



POSOLOGICAL DOSE OF THERAPEUTICS OVER PROPHYLAXIS IN TOXICOLOGICAL SERENDIPITY

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ABSTRACT

Drug, any chemical substance that affects the functioning of living things and the organisms (such as bacteria, fungi and viruses) that infect them. Pharmacology, the science of drugs, deals with all aspects of drugs in medicine, including their mode of action, physical and chemical properties, metabolism, therapeutics and toxicity. The therapeutic index of a drug is the ratio between the dosage that causes a toxic/lethal effect and the dosage that causes a therapeutic effect. The term pharmacotherapy refers to the use of drugs for treating diseases, whereas pharmacology is the study of drug action on living systems. It is the interaction of the drug molecules and drug receptors that brings about a therapeutic effect. However, it is extremely essential to administer the drug in the right dose to achieve such an effect. If taken in large doses, certain drugs could cause adverse effects. Thus, in order to reap the benefits, it is essential to assess the right dose. Therapeutic drug monitoring (TDM) becomes essential to determine the dose at which a drug will be safe and effective, especially with those with a narrow therapeutic index (NTI). Also, monitoring might be required when the patient is affected by a medical condition that has an adverse effect on the clearance of NTI drugs. There's no denying the fact that drug metabolism could vary from person to person. Thus, the key to avoid drug-related problems is to consider the drug's therapeutic index and other relevant factors for ensuring safety. Therapeutic index (TI) refers to any of the several indices that are used for measuring a drug's safety. The most common TI is the ratio of the median lethal dose to the median effective dose of a drug. The formula for TI is: LD_{50}/ED_{50} . LD_{50} stands for median lethal dose and ED_{50} stands for median effective dose (therapeutic dose). LD_{50} refers to the dose that would produce a lethal effect in 50% of the population, whereas ED_{50} refers to the dose that will produce the desired therapeutic effect in 50% of the population. This index is commonly used to measure a drug's safety. The therapeutic index formula is: TD_{50}/ED_{50} . TD_{50} stands for the median toxic dose, whereas ED_{50} stands for the median effective dose. TD_{50} refers to the minimum drug dose that would produce a toxic effect in 50% of the population, whereas ED_{50} refers to the minimum drug dose that will produce the desired therapeutic effect in 50% of the population. Another related concept is that of therapeutic range (TR). TR is the range of doses/concentrations at which a therapeutic agent or drug produces a therapeutic response without causing any significant adverse effect in patients. It can be measured by: MEC/MTC. MEC stands for minimum effective concentration, whereas MTC stands for minimum toxic concentration. MEC is the minimum concentration of the drug that is required for achieving the therapeutic effect, whereas MTC is the minimum concentration at which toxicity occurs. Basically, if at a particular dosage, a drug is above MTC, it would cause adverse effects. Similarly, a drug below MEC will not produce the desired therapeutic response.

Keywords: TDM, NTI, TI, ED_{50} , ED_{95} , LD_{50} , ID_{50} , IC_{50} , TD_{50} , LD_{Lo} , MEC, MTC, TD, TR, USFDA, OBD, MTD, Safety Ratio, USFDA, Protective index, Therapeutic window.

INTRODUCTION

A ratio that compares the blood concentration at which a drug becomes toxic and the concentration at which the drug is effective is known as therapeutic index (TI). The larger the therapeutic index (TI), the safer the drug is. If the TI is small (the difference between the two concentrations is very small), the drug must be dosed carefully and the person receiving the drug should be monitored closely for any signs of drug toxicity. The therapeutic index (TI) (also referred to as therapeutic window or safety window or sometimes as therapeutic ratio) is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity. Classically, in an established clinical indication setting of an approved drug, TI refers to the ratio of the dose of drug that causes adverse effects at an incidence/severity not compatible with the targeted indication (e.g. toxic dose in 50% of subjects, TD_{50}) to the dose that leads to the desired pharmacological effect (e.g. efficacious dose in 50% of subjects, ED_{50}). In contrast, in a drug development setting TI is calculated based

on plasma exposure levels. In the early days of pharmaceutical toxicology, TI was frequently determined in animals as lethal dose of a drug for 50% of the population (LD_{50}) divided by the minimum effective dose for 50% of the population (ED_{50}). Today, more sophisticated toxicity endpoints are used.^[1]



Figure-1: Therapeutics and Prophylaxis

Therapeutic Index= LD_{50}/ED_{50} in animal studies, or for humans, Therapeutic Index= TD_{50}/ED_{50} . For many drugs, there are severe toxicities that occur at sub-lethal doses in humans and these toxicities often limit the maximum dose of a drug. A higher therapeutic index is preferable to a lower one: a patient would have to take a much higher dose of such a drug to reach

the toxic threshold than the dose taken to elicit the therapeutic effect. Generally, a drug or other therapeutic agent with a narrow therapeutic range (i.e. having little difference between toxic and therapeutic doses) may have its dosage adjusted according to measurements of the actual blood levels achieved in the person taking it. This may be achieved through therapeutic drug monitoring (TDM) protocols. TDM is recommended for use in the treatment of psychiatric disorders with lithium due to its narrow therapeutic range.^[2]

An effective dose (ED) in pharmacology is the dose or amount of drug that produces a therapeutic response or desired effect in some fraction of the subjects taking it. ED=Effective Dose, TD=Toxic Dose, LD=Lethal Dose, TI=Therapeutic Index, TR=Therapeutic Ratio.

EFFECTIVE DOSE₅₀: It has been stated that any substance can be toxic at a high enough dose. The line between efficacy and toxicity is dependent upon the particular patient, although the dose administered by a physician should fall into the predetermined therapeutic window of the drug. The importance of determining the therapeutic range of a drug cannot be overstated. This is generally defined by the range between the minimum effective dose (MED) and the maximum tolerated dose (MTD). The MED is defined as the lowest dose level of a pharmaceutical product that provides a clinically significant response in average efficacy, which is also statistically significantly superior to the response provided by the placebo. Similarly, the MTD is the highest possible but still tolerable dose level with respect to a pre-specified clinical limiting toxicity. In general, these limits refer to the average patient population. For instances in which there is a large discrepancy between the MED and MTD, it is stated that the drug has a large therapeutic window. Conversely, if the range is relatively small, or if the MTD is less than the MED, then the pharmaceutical product will have little to no practical value.

The "median effective dose" is the dose that produces a quantal effect (all or nothing) in 50% of the population that takes it (median referring to the 50% population base). It is also sometimes abbreviated as the ED₅₀, meaning effective dose, for 50% of people receiving the drug. The ED₅₀ is commonly used as a measure of the reasonable expectancy of a drug effect, but does not necessarily represent the dose that a clinician might use. This depends on the need for the effect and also the toxicity. The toxicity and even the lethality of a drug can be quantified by the TD₅₀ and LD₅₀ respectively. Ideally, the effective dose would be substantially less than either the toxic or lethal dose for a drug to be therapeutically relevant.^[3]

EFFECTIVE DOSE₉₅: The ED₉₅ is the dose required for desired effect in 95% of the population exposed to it. In anesthesia pharmacology, the term ED₉₅ is used in a different context and thus, commonly misunderstood to correspond to the median dose required to achieve a quantal effect in 95% of the population. This is erroneous. Its correct use and definition is with reference to non-depolarizing neuromuscular blocking agents. ED₉₅ in this context is the median dose required to achieve a 95% reduction in maximal twitch response from baseline, in 50% of the population. The single twitch response occurs when applying a square wave supra-maximal current, using a nerve stimulator, to the ulnar nerve and measuring twitch of the adductor pollicis. Thus, the more accurate use of

the term in this regard would be ED₅₀ of 95%. Notwithstanding, usage of the term ED₉₅ has stuck in this context.^[4]

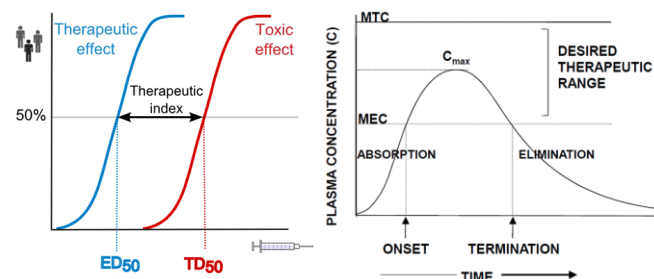


Figure-2: Graphical plot of therapeutic index

MEDIAN TOXIC DOSE: In toxicology, the median toxic dose (TD₅₀) of a drug or toxin is the dose at which toxicity occurs in 50% of cases. The type of toxicity should be specified for this value to have meaning for practical purposes. The median toxic dose encompasses the category of toxicity that is greater than half maximum effective concentration (ED₅₀) but less than the median lethal dose (LD₅₀). However, for some highly potent toxins (ex. lofentanil, botulinum toxin) the difference between the ED₅₀ and TD₅₀ is so minute that the values assigned to them may be approximated to equal doses. Since toxicity need not be lethal, the TD₅₀ is generally lower than the median lethal dose (LD₅₀) and the latter can be considered an upper bound for the former. However, since the toxicity is above the effective limit, the TD₅₀ is generally greater than the ED₅₀. If the result of a study is a toxic effect that does not result in death, it is classified as this form of toxicity. Toxic effects can be defined differently, sometimes considering the therapeutic effect of a substance to be toxic (such as with chemotherapeutics) which can lead to confusion and contention regarding a substance's TD₅₀. Examples of these toxic endpoints include cancer, blindness, anemia, birth defects, etc. Alternative definition: ED₉₅ (when referring to non depolarizing muscle relaxants) is the ED₅₀ from a cumulative log dose response curve where the quantum is a 95% reduction in twitch height.^[5]

LETHAL DOSE: In toxicology, the lethal dose (LD) is an indication of the lethality of a given substance or type of radiation. Because resistance varies from one individual to another, the lethal dose represents a dose (usually recorded as dose per kilogram of subject body weight) at which a given percentage of subjects will die. The lethal concentration is a lethal dose measurement used for gases or particulates. The LD may be based on the standard person concept, a theoretical individual that has perfectly normal characteristics and thus not apply to all sub-populations. The median lethal dose, LD₅₀ (abbreviation for lethal dose, 50%), LC₅₀ (lethal concentration, 50%) or LCt₅₀ (lethal concentration and time) of a toxin, radiation, or pathogen is the dose required to kill half the members of a tested population after a specified test duration. LD₅₀ figures are frequently used as a general indicator of a substance's acute toxicity. A lower LD₅₀ is indicative of increased toxicity.

The test was created by J.W. Trevan in 1927. The term "semi-lethal dose" is occasionally used with the same meaning, in particular in translations from non-English-language texts,

but can also refer to a sub-lethal dose; because of this ambiguity, it is usually avoided. LD₅₀ is usually determined by tests on animals such as laboratory mice. In 2011 the USFDA approved alternative methods to LD₅₀ for testing the cosmetic drug Botox without animal tests. LD values for humans are best estimated by extrapolating results from human cell cultures. One form of measuring LD is to use animals like mice or rats, converting to dosage per kilogram of biomass and extrapolating to human norms. The degree of error from animal-extrapolated LD values is large. The biology of test animals differs in important aspects to that of humans. For instance, mouse tissue is approximately fifty times less responsive than human tissue to the venom of the Sydney funnel-web spider. The square-cube law also complicates the scaling relationships involved. Researchers are shifting away from animal-based LD measurements in some instances. The USFDA has begun to approve more non-animal methods in response to animal welfare concerns. The LD₅₀ is usually expressed as the mass of substance administered per unit mass of test subject, typically as milligrams of substance per kilogram of body mass, but stated as nanograms (suitable for botulinum), micrograms, milligrams, or grams (suitable for paracetamol) per kilogram. Stating it this way allows the relative toxicity of different substances to be compared, and normalizes for the variation in the size of the animals exposed, although toxicity does not always scale simply with body mass.

The choice of 50% lethality as a benchmark avoids the potential for ambiguity of making measurements in the extremes and reduces the amount of testing required. However, this also means that LD₅₀ is not the lethal dose for all subjects; some may be killed by much less, while others survive doses far higher than the LD₅₀. Measures such as LD₁ and LD₉₉ (dosage required to kill 1% or 99%, respectively, of the test population) are occasionally used for specific purposes. Lethal dosage often varies depending on the method of administration; for instance, many substances are less toxic when administered orally than when intravenously administered. For this reason, LD₅₀ figures are often qualified with the mode of administration, e.g., LD₅₀ i.v. The related quantities LD₅₀/30 or LD₅₀/60 are used to refer to a dose that without treatment will be lethal to 50% of the population within (respectively) 30 or 60 days. These measures are used more commonly with radiation, as survival beyond 60 days usually results in recovery.^[6]

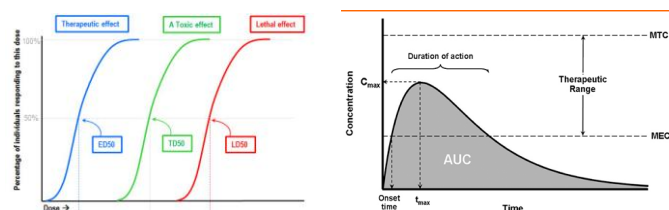


Figure-3: Comparison of ED₅₀, TD₅₀ & LD₅₀ with Therapeutic Range

MEDIAN INFECTIVE DOSE: The median infective dose (ID₅₀) is the number of organisms received by a person or test animal qualified by the route of administration (e.g., 1,200 organism/man per oral). Because of the difficulties in counting actual organisms in a dose, infective doses may be expressed in terms of biological assay, such as the number of LD₅₀'s to some test animal. In biological warfare infective dosage is the

number of infective doses per minute for a cubic meter (e.g., ICT₅₀ is 100 medium doses - min/m³).^[7]

LOWEST LETHAL DOSE: The lowest lethal dose (LD_{Lo}) is the least amount of drug that can produce death in a given animal species under controlled conditions. The dosage is given per unit of bodyweight (typically stated in milligrams per kilograms) of a substance known to have resulted in fatality in a particular species. When quoting an LD_{Lo}, the particular species and method of administration (e.g. ingested, inhaled and intravenous) are typically stated.^[8]

MEDIAN LETHAL CONCENTRATION: For gases and aerosols, lethal concentration (given in mg/m³ or ppm, parts per million) is the analogous concept, although this also depends on the duration of exposure, which has to be included in the definition. The term incipient lethal level is used to describe a LC₅₀ value that is independent of time. A comparable measurement is LCt₅₀, which relates to lethal dosage from exposure, where C is concentration and t is time. It is often expressed in terms of mg-min/m³. LCt₅₀ is the dose that will cause incapacitation rather than death. These measures are commonly used to indicate the comparative efficacy of chemical warfare agents and dosages are typically qualified by rates of breathing (e.g., resting=10l/min) for inhalation, or degree of clothing for skin penetration. The concept of Ct was first proposed by Fritz Haber and is sometimes referred to as Haber's Law, which assumes that exposure to 1 minute of 100 mg/m³ is equivalent to 10 minutes of 10 mg/m³ (1×100=100, as does 10×10=100). Some chemicals, such as hydrogen cyanide, are rapidly detoxified by the human body and do not follow Haber's Law. So, in these cases, the lethal concentration may be given simply as LC₅₀ and qualified by duration of exposure (e.g., 10 minutes). The Material Safety Data Sheets for toxic substances frequently use this form of the term even if the substance does follow Haber's Law.^[9]

LOWEST LETHAL CONCENTRATION: The LC_{Lo} is the lowest concentration of a chemical, given over a period of time, which results in the fatality of an individual animal. LC_{Lo} is typically for an acute (<24 hour) exposure. It is related to the LC₅₀, the median lethal concentration. The LC_{Lo} is used for gases and aerosolized material.^[10]

LIMITATIONS: As a measure of toxicity, lethal dose is somewhat unreliable and results may vary greatly between testing facilities due to factors such as the genetic characteristics of the sample population, animal species tested, environmental factors and mode of administration. There can be wide variability between species as well; what is relatively safe for rats may very well be extremely toxic for humans (i.e. paracetamol toxicity) and vice versa. For example, chocolate, comparatively harmless to humans, is known to be toxic to many animals. When used to test venom from venomous creatures, such as snakes, LD₅₀ results may be misleading due to the physiological differences between mice, rats and humans. Many venomous snakes are specialized predators of mice and their venom may be adapted specifically to incapacitate mice; and mongooses may be exceptionally resistant. While most mammals have a very similar physiology, LD₅₀ results may or may not have equal bearing upon every mammal species, including humans.

Animal-rights and animal-welfare groups, such as Animal Rights International, have campaigned against LD₅₀ testing on animals in particular as, in the case of some substances, causing the animals to die slow, painful deaths. Several countries, including the UK, have taken steps to ban the oral LD₅₀ and the Organization for Economic Cooperation and Development (OECD) abolished the requirement for the oral test in 2001.

THERAPEUTIC INDEX IN DRUG

DEVELOPMENT: A high Therapeutic Index (TI) is preferable for a drug to have a favorable safety profile. At early discovery/development stage, the clinical TI of a drug candidate is not known. However, understanding the preliminary TI of a drug candidate is of utmost importance as early as possible since TI is an important indicator of the probability of the successful development of a drug. Recognizing drug candidates with potentially suboptimal TI at earliest possible stage helps to initiate mitigation or potentially re-deploy resources. In a drug development setting, TI is the quantitative relationship between efficacy (pharmacology) and safety (toxicology), without considering the nature of pharmacological or toxicological endpoints themselves. However, to convert a calculated TI to something that is more than just a number, the nature and limitations of pharmacological and/or toxicological endpoints must be considered. Depending on the intended clinical indication, the associated unmet medical need and/or the competitive situation, more or less weight can be given to either the safety or efficacy of a drug candidate with the aim to create a well balanced indication-specific safety vs efficacy profile.

In general, it is the exposure of a given tissue to drug (i.e. drug concentration over time), rather than dose, that drives the pharmacological and toxicological effects. For example, at the same dose there may be marked inter-individual variability in exposure due to polymorphisms in metabolism, DDIs or differences in body weight or environmental factors. These considerations emphasize the importance of using exposure rather than dose for calculating TI. To account for delays between exposure and toxicity, the TI for toxicities that occur after multiple dose administrations should be calculated using the exposure to drug at steady state rather than after administration of a single dose.^[11]

VARIATION: The therapeutic index varies widely among substances: most forgiving among the opioid analgesics is remifentanyl, which offers a therapeutic index of 33,000:1 while diazepam, a benzodiazepine sedative-hypnotic and skeletal muscle relaxant has a less-forgiving index of 100:1 and morphine, a sedative, antidepressant and analgesic of herbal origin (genus Papaver) has an index of 70:1 (which, however, is still considered very safe). Less safe are cocaine, a stimulant and local anaesthetic and ethanol (colloquially, the alcohol in alcoholic beverages), a widely available sedative consumed world-wide – the therapeutic indices for these substances are 15:1 and 10:1, respectively. Even less-safe are drugs such as digoxin, a cardiac glycoside; its therapeutic index is approximately 2:1. Other examples of drugs with a narrow therapeutic range, which may require drug monitoring both to achieve therapeutic levels and to minimize toxicity, include: paracetamol (acetaminophen), dimercaprol, theophylline, warfarin and lithium carbonate. Some antibiotics require

monitoring to balance efficacy with minimizing adverse effects, including: gentamicin, vancomycin, Amphotericin B and polymyxin B.

CANCER RADIOTHERAPY: Radiotherapy aims to minimize the size of tumors and kill cancer cells with high energy. The source of high energy arises from x-rays, gamma rays, charged particles and heavy particles. The therapeutic ratio in radiotherapy for cancer treatment is related to the maximum radiation dose by which death of cancer cells is locally controlled and the minimum radiation dose by which cells in normal tissues have low acute and late morbidity. Both of parameters have sigmoidal dose-response curves. Thus, a favorable outcome in dose-response curve is the response of tumor tissue is greater than that of normal tissue to the same dose, meaning that the treatment is effective to tumors and does not cause serious morbidity to normal tissue. Reversely, overlapping response of two tissues is highly likely to cause serious morbidity to normal tissue and ineffective treatment to tumors. The mechanism of radiation therapy is categorized into direct and indirect radiation. Both of direct and indirect radiations induce DNAs to have a mutation or chromosomal rearrangement during its repair process. Direct radiation creates a free DNA radical from radiation energy deposition that damages DNA. Indirect radiation occurs from radiolysis of water, creating a free hydroxyl radical, hydronium and electron. Then, hydroxyl radical transfers its radical to DNA or together with hydronium and electron, a free hydroxyl radical can damage base region of DNA. Cancer cells have imbalance of signals in cell cycle. G1 and G2/M arrest are found to be major checkpoints by irradiation in human cells. G1 arrest delays repair mechanism before synthesis of DNA in S phase and mitosis in M phase, suggesting key checkpoint to lead survival of cells. G2/M arrest occurs when cells need to repair after S phase before the mitotic entry. It was also known that S phase is the most resistant to radiation and M phase was the most sensitive to radiation. p53, a tumor suppressor protein that plays a role in G1 and G2/M arrest, enabled the understanding of the cell cycle by radiation. For example, irradiation to myeloid leukemia cell leads to an increase in p53 and a decrease in the level of DNA synthesis. Patients with Ataxia telangiectasia delays have hypersensitivity to radiation due to the delay of accumulation of p53. In this case, cells are able to replicate without repair of their DNA, prone to incidence of cancer. Most cells are in G1 and S phase and irradiation at G2 phase showed increased radio-sensitivity and thus G1 arrest has been on focus for therapeutic treatment. Irradiation to a tissue creates response to both irradiated and non-irradiated cells. It was found that even cells up to 50-75 cell diameter distant from irradiated cells have phenotype of enhanced genetic instability such as micro-nucleation. This suggests the effect of cell-to-cell communication such as paracrine and juxtacrine signaling. Normal cells do not lose DNA repair mechanism whereas cancer cells often lose during radiotherapy. However, the nature of high energy radiation can override the ability of damaged normal cell to repair, leading to cause another risk for carcinogenesis. This suggests a significant risk associated with radiation therapy. Thus, it is desirable to improve the therapeutic ratio during radiotherapy. Employing IG-IMRT, protons and heavy ions are likely to minimize dose to normal tissues by altered fractionation. Molecular targeting to DNA

repair pathway can lead to radio-sensitization or radioprotection. Examples are direct and indirect inhibitors on DNA double-strand breaks. Direct inhibitors target proteins (PARP family) and kinases (ATM, DNA-PKCs) that are involved in DNA repair. Indirect inhibitors target proteins tumor cell signaling proteins such as EGFR and insulin growth factor. The effective therapeutic index can be affected by targeting, in which the therapeutic agent is concentrated in its area of effect. For example, in radiation therapy for cancerous tumors, shaping the radiation beam precisely to the profile of a tumor in the beam's eye view can increase the delivered dose without increasing toxic effects, though such shaping might not change the therapeutic index. Similarly, chemotherapy or radiotherapy with infused or injected agents can be made more efficacious by attaching the agent to an oncophilic substance, as is done in peptide receptor radionuclide therapy for neuro-endocrine tumors and in chemo-embolization or radioactive microspheres therapy for liver tumors and metastases. This concentrates the agent in the targeted tissues and lowers its concentration in others, increasing efficacy and lowering toxicity.^[12]

SAFETY RATIO: Sometimes the term **safety ratio** is used instead, particularly when referring to psychoactive drugs used for non-therapeutic purposes, e.g. recreational use. In such cases, the effective dose is the amount and frequency that produces the desired effect, which can vary and can be greater or less than the therapeutically effective dose. The Certain Safety Factor is the ratio of the lethal dose to 1% of population to the effective dose to 99% of the population (LD_1/ED_{99}). This is a better safety index than the LD_{50} for materials that have both desirable and undesirable effects, because it factors in the ends of the spectrum where doses may be necessary to produce a response in one person but can, at the same dose, be lethal in another.^[13]

SYNERGISTIC EFFECTS: A therapeutic index does not consider drug interactions or synergistic effects. For example, the risk associated with benzodiazepines increases significantly when taken with alcohol, opiates, or stimulants when compared with being taken alone. Therapeutic index also does not take into account the ease or difficulty of reaching a toxic or lethal dose. This is more of a consideration for recreational drug users, as the purity can be highly variable.^[14]

PROTECTIVE INDEX: Protective index is a similar concept, except that it uses TD_{50} (median toxic dose) in place of LD_{50} . For many substances, toxic effects can occur at levels far below those needed to cause death and thus the protective index (if toxicity is properly specified) is often more informative about a substance's relative safety. Nevertheless, the therapeutic index is still useful as it can be considered an upper bound for the protective index and the former also has the advantages of objectivity and easier comprehension. The protective index is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity. Quantitatively, it is the ratio given by the toxic dose divided by the therapeutic dose. A protective index is the toxic dose of a drug for 50% of the population (TD_{50}) divided by the minimum effective dose for 50% of the population (ED_{50}). A high protective index is preferable to a low one: this corresponds to a situation in which one would

have to take a much higher dose of a drug to reach the toxic threshold than the dose taken to elicit the therapeutic effect. A drug should ordinarily only be administered if the protective index is greater than one, indicating that the benefit outweighs the risk. $Protective\ Index = TD_{50}/ED_{50}$.

The protective index is similar to the therapeutic index, but concerns toxicity (TD_{50}) rather than lethality (LD_{50}); thus, the protective index is a smaller ratio. Toxicity can take many forms, as drugs typically have multiple side effects of varying severity, so a specific criterion of toxicity must be specified for the protective index to be meaningful. Ideally a choice is made such that the harm caused by the toxicity just outweighs the benefit of the drug's effect. Thus, the protective index is a more accurate measure of the benefit-to-risk ratio than the therapeutic index, but is less objectively defined. Nevertheless, the therapeutic index can be viewed as an upper bound to the protective index for a given substance. Protective index refers to the factor by which the dose of a toxicant must be multiplied to produce a defined level of toxicity in the presence of a nontoxic dose of another chemical. $Protective\ index = LD_{50}\ of\ A\ with\ B / LD_{50}\ of\ A\ alone$. The higher the protective index better is the antidotal value of a given substance. Sometimes the protective index is higher in the presence of two or more substances than in the presence of either of the substances alone. For example, the LD_{50} of potassium cyanide alone is 11 mg/kg, whereas it is 21 mg/kg in the presence of sodium nitrite, giving a protective index of 1.91. The LD_{50} of potassium cyanide in the presence of sodium thiosulfate is 35 mg/kg, giving a protective index of 3.2. The LD_{50} of potassium cyanide in the presence of both nitrite and thiosulfate is 52 mg/kg with a protective index of 4.73. Since the protective index is higher for the simultaneous use of nitrite and thiosulfate, the two chemicals constitute the antidote against cyanide intoxication.^[15]

THERAPEUTIC WINDOW: The therapeutic window (or pharmaceutical window) of a drug is the range of drug dosages which can treat disease effectively without having toxic effects. Medication with a small therapeutic window must be administered with care and control, frequently measuring blood concentration of the drug, to avoid harm. Medications with narrow therapeutic windows include digoxin, lithium and warfarin.^[16]

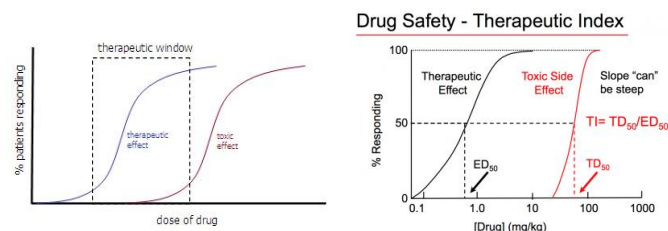


Figure-4: Therapeutic window & Drug safety

OPTIMAL BIOLOGICAL DOSE: Optimal Biological Dose (OBD) is a vague concept that refers to the quantity of a drug that will most effectively produce the desired effect while remaining in the range of acceptable toxicity.

MAXIMUM TOLERATED DOSE: MAXIMUM TOLERATED DOSE (MTD) refers to the highest dose of

a radiological or pharmacological treatment that will produce the desired effect without unacceptable toxicity. The purpose of administering MTD is to determine whether long-term exposure to a chemical might lead to unacceptable adverse health effects in a population, when the level of exposure is not sufficient to cause premature mortality due to short-term toxic effects. The maximum dose is used, rather than a lower dose, to reduce the number of test subjects (and, among other things, the cost of testing), to detect an effect that might occur only rarely. This type of analysis is also used in establishing chemical residue tolerances in foods. Maximum tolerated dose studies are also done in clinical trials.

MTD is an essential aspect of a drugs profile as all modern healthcare systems dictate a maximum safe dose for each drug, and generally have numerous safeguards (i.e. insurance quantity limits and government-enforced maximum quantity/time-frame limits) to prevent the prescription and dispensing of quantities exceeding the highest dosage which has been demonstrated to be safe for members of the general patient population. Patients are often unable to tolerate the theoretical MTD of a drug due to the occurrence of side-effects which are not innately a manifestation of toxicity (not considered to severely threaten a patient's health) but cause the patient sufficient distress and/or discomfort to result in non-compliance with treatment. Such examples include emotional blunting with antidepressants, pruritis with opiates and blurred vision with anticholinergics.^[17]

EC₅₀: The term half maximal effective concentration (EC₅₀) refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. It is commonly used as a measure of drug's potency. The EC₅₀ of a graded dose response curve therefore represents the concentration of a compound where 50% of its maximal effect is observed. The EC₅₀ of a quantal dose response curve represents the concentration of a compound where 50% of the population exhibits a response, after specified exposure duration. It is also related to IC₅₀ which is a measure of a compound's inhibition (50% inhibition). For competition binding assays and functional antagonist assays IC₅₀ is the most common summary measure of the dose-response curve. For agonist/stimulator assays the most common summary measure is the EC₅₀.

Sometimes it is also expressed as pEC₅₀ = -log(EC₅₀) (with EC₅₀ in mol/L). A small change in ligand concentration typically results in rapid changes in response in the biological system, following a sigmoidal function. The inflection point at which the increase in response with increasing ligand concentration begins to slow is the EC₅₀. Which can be mathematically determined by derivation of the best-fit line?

While relying on a graph for estimation is more convenient, this typical method yields less accurate results and less precise.

EQUATION: Many different equations can be used to derive an EC₅₀. One possible function is: $Y = \text{Bottom} + [\text{Top} - \text{Bottom}] / [1 + (X/EC_{50})^{-\text{Hill Coefficient}}]$. Where Y is the observed value, Bottom is the lowest observed value, Top is the highest observed value, and the Hill coefficient gives the largest absolute value of the slope of the curve.

Substance	Animal, Route	LD ₅₀ (mg/kg)	Substance	Animal, Route	LD ₅₀ (mg/kg)
Water	rat, oral	>90	Melamine cyanurate	rat, oral	4,100
Sucrose	rat, oral	29,700	Sodium molybdate	rat, oral	4,000
MSG	rat, oral	16,600	Sodium chloride	rat, oral	3,000
Vitamin C	rat, oral	11,900	Paracetamol	rat, oral	1,944
Urea	rat, oral	8,471	THC	rat, oral	1,270
Cyanuric acid	rat, oral	7,700	Arsenic	rat, oral	763
Cadmium sulfide	rat, oral	7,080	ADBAC	rat, oral	304.5
Ethanol	rat, oral	7,060	Coumarin	rat, oral	293
IMPA	rat, oral	6,860	Aspirin	rat, oral	200
Melamine	rat, oral	6,000	Caffeine	rat, oral	192
Arsenic trisulfide	rat, oral	185-6,400	LSD	rat, IV	16.5
Sodium nitrite	rat, oral	180	Arsenic trioxide	rat, oral	14
Uranyl acetate dihydrate	mouse, oral	136	Arsenic metal	rat, IP	13
Bisoprolol	mouse, oral	100	Nicotine	human, oral	6.5-13.0
Cobalt (II) chloride	rat, oral	80	Sodium cyanide	rat, oral	6.4
Cadmium oxide	rat, oral	72	White phosphorus	rat, oral	3.03
Sodium fluoride	rat, oral	52	Strychnine	human, oral	1-2
Pentaborane	human, oral	<50	Cantharidin	human, oral	0.5
Capsaicin	mouse, oral	47.2	Aflatoxin B1	rat, oral	0.48
Mercury (II) chloride	rat, dermal	41	Venom of the spider	rat, SC	134 µg/kg
Venom of the snake	rat, SC	25 µg/kg	Batrachotoxin	human, SC	2-7 µg/kg
Ricin	rat, IP rat, oral	22 µg/kg 20-30 mg/kg	Abrin	mice, IV	0.7 µg/kg
TCDD	rat, oral	20 µg/kg	Maitotoxin	mouse, IP	0.13 µg/kg
Sarin	mouse, SC injection	17.23 µg/kg	Polonium-210	human, inhalation	10 ng/kg
VX	human, oral	2.3 µg/kg	Botulinum toxin (Botox)	human, oral, injection, inhalation	1 ng/kg

Mercury (II) chloride	rat, dermal	41 mg/kg	Venom of the Brazilian wandering spider	rat, SC	134 µg/kg
Ionizing radiation	human, irradiation	5 Gy	Abbreviations: MSG=Monosodium glutamate, IMPA= Sodium isopropyl methyl phosphate, THC= Delta-9-tetrahydro cannabinol, Alkyl ADBAC=Dimethyl Benzalkonium Chloride, LSD=Lysergic acid diethylamide, 2,3,7,8-TCDD=Tetra chloro dibenzo dioxin, IP=Intraperitoneal, IV=Intravenous, SC=Subcutaneous		

Table-1: LD₅₀

LIMITATIONS: The effects of a stressor or drug generally depend on the exposure time. Therefore, the EC₅₀ (and similar statistics) will be a function of exposure time. The exact shape of this time function will depend upon the stressor (e.g., the specific toxicant), its mechanism of action, the organism exposed, etc. This time dependency hampers the comparison of potency or toxicity between compounds and between different organisms.

The half maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The values are typically expressed as molar concentration. It is commonly used as a measure of antagonist drug potency in pharmacological research. According to the FDA, IC₅₀ represents the concentration of a drug that is required for 50% inhibition *in-vitro*. It is comparable to an EC₅₀ for agonist drugs. EC₅₀ also represents the plasma concentration required for obtaining 50% of a maximum effect *in-vivo*.^[18]

FUNCTIONAL ANTAGONIST ASSAY: The IC₅₀ of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC₅₀ values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC₅₀ values can be used to compare the potency of two antagonists. IC₅₀ values are very dependent on conditions under which they are measured. In general, the higher the concentration of inhibitor, the more agonist activity will be lowered. IC₅₀ value increases as agonist concentration increases. Furthermore, depending on the type of inhibition other factors may influence IC₅₀ value; for ATP dependent enzymes IC₅₀ value has an interdependency with concentration of ATP, especially so if inhibition is all of it competitive.

COMPETITION BINDING ASSAYS: In this type of assay, a single concentration of radioligand (usually an agonist) is used in every assay tube. The ligand is used at a low concentration, usually at or below its K_d value. The level of specific binding of the radioligand is then determined in the presence of a range of concentrations of other competing non-radioactive compounds (usually antagonists), in order to measure the potency with which they compete for the binding of the radioligand. Competition curves may also be

computer-fitted to a logistic function as described under direct fit.

In this situation the IC₅₀ is the concentration of competing ligand which displaces 50% of the specific binding of the radioligand. The IC₅₀ value is converted to an absolute inhibition constant K_i using the **Cheng-Prusoff equation** formulated by Yung-Chi Cheng and William Prusoff.

IC₅₀ AND AFFINITY: IC₅₀ is not a direct indicator of affinity although the two can be related at least for competitive agonists and antagonists by the Cheng-Prusoff equation. For enzymatic reactions, this equation is: $K_i = IC_{50} / [1 + (S) / K_m]$

where K_i is the binding affinity of the inhibitor, IC₅₀ is the functional strength of the inhibitor, [S] is fixed substrate concentration and K_m is the concentration of substrate at which enzyme activity is at half maximal (but is frequently confused with substrate affinity for the enzyme, which it is not). Alternatively, for inhibition constants at cellular receptors: $K_i = IC_{50} / [1 + (A) / EC_{50}]$, where (A) is the fixed concentration of agonist and EC₅₀ is the concentration of agonist that results in half maximal activation of the receptor. Whereas the IC₅₀ value for a compound may vary between experiments depending on experimental conditions, (eg. substrate and enzyme concentrations) the K_i is an absolute value. K_i is the inhibition constant for a drug; the concentration of competing ligand in a competition assay which would occupy 50% of the receptors if no ligand was present. The Cheng-Prusoff equation produces good estimates at high agonist concentrations, but over- or under-estimates K_i at low agonist concentrations. In these conditions, other analyses have been recommended.^[19]

pIC₅₀: Sometimes, IC₅₀ values are converted to the pIC₅₀ scale. $pIC_{50} = -\log_{10}(IC_{50})$

Note the minus sign, which means that higher values of pIC₅₀ indicate exponentially greater potency. pIC₅₀ is usually given in terms of molar concentration (mol/L, or M). Therefore, to obtain a pIC₅₀, an IC₅₀ should be specified in units of M. When IC₅₀ is expressed in µM or nM, it will need to be converted to M before conversion to pIC₅₀.

Examples: Comparing substances (especially drugs) to each other by LD₅₀ can be misleading in many cases due (in part) to differences in effective dose (ED₅₀). Therefore, it is more useful to compare such substances by therapeutic index, which is simply the ratio of LD₅₀ to ED₅₀.

Animal-rights and animal-welfare groups, such as Animal Rights International, have campaigned against LD₅₀ testing on animals. Several countries, including the UK, have taken steps to ban the oral LD₅₀, and the Organization for Economic Cooperation and Development (OECD) abolished the requirement for the oral test in 2001.^[20]

CONCLUSION: While prescribing drugs, healthcare providers rely on their clinical experience and the results of drug trials that determine the TI of a drug. The larger value of TI indicates that there is a wide margin between the toxic and effective dose, whereas a smaller value indicates that there is a narrow margin between the effective and toxic dose. In case of drugs that have a low TI, even a small increase in the dosage can produce toxic effects. Additional care must be taken while

prescribing a drug with a narrow TI. Therefore, the pharmaceutical industry has been making efforts to replace NTI drugs (drugs that could be toxic at relatively low levels) with drugs with higher TIs. Healthcare providers mostly prescribe drugs that have a wide margin of safety. However, they might sometimes prescribe NTI drugs when the medical condition is of a serious nature and other safer options are not available. In such cases, monitoring the effects of the drug becomes essential. Initially, the ratio of the LD₅₀ and ED₅₀ was determined through animal studies. It must be noted that the ratio measured by animal studies might not be very accurate when it comes to humans. Also, human subjects cannot be used for determining a median lethal dose, for obvious reasons. To add to that, using animals for determining a lethal dose raises ethical issues. While this ratio might not give an accurate estimate of toxicity in humans, even defining an effective dose might not be a simple task. Also, median values for animals or healthy individuals might not be right for individuals of different age groups or those affected by diseases. According to the Food and Drug Administration of the United States (FDA), narrow therapeutic range (NTR) drug products are those 'containing certain drug substances subject to therapeutic drug concentration or pharmacodynamic monitoring, and/or where product labeling indicates a narrow therapeutic range designation'. According to the Therapeutic Products Directorate of Health, Canada, an NTR drug is that wherein the ratio of the lowest concentration at which clinical toxicity occurs to the median concentration providing a therapeutic effect is less than or equal to two. Some drugs that have a narrow therapeutic index are: Warfarin, Lithium, Digoxin, Phenytoin, Gentamicin, Amphotericin B, 5-fluorouracil, AZT (Zidovudine). Care must be taken to determine the right dose for the aforementioned drugs in individual cases, as administration of large doses could cause adverse effects. On a concluding note, the concept of therapeutic index has some limitations, but it is clinically significant, as it lays stress on the importance of the margin of safety of a drug. Drugs that have a narrow or relatively narrow TI are still used when safer alternatives are not available. Under such circumstances, therapeutic drug monitoring becomes extremely important. In such cases, the plasma levels of the drug should be monitored regularly.

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