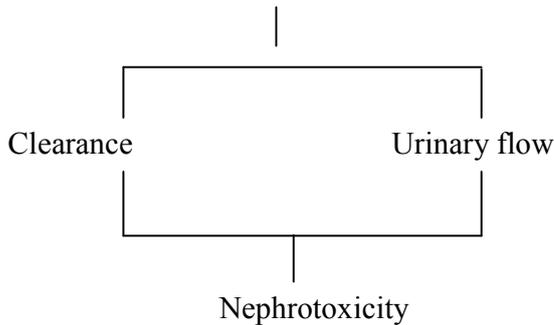
Administration of S phase active cytotoxic therapy } Decrease in tumour cell count
Methotrexate, 5FU, 6MCP, Cytarabine

6. Diabetes:

Cryptochrome → regulates gluconeogenesis according to diurnal activity & feeding levels so modulating Cryptochrome levels can help decrease diabetic effects on patients.

The additional function of the cryptochrome is the regulation of gluconeogenesis according to the diurnal activity and feeding levels.¹⁸

7. **Urinary system** : Plays important role in elimination of drug.
Circadian rhythm alters either



Egs:

- Aminoglycosids produce Nephrotoxicity

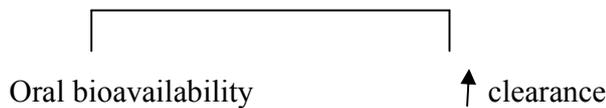
Chronic administration → drug accumulation → toxicity, hence
Monitoring a must in Renal impairment patients .

- Theophylline causes increase in renal blood flow by increase in clearance levels

↓
Increase in urine flow & excretion

- Carbamazepine → time dependence in its disposition

Repetitive oral administration
|



- The elimination of diazepam is slower of the multiple dosing than following a single dose.¹⁹

8. Hepatic system:

Antidepressant nor-tryptalline which is injected to significant presystemic hepatic metabolism accumulation in highly predictable manner on multiple oral dosing.

The clearance levels of acetaminophen are decreased due to effect on circadian → rhythm results in hepatotoxicity.

Conclusion:

The major objective of this review is to inform biologists, clinicians, pharmaceutical scientists and other professionals the importance of biological clocks and chronopharmacology to human health and disease. Other purpose is to trigger further experimental and clinical research in the field of chronopharmacotherapy. However, the most important issue of this article is to motivate

the investigation, and development of Chronotherapeutics as a practical means of improving the outcomes and safety of medical treatment with the older or already well established active pharmaceutical ingredients. Attention should be paid to the alteration of clock gene expression and to consider it an adverse effect when it leads to altered regulation of circadian system, which is a serious problem affecting basic functioning of living organism. One approach to increasing the efficiency of pharmacotherapy is administering drugs at times during which they are best tolerated. In addition, the discoveries of small compounds or new drug substances that act on the peripheral clock will help us to establish chronopharmacotherapeutic approaches to more appropriate extend.

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