Program	B. Pharmacy
Semester	VI
Subject /Course	Pharmacology-III
Subject/Course ID	BP602T
Module No.	05
Module Title	Chemotherapy and immunology
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Learning Outcome of Module-5

LO	Learning Outcome (LO)	Course Outcome Code
LO1	Know about the different types of toxicity studies (acute, subacute and chronic).	BP602.5
LO2	Learn about the terms: genotoxicity, carcinogenicity, teratogenicity and mutagenicity.	BP602.5
LO3	Learn about the poisoning, factors responsible, prevention methods and its management.	BP602.5
LO4	To Understand the concept of chronopharmacology: rhythm and cycles, biological clock and their significance.	BP602.6

Content Table

Topic
• Definition and basic knowledge of acute, subacute and chronic toxicity. • Definition and basic knowledge of genotoxicity, carcinogenicity, teratogenicity and mutagenicity. • General principles of treatment of poisoing. • Clinical symptoms and management of barbiturates, morphine, organophosphorus compound, lead, mercury and arsenic poisoning. • Definition of rhythm and cycles. • Biological clock and their significance leading to chronotherapy.

TOXICITY STUDY

Toxicology is a branch of science that deals with toxins and poisons and their effects and treatment. Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. The US Food and Drug Administration (FDA) states that it is essential to screen new molecules for pharmacological activity and toxicity potential in animals (21CFR Part 314). The toxic effects of chemicals, food substances, pharmaceuticals, etc., have attained great significance in the 21st century. This brief review focuses on the historical importance of toxicological screening and alternative and specific methods using various experimental animal models. Toxicity tests are mostly used to examine specific adverse events or specific end points such as cancer, cardiotoxicity, and skin/eye irritation. Toxicity testing also helps calculate the No Observed Adverse Effect Level (NOAEL) dose and is helpful for clinical studies.

What is Toxicology?

Toxicology is the study of how natural or man-made poisons cause undesirable effects in living organisms.

What are harmful or adverse effects?

Harmful or adverse effects are those that are damaging to either the survival or normal function of the individual.

What is Toxicity?

The word “toxicity” describes the degree to which a substance is poisonous or can cause injury. The toxicity depends on a variety of factors: dose, duration and route of exposure, shape and structure of the chemical itself, and individual human factors.

What is Toxic?

This term relates to poisonous or deadly effects on the body by inhalation (breathing), ingestion (eating), or absorption, or by direct contact with a chemical

What is a Toxicant?

A toxicant is any chemical that can injure or kill humans, animals, or plants; a poison. The term

"toxicant" is used when talking about toxic substances that are produced by or are a by-product of human-made activities. For example, dioxin (2,3-7,8-tetrachlorodibenzo-p-dioxin {TCDD}), produced as a by-product of certain chlorinated chemicals, is a toxicant. On the other hand, arsenic, a toxic metal, may occur as a natural contaminant of groundwater or may contaminate groundwater as a by-product of industrial activities. If the second case is true, such toxic substances are referred to as toxicants, rather than toxins.

What is a Toxin?

The term "toxin" usually is used when talking about toxic substances produced naturally. A toxin is any poisonous substance of microbial (bacteria or other tiny plants or animals), vegetable, or synthetic chemical origin that reacts with specific cellular components to kill cells, alter growth or development, or kill the organism.

What is a Toxic Symptom?

This term includes any feeling or sign indicating the presence of a poison in the system.

What are Toxic Effects?

This term refers to the health effects that occur due to exposure to a toxic substance; also known as a poisonous effect on the body.

What is Selective Toxicity?

"Selective toxicity" means that a chemical will produce injury to one kind of living matter without harming another form of life, even though the two may exist close together.

How Does Toxicity Develop?

Before toxicity can develop, a substance must come into contact with a body surface such as skin, eye or mucosa of the digestive or respiratory tract. The dose of the chemical, or the amount one comes into contact with, is important when discussing how "toxic" an substance can be.

What is a dose?

The dose is the actual amount of a chemical that enters the body. The dose received may be due to either acute (short) or chronic (long-term) exposure. An acute exposure occurs over a very short period of time, usually 24 hours. Chronic exposures occur over long periods of time such as

weeks, months, or years. The amount of exposure and the type of toxin will determine the toxic effect.

What is dose-response?

Dose-response is a relationship between exposure and health effect, that can be established by measuring the response relative to an increasing dose. This relationship is important in determining the toxicity of a particular substance. It relies on the concept that a dose, or a time of exposure (to a chemical, drug, or toxic substance), will cause an effect (response) on the exposed organism. Usually, the larger or more intense the dose, the greater the response, or the effect. This is the meaning behind the statement "the dose makes the poison."

What is the threshold dose?

Given the idea of a dose-response, there should be a dose or exposure level below which the harmful or adverse effects of a substance are not seen in a population. That dose is referred to as the 'threshold dose'. This dose is also referred to as the no observed adverse effect level (NOAEL), or the no effect level (NEL). These terms are often used by toxicologists when discussing the relationship between exposure and dose. However, for substances causing cancer (carcinogens), no safe level of exposure exists, since any exposure could result in cancer.

What is meant by 'individual susceptibility?'

This term describes the differences in types of responses to hazardous substances, between people. Each person is unique, and because of that, there may be great differences in the response to exposure. Exposure in one person may have no effect, while a second person may become seriously ill, and a third may develop cancer.

What is a "sensitive sub-population?"

A sensitive sub-population describes those persons who are more at risk from illness due to exposure to hazardous substances than the average, healthy person. These persons usually include the very young, the chronically ill, and the very old. It may also include pregnant women and women of childbearing age. Depending on the type of contaminant, other factors (e.g., age, weight, lifestyle, sex) could be used to describe the population.

The Field of Toxicology

Toxicology addresses a variety of questions. For example, in agriculture, toxicology determines the possible health effects from exposure to pesticides or herbicides, or the effect of animal feed additives, such as growth factors, on people. Toxicology is also used in laboratory experiments on animals to establish dose-response relationships. Toxicology also deals with the way chemicals and waste products affect the health of an individual.

Sub-disciplines of Toxicology

The field of toxicology can be further divided into the following sub-disciplines or sub-specialities:

Environmental Toxicology is concerned with the study of chemicals that contaminate food, water, soil, or the atmosphere. It also deals with toxic substances that enter bodies of waters such as lakes, streams, rivers, and oceans. This sub-discipline addresses the question of how various plants, animals, and humans are affected by exposure to toxic substances.

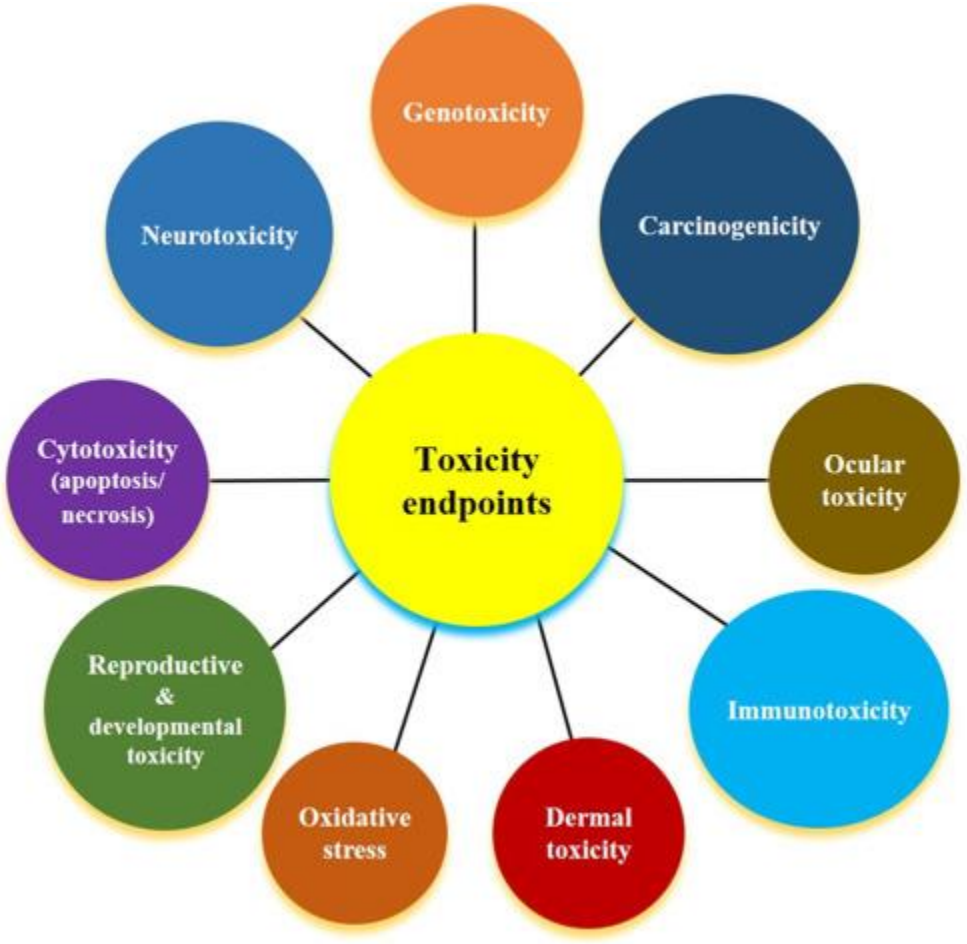
Occupational (Industrial) Toxicology is concerned with health effects from exposure to chemicals in the workplace. This field grew out of a need to protect workers from toxic substances and to make their work environment safe. Occupational diseases caused by industrial chemicals account for an estimated 50,000 to 70,000 deaths, and 350,000 new cases of illness each year in the United States.

Regulatory Toxicology gathers and evaluates existing toxicological information to establish concentration-based standards of “safe” exposure. The standard is the level of a chemical that a person can be exposed to without any harmful health effects.

Food Toxicology is involved in delivering a safe and edible supply of food to the consumer. During processing, a number of substances may be added to food to make it look, taste, or smell better. Fats, oils, sugars, starches and other substances may be added to change the texture and taste of food. All of these additives are studied to determine if and at what amount, they may produce adverse effects. A second area of interest includes food allergies. Almost 30% of the American people have some food allergy. For example, many people have trouble digesting milk, and are lactose intolerant. In addition, toxic substances such as pesticides may be applied to

a food crop in the field, while lead, arsenic, and cadmium are naturally present in soil and water, and may be absorbed by plants. Toxicologists must determine the acceptable daily intake level for those substances.

Clinical Toxicology is concerned with diseases and illnesses associated with short term or long term exposure to toxic chemicals. Clinical toxicologists include emergency room physicians who must be familiar with the symptoms associated with exposure to a wide variety of toxic substances in order to administer the appropriate treatment.



Descriptive Toxicology is concerned with gathering toxicological information from animal experimentation. These types of experiments are used to establish how much of a chemical would cause illness or death. The United States Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA), use information from these studies to set regulatory exposure limits.

Forensic Toxicology is used to help establish cause and effect relationships between exposure to a drug or chemical and the toxic or lethal effects that result from that exposure.

Analytical toxicology identifies the toxicant through analysis of body fluids, stomach content, excrement, or skin.

Mechanistic Toxicology makes observations on how toxic substances cause their effects. The effects of exposure can depend on a number of factors, including the size of the molecule, the specific tissue type or cellular components affected, whether the substance is easily dissolved in water or fatty tissues, all of which are important when trying to determine the way a toxic substance causes harm, and whether effects seen in animals can be expected in humans.

Classification of Toxic Agents:

Toxic substances are classified into the following:

A. Heavy Metals

Metals differ from other toxic substances in that they are neither created nor destroyed by humans. Their use by humans plays an important role in determining their potential for health effects. Their effect on health could occur through at least two mechanisms: first, by increasing the presence of heavy metals in air, water, soil, and food, and second, by changing the structure of the chemical. For example, chromium III can be converted to or from chromium VI, the more toxic form of the metal.

B. Solvents and Vapors

Nearly everyone is exposed to solvents. Occupational exposures can range from the use of “white-out” by administrative personnel, to the use of chemicals by technicians in a nail salon. When a solvent evaporates, the vapors may also pose a threat to the exposed population.

Have participants discuss possible solvents they use ormay be exposed to during the course of a typical day.

C. Radiation and Radioactive Materials

Radiation is the release and propagation of energy in space or through a material medium in the

form of waves, the transfer of heat or light by waves of energy, or the stream of particles from a nuclear reactor (3).

An example for discussion purposes would be the dropping of the atomic bomb during World War II, or the Chernobyl Accident in Russia. These items can be provided by the presenter.

D. Dioxin/Furans

Dioxin, (or TCDD) was originally discovered as a contaminant in the herbicide Agent Orange. Dioxin is also a by-product of chlorine processing in paper producing industries.

E. Pesticides

The EPA defines pesticide as any substance or mixture of substances intended to prevent, destroy, repel, or mitigate any pest. Pesticides may also be described as any physical, chemical, or biological agent that will kill an undesirable plant or animal pest.

Have participants list pesticides they are familiar with either through personal use or in relation to hazardous chemicals in their community.

F. Plant Toxins

Different portions of a plant may contain different concentrations of chemicals. Some chemicals made by plants can be lethal. For example, taxon, used in chemotherapy to kill cancer cells, is produced by a species of the yew plant.

G. Animal Toxins

These toxins can result from venomous or poisonous animal releases. Venomous animals are usually defined as those that are capable of producing a poison in a highly developed gland or group of cells, and can deliver that toxin through biting or stinging. Poisonous animals are generally regarded as those whose tissues, either in part or in their whole, are toxic.

Trainer may want to provide examples of venomous animals, such as snakes, spiders, etc., and poisonous animals, such as puffer fish, or oysters, which may be toxic to some individuals when contaminated with vibrio vulnificus.

H. Subcategories of Toxic Substance Classifications

All of these substances may also be further classified according to their:

- Effect on target organs (liver, kidney, hematopoietic system),
- Use (pesticide, solvent, food additive),
- Source of the agent (animal and plant toxins),
- Effects (cancer mutation, liver injury),
- Physical state (gas, dust, liquid),
- Labeling requirements (explosive, flammable, oxidizer),
- Chemistry (aromatic amine, halogenated hydrocarbon), or
- Poisoning potential (extremely toxic, very toxic, slightly toxic)

Acute toxicity testing

Acute toxicity testing is carried out to determine the effect of a single dose on a particular animal species. In general, it is recommended that acute toxicity testing be carried out with two different animal species (one rodent and one nonrodent). In acute toxicological testing, the investigational product is administered at different dose levels, and the effect is observed for 14 days. All mortalities caused by the investigational product during the experimental period are recorded and morphological, biochemical, pathological, and histological changes in the dead animals are investigated. Acute toxicity testing permits the 50% lethal dose (LD_{50}) of the investigational product to be determined. The LD_{50} was used as an indicator of acute toxicity previously. The determination of the LD_{50} involves large numbers of animals, and the mortality ratio is high. Because of these limitations, modified methods were developed:

- The fixed dose procedure (FDP)
- The acute toxic category (ATC) method
- The up-and-down (UDP) method.

✓ The FDP is used to assess the nonlethal toxicity rather than the lethal dose. The investigational product is administered at fixed dose levels of 5, 50, 500, and 2000 mg/kg and the experimental animal is observed for a specified period. The ATC method is a sequential procedure in which three animals of the same sex are used in each step. In the ATC screening method, four preidentified starting doses may be used, and the test dose should be selected based on the Globally Harmonized Classification system.

- The UDP testing approach is also known as the staircase design. This is the toxicological testing approach most recommended by various regulatory agencies because this method reduces the number of vertebrate animals in research. The UDP screening method involves dosing single animals sequentially at 48 h intervals. Female rodents are preferable for UDP testing. A dose less than the best-estimate LD50 dose is selected and administered to an animal, and the animal is observed for 48 h. If it survives, the study is continued with a higher dose (twice the original dose); if the animal dies, testing is conducted with a lower dose with another animal of the same sex as the original animal. UDP testing is limited to doses up to 2000 mg/kg. Testing procedures used for doses of 2000–5000 mg/kg are different.

In 1996, the Center for Drug Evaluation and Research (CDER) suggested a single dose acute toxicity testing procedure for pharmaceutical substances that uses a fixed safe dose that should not cause adverse events or threaten the life of an animal. The experiment must be carried out with a minimum of two mammalian species, including a nonrodent species, and the animals must be observed for 14 days.

Acute toxicity testing for inhalation

Acute inhalation toxicity testing is performed for aerosol-like preparations. Rats are the most preferred animal species. The animals are acclimatized to laboratory conditions (temperature preferably 22°C ± 2°C). They are maintained in an air flow of 12–15 air changes per hour with adequate oxygen (19%/h). The animal is exposed to the test substance for a minimum of 4 h, and then it is monitored for 14 days. Food is withheld during the exposure period, and water may be withheld under certain conditions. During the observation period, the animal is observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Mortality during the exposure and observation period is noted. Dead animals are examined for histological and pathological changes. At the end of the study, the animals are sacrificed, and pathological changes are evaluated.

Acute toxicity testing for topical preparations

The eye irritation test and skin irritation test are very important for topical preparations. Dermal and ophthalmic preparations can be tested using Draize tests. The Draize eye irritancy test and

the Draize skin irritancy test are used to measure the harmfulness of chemicals and pharmaceutical substances in rabbits and guinea pigs. In the eye irritation test, 0.5 ml of a test substance is administered to an animal's eyes, and the animal is restrained for 4 h. Redness, swelling, discharge, ulceration, hemorrhage, and blindness are assessed and monitored for 14 days.

In the skin irritation test, 0.5 g of a test substance is applied to the surface of an animal's skin. During the observation period (14 days), signs such as erythema and edema are assessed. Some alternative in vitro testing methods are available that can be used in place of the Draize eye irritancy test.[12,13] At the end of the study, the animals are sacrificed and pathological changes are evaluated.

Skin sensitization tests

Skin sensitization tests are carried out using the guinea pig as a model. Skin sensitization is assessed using the Draize test, open epicutaneous test, optimization test, split adjuvant test, guinea pig maximization test (GPMT), Buehler test, and murine local lymph node assay (LLNA). The LLNA method is used as an alternative to the guinea pig Draize test, and it is widely accepted that this method meets regulatory requirements. In the LLNA test, the test substance is applied on the surface of the ears of a mouse for three consecutive days, and the proliferation of lymphocytes in the draining lymph node is measured at the end.

Repeated dose toxicity testing

Repeated dose toxicity testing is carried out for a minimum of 28 days. The test substance is administered daily for a certain period through the oral route. If this route is not convenient, the test substance may be administered parenterally. The test substance is administered regularly at a specific time. Usually, a rodent of any gender and age 5–6 weeks is used for repeated dose toxicity testing. There should be little individual variation between the animals: the allowable variation in the weight is ±20%. A satellite group may be included in the study protocol. This group has both a control group and a high-dose group. Baseline parameters such as the behavioural and biochemical parameters of the animals should be recorded. These will be helpful in calculating percentage changes. The interpretation of human safety details is essential in repeated dose toxicity studies.[14] At the end of the study, tissues from most of the organs are removed, and histological changes are recorded. If possible, immunotoxicity (adverse effects on the immune system) studies are performed on the same animals. Immunotoxicological analysis is

not feasible beyond the period of 14 days. Parameters such as delayed-type hypersensitivity (DTH), mitogen- or antigen-stimulated lymphocyte proliferative responses, macrophage function, and primary antibody response to T-cell dependent antigen are assessed in immunotoxicological studies. The major difference between repeated dose and subchronic toxicity studies is the duration: repeated dose toxicity studies are conducted over a duration of 28 days,and subchronic toxicity studies are carried out over 90 days.

Mutagenicity testing

Mutagenicity testing is used to assess submicroscopic changes in the base sequence of DNA, chromosomal aberrations, and structural aberrations in DNA including duplications, insertions, inversions, and translocations. Certain types of mutations result in carcinogenesis (alteration in proto-oncogenes of tumor suppressor gene mutation), and so the determination of the mutagenicity is essential in the drug development process. In vitro testing is carried out in two or three different bacteria and mammalian cells to cover the end points of gene mutations, clastogenicity, and aneuploidy. The test generally includes a bacterial reverse mutation assay. The choice of an additional test depends on the chemical structure/class of the substance. In vivo mutagenicity which is dose dependent is used to determine the case-by-case basis risk assessment of the test substances. Mutagenicity studies with transgenic animals are more appropriate assay techniques to determine the toxicity of a test substance.

Subchronic oral toxicity testing (repeated dose 90-day oral toxicity testing)

Rodents and nonrodents are used to study the subchronic toxicity of a substance. The test substance is administered orally for 90 days, and weekly body weight variations, monthly biochemical and cardiovascular parameters changes, and behavioral changes are observed. At the end of the study, the experimental animals are sacrificed. Gross pathological changes are observed, and all the tissues are subjected to histopathological analyses. There should be little individual variation between the animals, and the allowed weight variation range is ±20%. A satellite group may be included in the study protocol, and this group has both a control group and a high-dose group.

Chronic oral toxicity testing

Chronic toxicity studies are conducted with a minimum of one rodent and one nonrodent species. The test compound is administered over more than 90 days, and the animals are observed periodically. A chronic toxicology study provides inferences about the long-term effect of a test

substance in animals, and it may be extrapolated to the human safety of the test substance. The report on chronic oral toxicity is essential for new drug entities. There should be little individual variation between the animals, and the allowable weight variation range is ±20%. A satellite group may be included in the study protocol. This group has both a control group and high-dose group. During the study period, the animals are observed for normal physiological functions, behavioral variations and alterations in biochemical parameters. At the end of the study, tissues are collected from all parts of the animal and subjected to histological analyses.

Carcinogenicity testing

Both rodents and nonrodent animal species may be used in carcinogenicity testing. The tests are carried out over the greater portion of an animal's lifespan. During and after exposure to test substances, the experimental animals are observed for signs of toxicity and development of tumors. If these are not found, a test may be terminated after 18 months in the case of mice and hamsters and after 24 months with rats. If the animals are healthy, hematological analysis is performed after the 12 months and the 18 months, respectively, and the study is terminated. The animals are sacrificed, and gross pathological changes are noted and histopathological studies are carried out on all the tissues.

One-generation reproduction toxicity testing

The test compound is administered to both male and female animals. Administration is for the duration of one complete spermatogenic cycle in male animals and for two complete estrous cycles for female animals. Rodents are preferred for the one-generation reproduction toxicity testing. After the completion of the specified duration of drug administration, the animals are allowed to mate. The test compound is administered to the female animals during the period of pregnancy and nursing. The sperms of male animals are collected, and the sperm morphology and motility are analyzed. During the study period, the animals are observed for signs of toxicity. Parturition, the number of offspring and their sexes are recorded. The number of dead and live pups are noted, and live pups are weighed in the morning and evening each day during the first 4 days. After the termination of the study, the animals and pups are sacrificed and subjected to a histopathological examination.

Two-generation reproduction toxicity studies

Both male and female rodents are administered the test substance. The duration of administration extends to one complete spermatogenic cycle for males and two complete estrous cycles for

females. After the administration period, the animals are intertwined (parental mating), after which the female animals are separated. Sperms are collected from male animals, and the sperm morphology and motility are analyzed. The test substance is administered continuously to pregnant female animals, which are monitored regularly for mortality and signs of toxicity. After parturition, nursing rats are administered the test drug, and the mortality of the pups (F1 generation) is observed. From the F1 generation, one male and one female animal are selected. The same procedure is repeated to get the F2 generation offspring. F1 offsprings are not allowed to mate until they have attained full sexual maturity, and pairs without a pregnancy are evaluated for infertility. Necropsies and histological examinations are carried out. At the end of the study, the animals are sacrificed and gross pathological and histological examinations are carried out on all the animals.

Genetic toxicity testing

Genetic toxicity tests are used to identify gene mutations, chromosome changes, and alterations in the DNA sequencing. These tests are usually conducted in various species including whole animals, plants, micro-organisms, and mammalian cells. In the whole animal model, rodents are preferred. Genetic toxicity is assessed using the rodent chromosome assay, dominant lethal assay, mouse-specific locus test, micronucleus test, heritable translocation assay, and sister chromatid exchange assay.

Regulatory requirements

Before conducting any clinical study, the safety of the test substance should be assessed using animals. The target organ toxicity, relationship between the dose and response, relevant human effects, and any complications arising during treatment (adverse drug reactions) should be established through preclinical evaluations. The toxicity study should be carried out with a minimum of three doses viz. low, medium, and high doses in the experimental animals and the toxic effect compared with data from a control group of animals. The Committee for Proprietary Medicinal Products (CPMP) has set guidelines on the toxicological experiment on various animal species. The guideline instructs that the maximum selected dose should be sufficient to identify the target organ toxicity. From the toxicological evaluation, the no observed effect level (NOEL) or NOAEL, which may be useful for human studies, may be established. The low dose, intermediate dose, and high dose used in the toxicity test provide the NOEL, dose–response relationship, and target organ toxicity in animals, respectively.

Genotoxicity covers a broader spectrum of endpoints than mutagenicity, includes DNA damage assessments. DNA damage is not themselves necessarily transmissible to the next generation of cells, pre-mutagenic

Mutagenicity refers to the production of transmissible genetic alterations. Somatic cell genotoxicity may lead to cancer. Germ cell genotoxicity may lead to infertility or diseased children

Teratogenicity

Capacity of a drug to cause foetal abnormalitites when administered to the pregnant mother.

Placenta does not consider a strict barrier and any drug can cross it to a greater or lesser extent.

The embryo is one of the most dynamic biological systems

Genotoxicity

- Genotoxicity tests can be defined as in vitro and in vivo tests designed to detect compounds that induce genetic damage by various mechanisms.
- These tests enable hazard identification with respect to damage to DNA and its fixation. Genotoxins can be of the following category depending on its effects

  1) Carcinogens or cancer causing agents

  2)Mutagens or mutation causing agents

  3)Teratogens or birth defect causing agents. Agents that can cause direct or indirect damage to the DNA

  • Reactive oxygen species. • UV and ionizing radiations. • Nucleoside analogues. •Topoisomerase inhibitors.

  • Protein synthesis inhibitors.

OECD GUIDELINES

• Genetic Toxicology : was first published in 1987

Following a global update of the Genetic Toxicology

Latest revision provides :

(1) General background and historical information on the OECD genetic toxicology.

(2) A brief overview of the important types of genetic damage evaluated by these tests.

(3) A description of the specific tests.

SCHEDULE –Y • Gene mutation in bacteria

- An in-vitro test with cytogenic evaluation of chromosomal damage

- An in-vivo test for chromosomal damage using rodent hematopoietic cells (chromosomal aberration , micronucleus
- DNA adduct tests
- DNA strands break , DNA repair /recombination .
- ICH

S2A: Guidance on Specific aspects of Regulatory Tests for Pharmaceuticals

S2B: Standard Battery for Testing of Pharmaceuticals • M3:Timing of Pre- Clinical Studies in

Relation to Clinical Trials

Importance

Genotoxicity assays have become an integral component of regulatory requirement.

Compounds which are positive in these tests have the potential to be human carcinogens and/or mutagens. So it's used in prediction.

Aim

- To identify substances that can cause genetic alterations in somatic and/or germ cells.
- To identify substances that causes genetic alterations and thus uses this information in regulatory decisions.

Mechanism of Genotoxicity

The damage to the genetic material is caused by the interactions of the genotoxic substance with the DNA structure and sequence.

These genotoxic substance interact at a specific location or base sequence of the DNA structure causing lesions, breakage, fusion, deletion, mis-segregation or non- disjunction leading to damage and mutation.

Standard test battery for genotoxicity

AMES TEST (Bacterial reverse mutation test) Bacteria: Salmonella typhimurium or strains E.coli.

Ames test was brought forward by Bruce Ames in 1970.

• He is professor in university of California , berkely . In department of biochemistry.

• He developed this method because previous methods were expensive and time consuming.

Principle

- Identifies substances that induce gene mutations by base substitutions or frame-shifts.

- Two species of bacteria Salmonella typhimurium and Escherichia coli with identified mutations in an amino acid i.e. His or Trp as the reporter locus.
- It detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid

PROCEDURE

1. Plate incorporation method.
2. Pre-incubation method.

STEPS OF AMES TEST:

• Prepare the culture of Salmonella histidine auxotroph's (His-).

• Mix the bacterial cells and test substance in dilute molten top agar with a small amount of histidine in one set, and control with complete medium plus large amount of histidine .

• Pour the molten mixture on the top of minimal agar plates and incubate at 37°C for 2-3 days. Until histidine is depleted all the His- cells will grow in the presence of test mutagen.

• When the histidine is completely exhausted only the revertants will grow on the plate.

• High number of colonies represent the greater mutagenicity

INVITRO MAMALIAN CELL MICRONUCLEUS TEST -2010

Micronuclei are the small nucleus that forms whenever a chromosome or its fragment is incorporated with daughter nuclei during cell division.

Principle

• Micronuclei are the product of fragmented chromosomes or mitotic spindle failure in a cell. Micronuclei are formed by condensation of acentric chromosomes that are not included in the main nuclei following the anaphase.

• Micronuclei are formed in the cytoplasm through the following events:

• In anaphase, a chromatid and chromosomal fragments lag behind when the centric elements move towards the spindle poles. Micronucleus arises from chromosomal fragments or acentric chromosomes that are not incorporated into daughter nuclei at mitosis because they lack a centromere.

Mammalian Erythrocyte Micronucleus Test

• Animals are exposed to the test substance by an appropriate route

• If bone marrow > the animals are sacrificed, bone marrow extracted, and preparations made and stained

• If peripheral blood > the blood is collected at appropriate times after treatment and smear preparations are made and stained.

• Preparations are analysed for the presence of micronuclei

• For the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts (rodents)

• Identifies micronuclei containing lagging chromosome fragments or whole chromosomes.

• An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage because they lack main nucleus.

INVITRO MAMMALIAN CHROMASOMALABBERATION TEST

Principle

• After exposure of cell cultures, treated with a metaphase- arresting substance colchicine . with and without metabolic activation

• Harvested, stained and metaphase cells are analysed microscopically for the presence of chromosome aberrations.

Cell lines: CHO, CHL, V79, TK6.

• Structural aberrations may be of two types: chromosome or chromatid.

MAMMALIAN BONE MARROW CHROMOSOME ABERRATION TEST

Principle

• For the detection of structural chromosome aberrations induced by test compounds only in bone marrow cells of animals (rodents).

• Animals are exposed to the test substance , metaphase-arresting agent , sacrificed at appropriate times after treatment.

Bone marrow cells are usually obtained from the femurs or tibias immediately after sacrifice, and stained using established methods.

• Blood: tail vein or other appropriate blood vessel, smear preparations are made and then stained

• DNA specific stain [e.g acridine orange or Hoechst 33258 plus pyronin-Y]

Prior to sacrifice, animals are injected i.p with an appropriate dose of a metaphase arresting agent, sampled thereafter. Cells are harvested from the bone marrow and analysed from chromosome aberrations.

• Chromosome preparation: bone marrow in hypotonic solution, spread on slides and stained

GENERAL PRINCIPLES OF POISONING

Poisoning is contact with a substance that results in toxicity. Symptoms vary, but certain common syndromes may suggest particular classes of poisons. Diagnosis is primarily clinical, but for some poisonings, blood and urine tests can help. Treatment is supportive for most poisonings; specific antidotes are necessary for a few. Prevention includes labeling drug containers clearly and keeping poisons out of the reach of children.

Most poisonings are dose-related. Dose is determined by concentration over time. Toxicity may result from exposure to excess amounts of normally nontoxic substances. Some poisonings result from exposure to substances that are poisonous at all doses. Poisoning is distinguished from hypersensitivity and idiosyncratic reactions, which are unpredictable and not dose-related, and from intolerance, which is a toxic reaction to a usually nontoxic dose of a substance.

Poisoning is commonly due to ingestion but can result from injection, inhalation, or exposure of body surfaces (eg, skin, eye, mucous membranes). Many commonly ingested nonfood substances are generally nontoxic (see Table: Substances Usually Not Dangerous When Ingested*); however, almost any substance can be toxic if ingested in excessive amounts.

Accidental poisoning is common among young children, who are curious and ingest items indiscriminately despite noxious tastes and odors; usually, only a single substance is involved. Poisoning is also common among older children, adolescents, and adults attempting suicide; multiple drugs, including alcohol, acetaminophen, and other OTC drugs, may be involved. Accidental poisoning may occur in the elderly because of confusion, poor eyesight, mental impairment, or multiple prescriptions of the same drug by different physicians (see also

After exposure or ingestion and absorption, most poisons are metabolized, pass through the GI tract, or are excreted. Occasionally, tablets (eg, aspirin, iron, enteric-coated drugs) form large concretions (bezoars) in the GI tract, where they tend to remain, continuing to be absorbed and causing toxicity.

Symptoms and Signs

Symptoms and signs of poisoning vary depending on the substance (see Table: Symptoms and Treatment of Specific Poisons). Also, different patients poisoned with the same substance may present with very different symptoms. However, 6 clusters of symptoms (toxic syndromes, or toxidromes) occur commonly and may suggest particular classes of substances (see Table: Common Toxic Syndromes (Toxidromes)). Patients who ingest multiple substances are less likely to have symptoms characteristic of a single substance.

Symptoms typically begin soon after contact but, with certain poisons, are delayed. The delay may occur because only a metabolite is toxic rather than the parent substance (eg, methanol, ethylene glycol, hepatotoxins). Ingestion of hepatotoxins (eg, acetaminophen, iron, *Amanita phalloides* mushrooms) may cause acute liver failure that occurs one to a few days later. With metals or hydrocarbon solvents, symptoms typically occur only after chronic exposure to the toxin.

Ingested and absorbed toxins generally cause systemic symptoms. Caustics and corrosive liquids damage mainly the mucous membranes of the GI tract, causing stomatitis, enteritis, or perforation. Some toxins (eg, alcohol, hydrocarbons) cause characteristic breath odors. Skin contact with toxins can cause various acute cutaneous symptoms (eg, rashes, pain, blistering); chronic exposure may cause dermatitis.

Inhaled toxins are likely to cause symptoms of upper airway injury if they are water-soluble (eg, chlorine, ammonia) and symptoms of lower airway injury and noncardiogenic pulmonary edema if they are less water-soluble (eg, phosgene). Inhalation of carbon monoxide, cyanide, or hydrogen sulfide gas can cause organ ischemia or cardiac or respiratory arrest. Eye contact with toxins (solid, liquid, or vapor) may damage the cornea, sclera, and lens, causing eye pain, redness, and loss of vision.

Some substances (eg, cocaine, phencyclidine, amphetamine) can cause severe agitation, which can result in hyperthermia, acidosis, and rhabdomyolysis.

Diagnosis

Consideration of poisoning in patients with altered consciousness or unexplained symptoms

History from all available sources

Selective, directed testing

The first step of diagnosis of poisoning is to assess the overall status of the patient. Severe poisoning may require rapid intervention to treat airway compromise or cardiopulmonary collapse.

Poisoning may be known at presentation. It should be suspected if patients have unexplained symptoms, especially altered consciousness (which can range from agitation to somnolence to coma). If purposeful self-poisoning occurs in adults, multiple substances should be suspected.

History is often the most valuable tool. Because many patients (eg, preverbal children, suicidal or psychotic adults, patients with altered consciousness) cannot provide reliable information, friends, relatives, and rescue personnel should be questioned. Even seemingly reliable patients may incorrectly report the amount or time of ingestion. When possible, the patient's living quarters should be inspected for clues (eg, partially empty pill containers, a suicide note, evidence of recreational drug use). Pharmacy and medical records may provide useful information. In potential workplace poisonings, coworkers and supervisors should be questioned. All industrial chemicals must have a material safety data sheet

(MSDS) readily available at the workplace; the MSDS provides detailed information about toxicity and any specific treatment.

Testing

In most cases, laboratory testing provides limited help. Standard, readily available tests to identify common drugs of abuse (often called toxic screens) are qualitative, not quantitative. These tests may provide false-positive or false- negative results, and they check for only a limited number of substances. Also, the presence of a drug of abuse does not necessarily indicate that the drug caused the patient's symptoms or signs. Urine drug screening is used most often but has limited value and usually detects classes of drugs or metabolites rather than specific drugs.

For example, an opioid urine immunoassay test does not detect fentanyl or methadone but does react with very small amounts of morphine or codeine analogues. The test used to identify cocaine detects a metabolite rather than cocaine itself.

For most substances, blood levels cannot be easily determined or do not help guide treatment. For a few substances (eg, acetaminophen, aspirin, carbon monoxide, digoxin, ethylene glycol, iron, lithium, methanol, phenobarbital, phenytoin, theophylline), blood levels may help guide treatment. Many authorities recommend measuring acetaminophen levels in all patients with mixed ingestions because acetaminophen ingestion is common, is often asymptomatic during the early stages, and can cause serious delayed toxicity that can be prevented by an antidote. For some substances, other blood tests (eg, PT for warfarin overdose, methemoglobin levels for certain substances) help guide treatment. For patients who have altered consciousness or abnormal vital signs or who have ingested certain substances, tests should include serum electrolytes, BUN, creatinine, serum osmolality, glucose, coagulation studies, and ABGs. Other tests (eg, methemoglobin level, carbon monoxide level, brain CT) may be indicated for certain suspected poisons or in certain clinical situations.

For certain poisonings (eg, due to iron, lead, arsenic, other metals, or to packets of cocaine or other illicit drugs ingested by so-called body packers), plain abdominal x-rays may show the presence and location of ingested substances.

For poisonings with drugs that have cardiovascular effects or with an unknown substance, ECG and cardiac monitoring are indicated.

If blood levels of a substance or symptoms of toxicity increase after initially decreasing or persist for an unusually long time, a bezoar, a sustained-release preparation, or reexposure (ie, repeated covert exposure to a recreationally used drug) should be suspected.

Treatment

Supportive care

- ✓ Activated charcoal for serious oral poisonings
- ✓ Occasional use of specific antidotes or dialysis
- ✓ Only rare use of gastric emptying

Seriously poisoned patients may require assisted ventilation or treatment of cardiovascular collapse. Patients with impaired consciousness may require continuous monitoring or restraints.

The discussion of treatment for specific poisonings, below and in see Table: Common Specific Antidotes, see Table: Guidelines for Chelation Therapy, and see Table: Symptoms and Treatment of Specific Poisons , is general and does not include specific complexities and details. Consultation with a poison control center is recommended for any poisonings except the mildest and most routine.

Initial stabilization

- Maintain airway, breathing, and circulation
- IV naloxone
- IV dextrose and thiamine
- IV fluids, sometimes vasopressors

Airway, breathing, and circulation must be maintained in patients suspected of a systemic poisoning. Patients without a pulse or BP require emergency cardiopulmonary resuscitation.

If patients have apnea or compromised airways (eg, foreign material in the oropharynx, decreased gag reflex), an endotracheal tube should be inserted (see Tracheal Intubation). If patients have respiratory depression or hypoxia, supplemental oxygen or mechanical ventilation should be provided as needed.

IV naloxone (2 mg in adults; 0.1 mg/kg in children; doses as high as 10 mg may be necessary in some cases) should be tried in patients with apnea or severe respiratory depression while maintaining airway support. In opioid addicts, naloxone may precipitate withdrawal, but withdrawal is preferable to severe respiratory depression. If respiratory depression persists despite use of naloxone, endotracheal intubation and continuous mechanical ventilation are required. If naloxone relieves respiratory depression, patients are monitored; if respiratory depression recurs, patients should be treated with another bolus of IV naloxone or endotracheal intubation and mechanical ventilation. Using a low-dose continuous

naloxone infusion to maintain respiratory drive without precipitating withdrawal has been suggested but in reality can be very difficult to accomplish.

IV dextrose (50 mL of a 50% solution for adults; 2 to 4 mL/kg of a 25% solution for children) should be given to patients with altered consciousness or CNS depression, unless hypoglycemia has been ruled out by immediate bedside determination of blood glucose.

Thiamine (100 mg IV) is given with or before glucose to adults with suspected thiamine deficiency (eg, alcoholics, undernourished patients).

IV fluids are given for hypotension. If fluids are ineffective, invasive hemodynamic monitoring may be necessary to guide fluid and vasopressor therapy. The first-choice vasopressor for most poison-induced hypotension is norepinephrine 0.5 to 1 mg/min IV infusion, but treatment should not be delayed if another vasopressor is more immediately available.

Topical decontamination

Any body surface (including the eyes) exposed to a toxin is flushed with large amounts of water or saline. Contaminated clothing, including shoes and socks, and jewelry should be removed. Topical patches and transdermal delivery systems are removed.

Activated charcoal

Charcoal is usually given, particularly when multiple or unknown substances have been ingested. Use of charcoal adds little risk (unless patients are at risk of vomiting and aspiration) but has not been proved to reduce overall morbidity or mortality. When used, charcoal is given as soon as possible. Activated charcoal adsorbs most toxins because of its molecular configuration and large surface area. Multiple doses of activated charcoal may be effective for substances that undergo enterohepatic recirculation (eg, phenobarbital, theophylline) and for sustained- release preparations. Charcoal may be given at 4- to 6-h intervals for serious poisoning with such substances unless bowel sounds are hypoactive. Charcoal is ineffective for caustics, alcohols, and simple ions (eg, cyanide, iron, other metals, lithium).

The recommended dose is 5 to 10 times that of the suspected toxin ingested. However, because the amount of toxin ingested is usually unknown, the usual dose is 1 to 2 g/kg, which is about 10 to 25 g for children < 5 yr and 50 to 100 g for older children and adults. Charcoal is given as a slurry in water or soft drinks. It may be unpalatable and results in vomiting in 30% of patients. Administration via a gastric tube may be considered, but caution should be used to prevent trauma caused by tube insertion or aspiration of charcoal; potential benefits must outweigh risks. Activated charcoal should probably be used without sorbitol or other cathartics, which have no clear benefit and can cause dehydration and electrolyte abnormalities.

Gastric emptying

Gastric emptying, which used to be well-accepted and seems intuitively beneficial, should not be routinely done. It does not clearly reduce overall morbidity or mortality and has risks. Gastric

emptying is considered if it can be done within 1 h of a life-threatening ingestion. However, many poisonings manifest too late, and whether a poisoning is life threatening is not always clear.

Thus, gastric emptying is seldom indicated and, if a caustic substance has been ingested, is contraindicated (see Caustic Ingestion).

If gastric emptying is used, gastric lavage is the preferred method. Gastric lavage may cause complications such as epistaxis, aspiration, or, rarely, oropharyngeal or esophageal injury. Syrup of ipecac has unpredictable effects, often causes prolonged vomiting, and may not remove substantial amounts of poison from the stomach. Syrup of ipecac may be warranted if the ingested agent is highly toxic and transport time to the emergency department is unusually long, but this is uncommon in the US.

For gastric lavage, tap water is instilled and withdrawn from the stomach via a tube. The largest tube possible (usually > 36 French for adults or 24 French for children) is used so that tablet fragments can be retrieved. If patients have altered consciousness or a weak gag reflex, endotracheal intubation should be done before lavage to prevent aspiration. Patients are placed in the left lateral decubitus position to prevent aspiration, and the tube is inserted orally. Because lavage sometimes forces substances farther into the GI tract, stomach contents should be aspirated and a 25-g dose of charcoal should be instilled through the tube immediately after insertion. Then aliquots (about 3 mL/kg) of tap water are instilled, and the gastric contents are withdrawn by gravity or syringe. Lavage continues until the withdrawn fluids appear free of the substance; usually, 500 to 3000 mL of fluid must be instilled. After lavage, a 2nd 25-g dose of charcoal is instilled.

Whole-bowel irrigation

This procedure flushes the GI tract and theoretically decreases GI transit time for pills and tablets. Irrigation has not been proved to reduce morbidity or mortality. Irrigation is indicated for any of the following:

Some serious poisonings due to sustained-release preparations or substances that are not adsorbed by charcoal (eg, heavy metals)

Drug packets (eg, latex-coated packets of heroin or cocaine ingested by body packers)

A suspected bezoar

A commercially prepared solution of polyethylene glycol (which is nonabsorbable) and electrolytes is given at a rate of 1 to 2 L/h for adults or at 25 to 40 mL/kg/h for children until the rectal effluent is clear; this process may require many hours or even days. The solution is usually given via a gastric tube, although some motivated patients can drink these large volumes.

Alkaline diuresis

Alkaline diuresis enhances elimination of weak acids (eg, salicylates, phenobarbital). A solution made by combining 1 L of 5% D/W with 3 50-mEq ampules of $NaHCO_3$ and 20 to 40 mEq of K can be given at a rate of 250 mL/h in adults and 2 to 3 mL/kg/h in children. Urine pH is kept at > 8, and K must be repleted. Hypernatremia, alkalemia, and fluid overload may occur but are usually not serious. However, alkaline diuresis is contraindicated in patients with renal insufficiency.

Dialysis

Common toxins that may require dialysis or hemoperfusion include

- Ethylene glycol
- Lithium
- Methanol
- Salicylates
- Theophylline

These therapies are less useful if the poison is a large or charged (polar) molecule, has a large volume of distribution (ie, if it is stored in fatty tissue), or is extensively bound to tissue protein (as with digoxin, phencyclidine, phenothiazines, or tricyclic antidepressants). The need for dialysis is usually determined by both laboratory values and clinical status. Methods of dialysis include hemodialysis, peritoneal dialysis, and lipid dialysis (which removes lipid- soluble substances from the blood), as well as hemoperfusion (which more rapidly and efficiently clears specific poisons—see Overview of Renal Replacement Therapy).

Specific antidotes

For the most commonly used antidotes, see Table: Common Specific Antidotes. Chelating drugs are used for poisoning with heavy metals and occasionally with other drugs (see Table: Guidelines for Chelation Therapy). IV fat emulsions in 10% and 20% concentrations and high-

dose insulin therapy have been used to successfully treat several different cardiac toxins (eg, bupivacaine, verapamil).

gives maximum protection against hepatotoxicity when administered within 10 hours of paracetamol overdose, but can be given with (lesser) benefit upto 36 hours Indications 1. Paracetamol ingested is more than

100 mg/kg. 2. Likelihood exists of paracetamol-induced hepatic failure General Principles in Rx of Poisoning & common drug poisoning

Salicylates Acute Poisoning: **a. Early**: Nausea, vomiting, sweating, tinnitus, vertigo & hyperventilation due to respiratory alkalosis. Disorientation, hyperactivity, slurred speech, ataxia, and restlessness may be early findings in patients with severe toxicity b. Late— Deafness, hyperactivity, agitation, delirium, convulsions, hallucinations, hyperpyrexia. Coma is unusual c. Complications—Metabolic acidosis, pulmonary oedema, rhabdomyolysis, cardiac depression, thrombocytopenic purpura General Principles in Rx of Poisoning & common drug poisoning

Chronic Poisoning (Salicylism): This is characterised by slow onset of confusion, agitation, lethargy, disorientation, slurred speech, hallucinations, convulsions, and coma.

Sometimes “salicylism” presents as pseudosepsis syndrome characterised by fever, leukocytosis, hypotension, and multi-organ system failure: ARDS, acute renal failure and coagulopathy (DIC) General Principles in Rx of Poisoning & common drug poisoning Salicylates must not be therapeutically administered to children under 15 years of age, especially if they are suffering from chicken pox or influenza. There is a serious risk of precipitating Reye’s syndrome which can be fatal.

Main feature: onset of hepatic failure & encephalopathy General Principles in Rx of Poisoning & common drug poisoning

Treatment

• Patients with major signs or symptoms (metabolic acidosis,dehydration, mental status changes, seizures, pulmonary oedema) should be admitted to the Intensive Care Unit regardless of serum salicylate level

• Minor symptoms only (i.e. nausea, tinnitus) following acute overdose may be managed in the emergency department with decontamination and alkaline diuresis if the salicylate level is shown to be declining General Principles in Rx of Poisoning & common drug poisoning Stomach wash

may be beneficial upto 12 hours after ingestion, since toxic doses of salicylates often cause pylorospasm and delayed gastric emptying.

• Activated charcoal (AC): It is said to be very efficacious in the treatment of salicylate poisoning since each gram of AC can adsorb 550 mg of the drug. A 10:1 ratio of AC to salicylate ingested appears to result in maximum efficiency. The initial dose of AC can be combined with a cathartic to enhance elimination. General Principles in Rx of Poisoning & common drug poisoning.

Atropine

• Belladonna poisoning may occur due to drug overdose or consumption of seeds & berries of belladonna/datura plant

• Dry mouth, difficulty in swallowing & talking Dilated pupil, photophobia, blurring of near vision, palpitation, psychotic behaviour, ataxia, delirium, visual hallucinations,Hypotension, weak & rapid pulse, cardiovascular collapse with respiratory depression

• Convulsions & coma occur only in severe poisoning General Principles in Rx of Poisoning & common drug poisoning

Iron

• Has a direct corrosive action on the stomach & proximal small bowel

• Once absorbed, produces shock, metabolic acidosis, liver failure& death

• Initially, GI symptoms prevail with persistent vomiting, abdominal pain& hemorrhage

• A quiescent phase may be observed, followed by shock, coma, metabolic acidosis& liver failure General Principles in Rx of Poisoning & common drug poisoning

Treatment

• Management of iron poisoning includes gastric lavage with normal saline

• The treatment of choice is the antidote desferrioxamine, which chelates free serum iron in the plasma to form ferrioxamine

Indications :

- ✓ All critical patients who present with coma, shock, or hemorrhage
- ✓ All patients with a serum iron level higher than 500 mg/dL
- ✓ Patients who aresymptomatic with a serum iron > 300 mg/dL

Morphine • It may be accidental, suicidal or seen in drug abusers. The human lethal dose is estimated to be about 250 mg • Stupor or coma, flaccidity, shallow & occasional breathing, cyanosis, pinpoint pupil,fall in BP & shock; convulsions may be seen in few, pulmonary edema occurs at terminal stages, death is due to respiratory failure General Principles in Rx of Poisoning & common drug poisoning 54

Treatment • Consists of respiratory support & maintenance of BP (i.v.fluids, vasoconstrictors) • Gastric lavage should be done with pot. permanganate to remove unabsorbed drug • Specific antidote: Naloxone 0.4–0.8 mg i.v. repeated every 2–3 min till respiration picks up, is the specific antagonist of choice Due to short duration of action, naloxone should be repeated every 1–4 hours, according to the response.

Barbiturate poisoning and management

Barbiturates are a leading cause of acute poisoning because of their ready availability. Most of the cases are suicidal but some occur through error or ungraded exploration in children. The poisoning is characterized by stupor or coma, areflexia and in late cases, severe respiratory depression and cardiovascular insufficiency. It is a potentially fatal form of poisoning with overall mortality of about7%

Barbiturates are chemical derivatives of barbituric acid and depending on their duration of action, they can be classified as long acting (>6 hours), intermediate acting (3-6 hours) and short acting (<3 hours). All barbiturates bind to Gamma amino butyric acid receptors and prolong the opening of chloride channels, thus inhibiting excitable cells of the central nervous system.

Management of barbiturate poisoning

Cardiorespiratory support: A clear airway is ensured by thorough suctioning and insertion of oral airway.

Gastric lavage: If no more than 2-4 hours have passed since ingestion of the barbiturate, gastric lavage is done.

Activated charcoal: inert nontoxic adsorbent which binds high molecular weight compounds due to intermolecular attractions. 1gKg-1 is administered through nasogastric tube. Cathartic like magnesium sulphate can be used along with it for further removal of barbiturates but hypermagnesemia can occur.

Forced diuresis with alkalinisation of urine: This is especially useful in long barbiturates which are largely excreted by the kidney. At high rates ofurine flow (by the use of diuretics), the renal clearance of barbiturates is increased. Thus, it shortens the duration of coma and decreases plasma concentration of barbiturates. This should be avoided in older patients as it can cause pulmonary oedema, hyponatraemia.

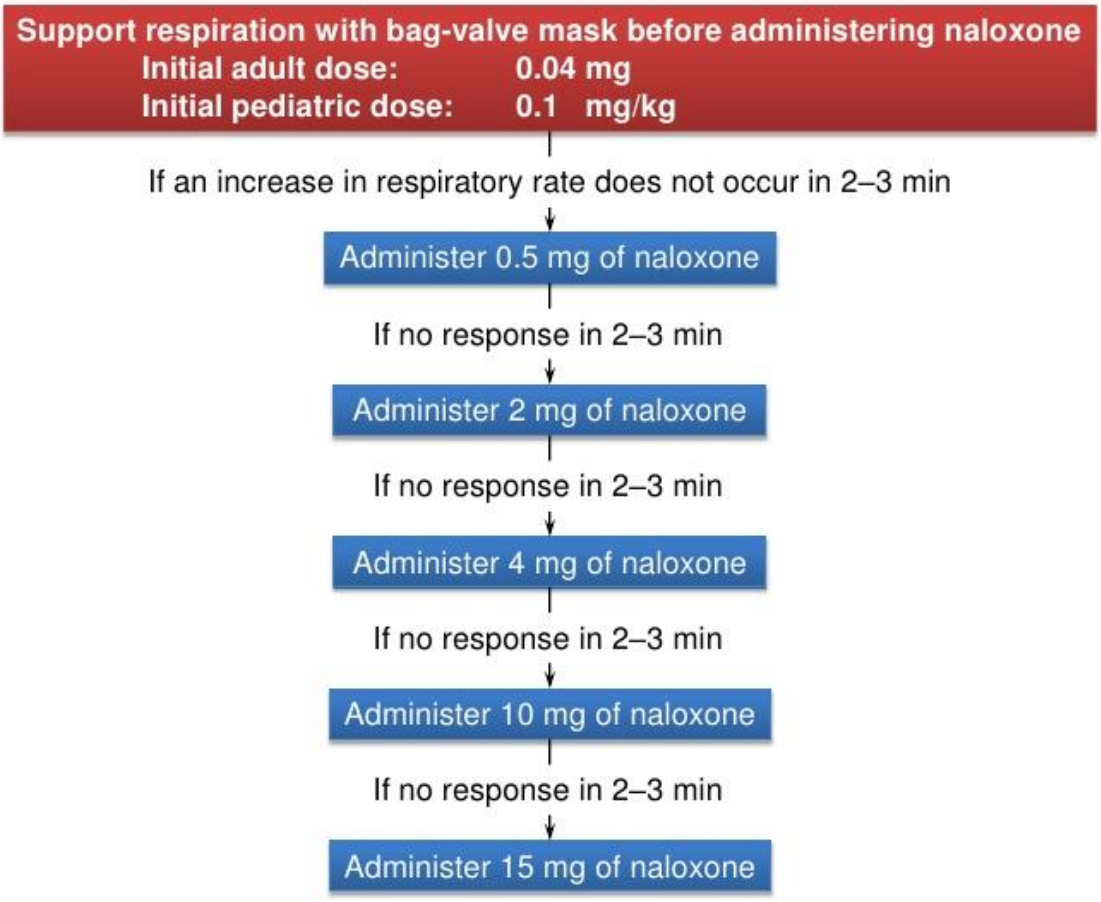
Supportive care: The most important aspect of management in these cases is closeobservation and quality nursing care. Prophylactic antibiotics shouldbe started. Good oral hygiene, temperature maintenance and posture change atregular intervals.

Opioid poisoning and management

Opioids are widely used for analgesic purposes.If taken as prescribed, they are safe and effective. Overdosing,however, can cause coma and life-threatening respiratorydepression. In the acute care setting, physicians often basetreatment on the presence of classic "opioid syndrome"characteristics—mental status depression, hypoventilation,miosis (pinpoint pupils), and reduced bowel motility.

Opioids find use in many situations including surgicaland medical emergencies. They are used for anesthesia, sedation, and postoperative analgesia and to treat trauma and burns, pain in orthopedic and terminal illness, cough and diarrhea. The major effects of opioids are on the central nervous system. Opioids provide analgesia without sedation, euphoria, or loss of consciousness. Theyalso affect the pulmonary, cardiovascular, and gastrointestinal systems by causing their classic symptoms associated with intoxication or withdrawal respiratory depression, bradycardia, nausea, vomiting and other classic symptoms associated with intoxication or withdrawal.

Naloxone, the antidote for opioid overdose, is a competitive mu opioid–receptor antagonist that reverses all signs of opioid intoxication. It is active when the parenteral, intranasal, or pulmonary route of administration is used but has negligible bioavailability after oral administration because of extensive first-pass metabolism. The onset of action is less than 2 minutes when naloxone for adults is administered intravenously and its apparent duration of action is 20 to 90 minutes, a much shorter period.An alternative to the administration of naloxone is orotracheal intubation, a procedure that safely ensures oxygenation and ventilation while providing protection against aspiration.


Support respiration with bag-valve mask before administering naloxone
Initial adult dose: 0.04 mg
Initial pediatric dose: 0.1 mg/kg
If an increase in respiratory rate does not occur in 2–3 min
Administer 0.5 mg of naloxone
If no response in 2–3 min
Administer 2 mg of naloxone
If no response in 2–3 min
Administer 4 mg of naloxone
If no response in 2–3 min
Administer 10 mg of naloxone
If no response in 2–3 min
Administer 15 mg of naloxone

CHRONOPHARMACOLOGY

Chronopharmacology is the branch of chronobiology concerned in this effect of drug upon the timing of biological events and cycles related to biological timing and endogenous periodicities to the effect of drugs. When a drug administered the pharmacological action of the drug can be predicted based on the body circadian rhythm. It is feasible correlate with body functions and time. In the same way as sleep – awake, feed hunger, joy depression are regulated by the biological clocks, the maximum efficacy and minimum toxicity of a drug can be achieved if it is administered at appropriate time i.e. right drug in the right form at right dose at right time.So the given drug acts synergistically with biological clock. Pharmacology is based on circadian rhythms which are important in medicine. A circadian clock in the brain coordinates daily physiological cycle.

Choice of Chronopharmacology

Auto induction:-

Multiple, repeated dose increases enzymes responsible for its elimination there will be is known as auto induction increasing its clearance. This is called as auto induction. It depends on dose and concentration of the drug. It has a number of therapeutic consequences. It impacts the time to achieve steady state and limits one's ability to use information from a single dose to multiple doses taken kinetics 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 withtime.

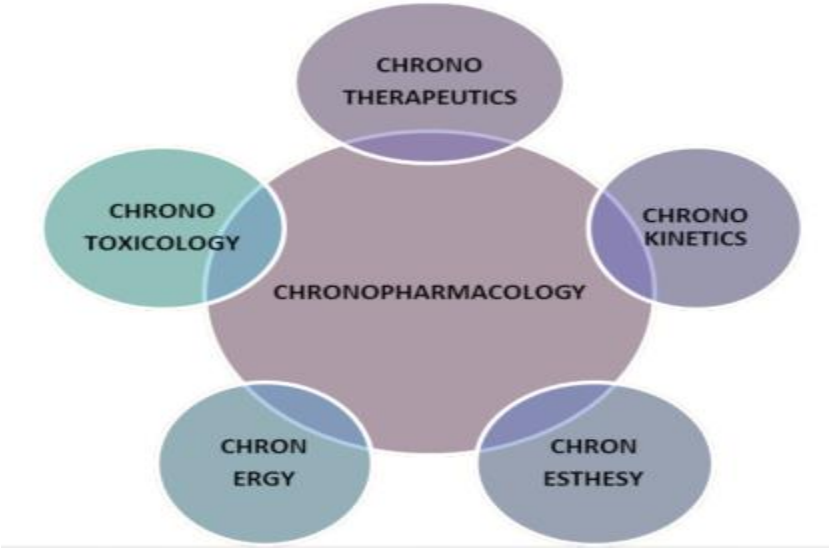
Auto inhibition:-

It may occur during the metabolism of certain drugs. The metabolites formed from drug firstly increase in concentration and further inhibit metabolism of the parent drug. This phenomenon is called as product inhibition or allosteric inhibition or feedback inhibition.

Chronotherapeutics:

In this basically refers to the observation that every metabolic event undergoes rhythmic changes in time. Researchers have concluded that all living organisms are composites of rhythms with varying frequencies that may range from seconds. It is based on the observation that there is an interdependent relationship between the peak-to-trough rhythmic activity in disease symptoms and risk factors, pharmacologic sensitivity, and pharmacokinetics of many drugs. As more

continues to be learned about chronobiology and chronotherapeutics, it is becoming increasingly more evident that the specific time that patients take the medication may be even more significant than was recognized in the past.



The tradition of prescribing medication at evenly spaced time intervals throughout the day, attempt to maintain constant drug levels throughout a 24-hour period, may bechanging as researchers' report that some medications may work better if their administration is coordinated with day-night patterns and biological rhythms. One approach to increase the efficiency of pharmacotherapy is the administration of drugs at times at which they are most effective and best tolerated.

Advantages of Chronotherapeutics:

1. It prevents an overdosing of drug.
2. It makes the utilization of the drug more appropriate.
3. It reduces unnecessary side effects of a drug and helps in caring out the treatment for only a particular or limited period of time.

Need for Chronotherapeutics: -

It is required to observe therapy to limit the duration of therapy especially in cases where patients are already having renal, cardiac and hepatic other function of the body. Accumulation of drugs in these organs causes greater toxicity which may diminish function of the organ. Thus, the chronotherapeutics becomes a very important part of treatment of diseases particularly those effecting targeted body parts. According to the 1996 American medical association review,

consideration of chronotherapy clinical trials is highly welcomed by the whole medical community.

Chronopharmacokinetics

Chronopharmacokinetics the study changes in drug absorption, distribution, metabolism and excretion. Circadian changes in gastric acid secretion, gastrointestinal motility, gastrointestinal blood flow, drug protein binding, liver enzyme activity. Numerous chronopharmacokinetic studies been conducted over the last 20 years. The results of these studies demonstrate that time of administration affects drug kinetics. Studies in man have been reported, particularly in relation to cardiovascular active drugs, nonsteroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, anticancer drugs, psychotropic drugs, antibiotics anti-asthmatic drugs. Most of the drugs seem to have a higher rate or extent of bioavailability when they are taken in morning than when they are taken in the evening.

Chronesthesy:

It deals with circadian or other systemic changes in the susceptibility and sensitivity of the target system to a drug.

Chronergy:

It deals with rhythmic difference in effects of drug on the organism as a whole which includes both desired and undesired effects.

Chrono toxicology:

It is an aspect of chrono dynamics; it refers specifically to dosing time i.e. rhythm dependant differences in the manifestations and severity of adverse effects and thus intolerance of patients to medication. The term circadian comes from Latin word circa means ‘about’ and dian means ‘day’. Circadian rhythms are most important type of biological rhythms and are most significant for humans and animals. They play an important role in maintaining body temperature, heart rate, blood pressure, organ blood flow, pulmonary and kidney functions as well as for concentration of neurotransmitters, hormones, enzymes, electrolytes and glucose. Study of rhythms is important for pharmacotherapy. Chronotherapy coordinate drug delivery with human biological rhythms and holds huge promise in areas of pain management and treatment of asthma, heart diseases and cancer.

Biological clocks and circadian rhythm

Biological rhythms are innately determined rhythmic biological process or function and self-sustaining oscillation with the duration of time between successive repetitions (i.e., the period) being rather nonvarying under normal conditions.

Rhythms affecting our body are ultradian cycles shorter than a day e.g. msec. for a neuron to fire;

Circadian-Circa- about a day, lasting for about 24 hours, e.g.,sleep and wake cycles;

Infradian- cycles longer than 24 hours e.g. menstrual cycle.

Seasonal-like seasonal affective disorder causing depression in people during the short day's of winter. While 24-hour clock times and sleep/wake rhythms frequently overlap with the internal clock, they do not always match the circadian rhythm.

There are a variety of methods to ascertain the timing of biological clocks.

- ✓ Melatonin provides the most reliable and consistent measure of the circadian pattern and can be measured in the plasma, saliva, or urine. Because secretion of the hormone is acutely suppressed by light exposure, the measurement of the time of onset of the daily melatonin rise during low-light exposure is a more reliable measure of the circadian phase.
- ✓ The dim-light melatonin onset (DLMO) has been used to assess alterations of circadian phase in a variety of diseases.
- ✓ Other markers, such as core body temperature, and cortisol may also serve as biomarkers for circadian rhythms.

Circadian rhythms

These are particularly important in medicine. Physiological day is about 25 hours where the clock is reset daily by the environment night day social schedules.

Biologic rhythms are endogenous nature of circadian. Lack of external synchronizers leads to free running rhythms.

The period of free-running rhythms is longer or shorter than 24 hours and is characteristic for each species. Our internal clocks are genetically determined. An internal biological clock is located in mammals, in the suprachiasmatic nucleus of the hypothalamus (SCN), delivering its message of time throughout the body. It is responsible for circadian rhythms and annual / seasonal rhythms.

The SCN uses its connections with the autonomic nervous system for spreading its time-of-day message, either by setting the sensitivity of endocrine glands i.e., thyroid, adrenal, ovary) or by directly controlling an endocrine output of pineal gland i.e., melatonin.

Mechanism of Chronopharmacology

The basic unit of circadian timekeeping is the cell. Even in very complex organisms, most cells contain autonomous circuitry for circadian oscillations. Generally speaking, this mechanism is comprised of negative feedback loops of transcription and translation: activation of a repressor gene results in its later repression by its own protein product, and the instability of this repressor insures this repression is short lived, so that a new cycle can begin.

- In mammals, the principal activators within this system are the CLOCK and BMAL1 proteins and their homologs, which dimerize and bind to certain elements to activate transcription of a large number of circadian genes.
- Among these circadian genes are loci encoding the PERIOD and CRYPTOCHROME families of repressor proteins (PER1-3 and CRY1-2), who's products multimerize and suppress the CLOCK: BMAL1 activating complex.

At each of these steps, additional precision and regulatory finesse is achieved through interaction with a wide range of auxiliary proteins: kinases that phosphorylate clock proteins to modify their stability or activity. Chronopharmacological techniques ensure that drug levels in the blood are within therapeutic ranges during periods of maximal disease severity.

An example of this is seen in how evening doses of antihypertensive therapy can be used to prevent morning rises in blood pressure. The evening dose of the drug may thus be well timed with diurnal changes in blood pressure, preventing diurnal worsening of hypertension. In addition, medications may have a different effect based on the timing of the dose. for example, efficacy of ketamine, , has been shown to have varying efficacy based on the timing of dose despite reaching equivalent plasma concentrations, giving rise to the theory that some of these diurnal effects may be due to changes in receptors or secondary messenger systems.

Chronotherapy may prevent up- or down-regulation of receptors during periods of lesser need allowing optimal efficacy during periods of disease exacerbation.

Mechanism of Circadian Rhythms

Circadian clock present in brain coordinates daily physiological cycle like

● Sleep wake cycle

●Digestion and temperature

● Hormones

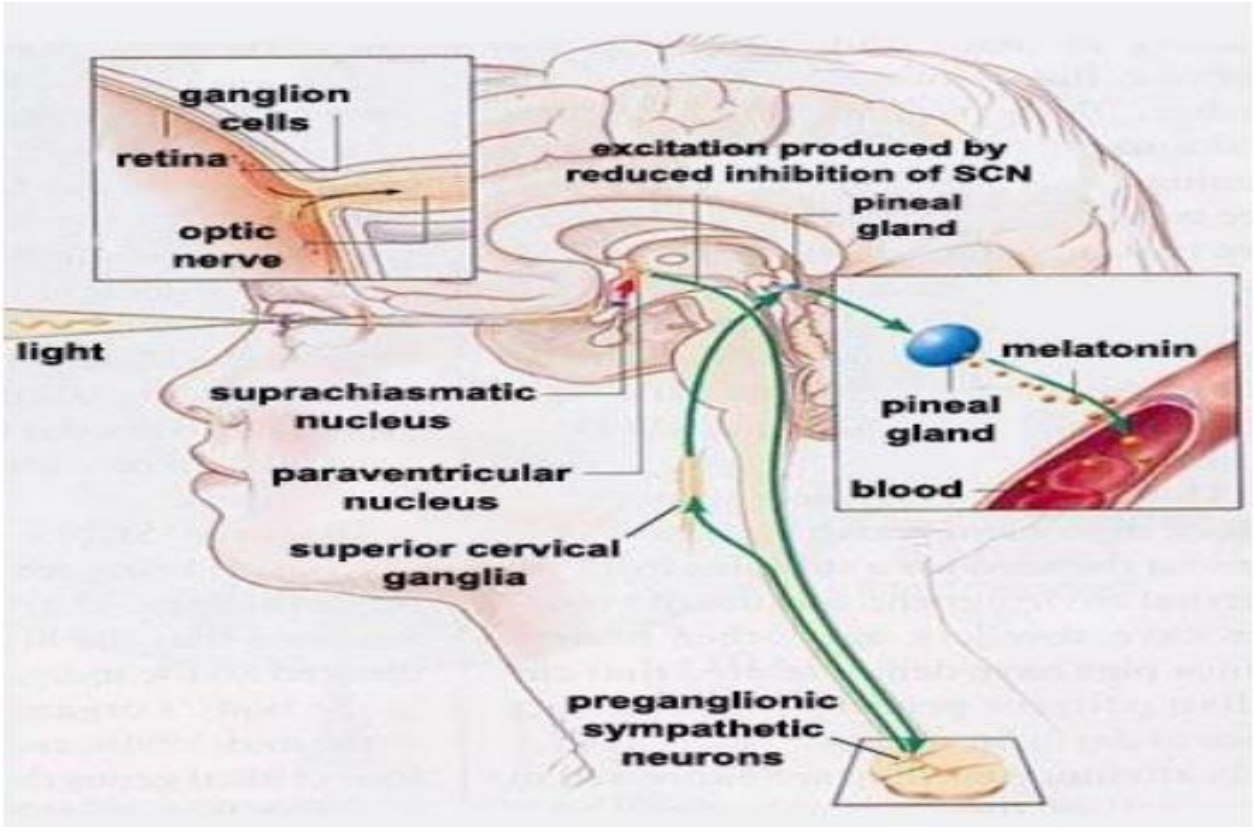


Figure: Supra chiasmatic nuclei (SCN) and pineal gland location.

Biological Rhythms and Rhythmic Components

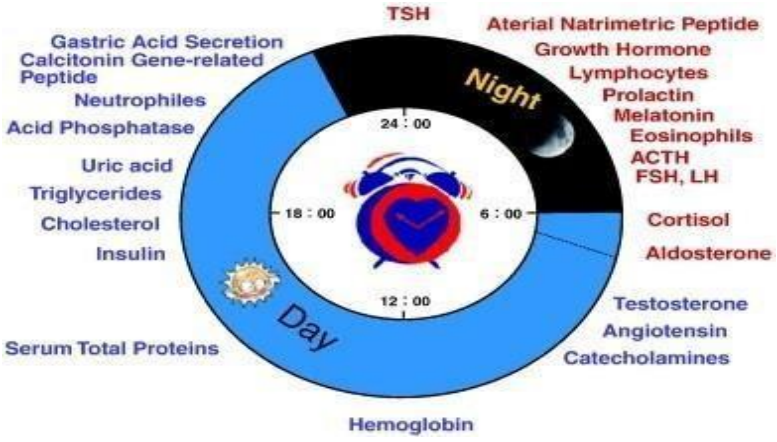
Circadian implies approximately a day, major periodic components of biological rhythms are found around 24 hours (circadian) and 30 days (Circamensual) and one year (Circannual). Circadian rhythms are found in all the organisms, infact the existence of circadian rhythms in living organisms was first established during a detailed study of leaf movement in plants more than 200 year ago. Biological rhythms posses both an internal as well as external components. Rhythmicity has been detected for a numbers of physiological variables like pulse, temperature, blood pressure, hormonal secretion via diurnal variation in effects of insulin on blood glucose.

Human Circadian Time Structure

release should also vary over time. Chronopharmaceutical drug delivery system are gaining importance in the field of pharmaceutical technology as these system deliver right dose at specific time at a specific site. Various technologies such as time- controlled, pulsed, triggered

and programmed drug delivery devices have been developed and extensively studied in recent years for Chronopharmaceutical drug delivery.Many functions of the human body vary considerably in a day. These variations cause changes both in disease state and in plasma drug concentrations. Human circadian rhythm is based on sleep-activity cycle, is influenced by our genetic makeup and hence, affects the body's functions day and night (24- hours period. Research in the chronopharmacological field has demonstrated the importance of biological rhythms in drug therapy and this brought a new approach to the development of drug delivery systems.

Circadian rhythm in the pathogenesis of diseases- From the various studies, it is formed that the many cardiovascular events including myocardial infarction, stroke and sudden death occur



during the initial hours of morning activity between 6 A.M. and 12 noon. And this is much higher during this period that other timing during the day. BP rises rapidly in the early morning hours, the time when most individuals wake and begin their day. This rise in BP corresponds to increased secretion of catecholamine's and increased plasma rennin activity. Thus, vascular tone and total peripheral resistance increase in the morning hours, and rises as a result. At the same time, heart rate increases in the late morning or early afternoon.

Chronotherapy of cardiovascular diseases

The differences in patterns of illness between day and night for cardiovascular disorders such as hypertension, angina, heart attack, sudden cardiac death and stroke have been documented. Chronotherapeutics approach gives more accurate determination of the time when patients are at highest risk and in greatest need of therapy. For example – it has often been found that the blood pressure of hypertensive patient increases rapidly in the morning after awakening, typically

peaks in the middle to late time of the day, decreases in the evening and is lowest while the patient sleeps at night. It may also be important to recognize that the risk of heart attack appears to be greatest during the early morning hours after awakening. For instance, capillary resistance and vascular reactivity are higher in the morning and decreases later in the day. Platelet agreeability is increased and fibrnolytic activity is deceased in the morning, leading to a state of relative hyper coagulability of the blood. Blood Pressure is at its lowest during sleeping period and rises steeply during the early morning period. Many anti- hypertensive drugs do not control the early morning blood pressure, when given once daily early in the morning.

VARIOUS CARDIOVASCULAR DISEASES

Blood pressure (B.P) / Hypertension Blood Pressure is well known to exhibit 24 h variation with a peak in the morning. A number of factors influence diurnal variation of blood pressure which is internal factors such as the autonomic nervous system, vasoactive intestinal peptide, plasma cortisol, plasma rennin activity, aldosterone, plasma atrial natriuretic peptide. Both sympathetic activity and the rennin-angiotensin–aldosterone access peak in the early morning hours. In addition, b.p is affected by a variety of external factors including physical activity, emotional state, meal and sleep/wake routine. These extrinsic stimuli alsoaffect the autonomic nervous system thus the 24 h variation in the B.P is representative of both endogenous diurnal rhythms and exogenous factors. Blood pressure is characterized by a circadian rhythm, both in hypertensive and in normotensive subjects; this pattern is associates with lower B.P values during sleeping time and periods of minimal activity and higher B.P levels during wakefulness and mental and physical activity. Various, researchers reported that blood pressure changed depending on whether the subjects was sleeping, resting or working. Blood pressure fluctuates throughout the day and night. The duration of the fluctuations may be short, from seconds to minutes, or long from day to night and season to season. The most easily noted and significant blood pressure variations are the diurnal changes related to the sleep-wake cycle. The pattern of blood pressure values obtained during the sleep-wake cycle from characteristic circadian rhythm. The Continuous monitoring of blood pressure throughout the day and night reveals a pattern with minimum values of systolic & diastolic pressure between midnight & 4 am. The pressure increases during waking hours remaining at a plateau for several hours & then reaching a maximum values early in the morning. This diurnal blood pressure fluctuation is altered in certain disease states, such as preeclampsia & chronic hypertension. Changing paradigm for

targeting blood pressure control multiple daily dosing of medication once daily dosing of long acting medication Evening dosing of long acting chronotherapeutics medication

Acute myocardial infraction (AMI) / pulmonary embolism (PE)-

It is well known that AMI or PE frequently occurs in the early morning. A number of physiological functions exhibit diurnal variation including BP, heart rate, coronary blood flow, platelet function, blood coagulability and fibrnolytic activity. In the early morning, systemic BP & heart rate increases and augment the oxygen demand of the heart. In addition, the vascular tone of the coronary artery rises and coronary blood flow decreases in the morning. This increases in oxygen demand & decreases in oxygen supply exaggerate a mismatch of oxygen demand and supply in the morning. In addition, platelet function & blood coagulability also increases in the morning. A reduction in fibrnolytic activity resulting in a hypercoagulable state that could elicit the morning onset of thromboimbolic events. Accumulating evidences suggests that the autonomic nervous system plays a major role in the circadian variation of the onset of AMI. A morning increase in the frequency of ischemic episodes is absent in diabetic patients with autonomic nervous dysfunction. Patients receiving beta-blocker do not show morning increase in the incidence of angina, AMI & sudden death. Heart rate variability which reflects sympathetic/vagal balance is also associated with the onset of ischemic episode in the chronic stable angina. Platelets are not involved in the variation of AMI or thromboimbolic numbers & their aggregation activity possess circadian oscillation. Platelet activation in vivo is induced by catecholamine secreted from the sympathetic nervous system in a circadian fashion. However studies regarding platelet activation do not show clear circadian expression of any surface marker characteristic of platelet activation, therefore it is unclear whether the internal clock system directly affects the circadian functions of platelets.

Arrhythmia

A number of reports demonstrated the presence of circadian variation of cardiac arrhythmia. Evidences suggest that basic electrophysiological parameters have circadian variations. Atrial & ventricular refractory periods are strongly affected by the autonomic nervous system, in which sympathetic activity shortens it and parasympathetic activity elongates the period. Therefore fluctuations in the activity of autonomic nervous system within a day can be a major trigger of circadian onset of cardiac arrhythmia. Each parameter of ECG was analyzed as to whether it has

diurnal variations. ECG, AV nodal function, QT interval, R&T wave voltage & QT interval have been shown to exhibit circadian variations.

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.

Urinary system- The urinary system which plays a pivotal role in the elimination of a drug has many instances of circadian rhythms altering either the clearance or the urinary flow causing nephrotoxicity. Amino glycosides can produce renal toxicity with chronic administration. Because these antibiotics are primarily eliminated by renal excretion, diminishing renal function with time may cause greater drug accumulation and more toxicity. There is clearly a need to monitor therapy to limit the duration of therapy, especially in patients who already have compromised renal function. Theophylline causes increase in the renal flow by increasing the clearance levels and thereby increase in the urine flow and renal excretion. Carbamazepine shows time dependence in its disposition.

Gastrointestinal system- The gastrointestinal motility, the intraluminal pH, blood flow to stomach and enzymatic action are not the only factors that influence the gastro intestinal absorption of the drug. It even depends on the circadian rhythms and all the above mentioned factors are also influenced by the time of the day. Most of the drugs we generally take are lipophilic and they are found to have more rate of absorption in early mornings rather than any hour of the day.

Hepatic system- The anti-depressant nartryptalline which is injected to significant presystemic hepatic metabolism accumulates in a highly predictable manner on multiple oral dosing. The clearance levels of acetaminophen are decreased due to the effect of circadian rhythms and thus resulting in the hepatotoxicity.

Diseases Showing Dependence on Biological Rhythms Asthma-

Chronic airway inflammation and limitation of airflow in the airways characterize bronchial asthma, and attacks begin with paroxysms of coughing, wheezing, and dyspnoea. Chronopharmacological studies statistically show that the development of asthma symptoms and many types of bronchospastic attacks is clearly more common from midnight to early morning from 2 A.M. and 6am every day. Chronopharmacotherapy for asthma is aimed at getting

maximal effect from bronchodilator medications during the early morning hours. Several drugs for asthma have beendeveloped based on chronopharmacology. One example is the bronchodilator uniphyl, a long-acting theophylline taken once a day in the evening causes theophylline blood levels to reach their peak and improve lung function during the difficult early morning hours. Some studies have even proved that a single dose administered in those early hours is equally effective as four doses given in a day. In addition to bronchodilators, the inhaled glucocorticosteroid ciclesonide is now available with once-daily dosing, which also improves patient's compliance. Numerous investigations have demonstrated the usefulness of chronotherapy for asthma, especially for patients with nocturnal asthma.

Diabetes- Biologists have found that a key protein that regulates the biological clocks of mammals also regulates glucose production in the liver and altering the levels of this protein can improve the health of diabetic mice. The additional function of the cytochrome is the regulation of gluconeogenesis according to the diurnal activity and feeding levels. So modulating cytochrome levels can also help decrease the diabetic effect on the patients.

Arthritis- Chronobiological patterns have been observed with arthritis pain. The symptoms of rheumatoid arthritis are always worse in the morning. Taking long-acting NSAIDs like flubiprofen, ketoprofen and indomethacin at bedtime optimizes their therapeutic effect and minimizes or averts their side effects. People with osteoarthritis, the most common form of the disease, tend to have less pain in the morning and more at night. For osteoarthritis sufferers, the optimal time for a non-steroidal anti-inflammatory drug such as ibuprofen would be around noon or mid-afternoon. Ankylosing spondylitis is characterized by swelling and discomfort of the joints of the back. The overall, back stiffness and pain were a problem throughout the 24 hours, but pain intensity was rated 2 to 3 times higher and stiffness about 8 times greater between 06:00 and 09:00 than between noon and 15:00.10

Cancer- The tumour cells and the normal cells differ in their Chronobiological cycles. This fact is the basis for the chronopharmacotherapy of cancer. Based on study which suggested that the DNA synthesis in the normal human bone marrow cells has a peak around noon while the peak of DNA synthesis in lymphoma cells is near midnight, a s-phase active cytotoxic therapy at late nights was administered and it was found that there is a decrease in the tumour cell count with a little effect on normal cells.

Allergy- The allergic reactions both local and systemic are mediated through interactions of immune and inflammatory responses. Such responses during the day are usually coordinated by adrenocortical function and steroid release with high amplitude daily rhythms. Scientists now believe that the symptoms of allergic rhinitis, and even the skin testing results, can vary according to the time of day.

New Techniques of Time Controlled Pulsatile Technology Currently pharmaceutical companies have been focused on developing and commercializing PDDS that fulfil unmet medical needs in the treatment of various diseases. Recently developed technologies are SODAS ® technology, IPDAS ® technology, CODAS technology, CONTINR, OROSR, CEFORMR, DIFFUCAPSR, chronomodulating infusion pumps, TIMERx R and physic-chemical modification of API.

Spheroidal Oral Drug Absorption System (SODAS) - This technology is based on the production of controlled release beads and it is characterised by its inherent flexibility, enabling the production of customized dosage forms that respond directly to individual drug candidate needs. SODAS can provide a number of tailored drug release profiles, including immediate release of drug followed by sustained release to give to a fast onset of action, which is maintained for 24 hrs. An additional option is pulsatile release, where a once daily dosage form can resemble multiple daily doses by releasing drug in discrete bursts throughout the day.

Chronotherapeutics Oral Drug Absorption System (CODAS)-

The Chronotherapeutics oral drug absorption system (CODAS) is a multiparticular system which is designed for bedtime drug dosing, incorporating a 4-5 hrs delay in drug delivery. This delay is introduced by the level of non enteric release – controlling polymer applied to drug loaded beads. This technology was designed to release its drug component after a prolonged period of time when administered. A good example is Verelan PM, which was designed to release verapamil approximately four to five hours after ingestion. This delay is introduced by the level of release – controlling polymer applied to the drug –loaded beads. The release controlling polymer is a combination of water-soluble and water –insoluble polymers. When fluid from the gastrointestinal tract contacts the polymer coat beads, the water-soluble polymer slowly dissolves and the drug diffuses through the resulting pores in the coating. The water- insoluble polymer continues to act as a barrier, maintaining the controlled-release of the drug. controlled onset extended release delivery system enables a maximum Plasma concentration of verapamil in the morning hours, when blood pressure normally is high.

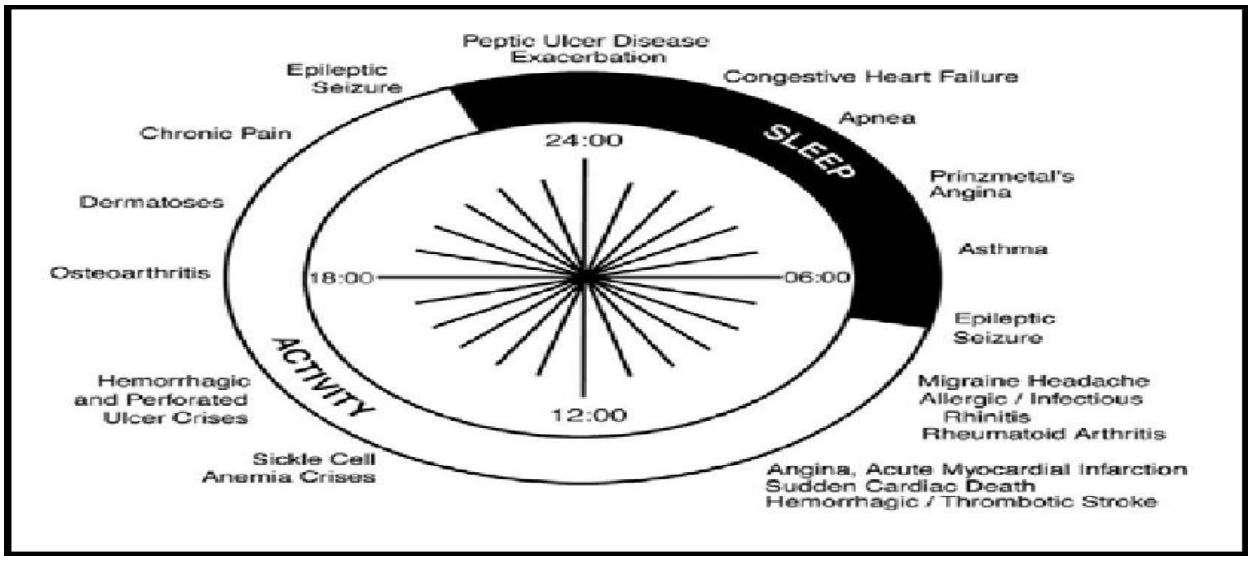
Continr Technology- In this technology, molecular coordination complexes are formed between a cellulose polymer & a non polar aliphatic alcohol optionally substituted with a aliphatic group by solvating the polymer with a volatile polar solvent & reacting the solvated cellulose polymer directly with the aliphatic alcohol . This constitutes the complex having utility as matrix in controlled release formulations since it has a uniform porosity (Semi permeable matrix).

Chronomodulating Infusion Pumps- Externally and internally controlled systems across a range of technologies including pre-programmed systems, as well as systems that are sensitive to modulated enzymatic or hydrolytic degradation , ph, magnetic fields, ultrasound, electronic field, temperature, light, & mechanical stimulation have been developed .

TIMERx Technology- The TIMERx Technology (hydrophilic system) combines primarily Xanthan & Locust bean gums mixed with dextrose .The physical interaction between these components works to form a strong binding gel in the presence of water. Drug release is controlled by the rate of water penetration from the gastrointestinal tract into the timer x gum matrix which expands to form a gel & subsequently releases the active drug Substances.

The major objective of this article is to inform biologists, clinicians, pharmaceutical scientists and other professional about the importance of biological clocks & Chronopharmacology to

human health and disease also motivate the investigator to develop new tools for the treatment of cardiovascular diseases such as cardiac arrhythmia, myocardial infarction etc. this article also provide a new ideas to use of older or already well-established active pharmaceutical ingredients for the treatment of various diseases.



IMPORTANT QUESTIONS

Very short question (2MARKS)

1. What is the treatment of poisoning?
2. Name antidote for heavy metal poisoning.
3. Name the antidote for barbiturate, atropine and organophosphorus poisoning.
4. What is morphine poisoning?
5. What are teratogens?
6. Name antidote for lead poisoning.
7. What is thalidomide tragedy?
8. Define mutagenicity.
9. Define teratogenicity.
10. Define LD50 and LC50.
11. Define acute and chronic toxicity.
12. Define genotoxicity.
13. What is carcinogenicity?
14. Define chronobiology and chronopharmacology.
15. Define biological, infardian and ciradian rhythms.
16. Define chronotherapeutics.
17. What is melatonin and pineal gland?
18. What are disorders of ciradian rhythms?
19. Define diurnal rhythms.
20. What should be the time for administration of diuretics?

Short questions (5MARKS)

21. Discuss barbiturate poisioning and treatment in detail.
22. Write a note on organophosphorus poisoning.
23. Discuss the genral principles of poisoning.
24. Write a descriptive note on heavy metal poisoning.
25. Discuss the rhythm and cycles in chrnopharmacology.
26. Describe the principles of toxicology.
27. What are the methods to evaluate on acute and chronic toxicity?

28. Discuss in detail about mutagenicity.

Long questions (10 MARKS)

29. Discuss the principles for treatment of poisoning with reference to heavy metals and barbiturates.
30. Explain clinical symptoms and management of opoid and atropine poisoning.
31. Discuss in detail about genotoxicity and teratogenecity.
32. Explain the methods to evaluate mutagenecity and carcinogenicity.
33. Discuss the Biological clock and their significance leading to chronotherapy.
34. Give detail about circadian rhythms in the pathogenesis of diseases.
35. Discuss new techniques of time controlled pulsatile technology.