22. MTD (Maximum Tolerated Dose) and NOAEL (No-Observed Adverse Effect Level)

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During the drug discovery and development process, early general toxicity studies in experimental animals aim to estimate the Maximum Tolerated Dose (MTD). The **MTD** is defined as the highest dose that does not cause unacceptable adverse effects, that is, the highest dose tolerated by most animals and that generally produces toxicity equivalent to grade 3 or higher adverse events in fewer than 33% of the experimental animals. In humans, grade 3 corresponds to adverse events that limit or prevent daily activities and/or require treatment, whereas grade 4 events are severe enough to cause serious injury and threaten the individual's survival (Sibille et al., 2010). In this preliminary assessment of tolerability, one or a few prototypes are evaluated using a single-dose escalation study (for example, four different doses and one control), in which experimental animals receive doses expected to reach relevant multiples of the effective dose (Herlich et al., 2009). Several parameters are recorded, including changes in mobility, body weight, food consumption, behavioral alterations, and organ/system-specific clinical signs related to the skin, gastrointestinal tract, ocular, respiratory, and urogenital systems, as well as mortality. In addition, hematological and biochemical analyses and histopathological examinations of target organs may be performed to better characterize the degree of toxicity associated with the tested doses.

According to the definition adopted in the "Agência Nacional de Vigilância Sanitária" (Anvisa-Brazil) guideline (2013), "*acute toxicity studies (single dose) are those used to evaluate the toxicity produced by a test substance when it is administered in one or more doses over a period not exceeding 24 hours, followed by observation of the animals for 14 days after administration*" and they "*must be conducted in at least two mammalian species*" usually starting with rodents. It is noteworthy that the trend among international regulatory agencies, such as the FDA, has been to relax requirements regarding the design of acute toxicology studies, accepting dose-escalation studies (instead of the classic parallel testing of multiple doses), and even short-duration dose-ranging studies to define the MTD (FDA, 2010).

It should be emphasized that the MTD value is of great importance, as it serves as the reference for selecting the doses to be used in the subsequent study, in which repeated doses of the selected optimized prototype will be administered, necessarily in a laboratory certified for compliance with Good Laboratory Practice (GLP). The nonclinical repeated-dose toxicity study (whose duration depends on the intended duration of administration in clinical trial protocols) is one of the nonclinical safety studies required by regulatory agencies to authorize the initiation of Phase 1 clinical trials of a drug candidate (Andrade et al., 2016).

According to the Anvisa guideline (2013), repeated-dose studies generally involve the use of "*three doses, with the highest dose selected with the expectation of producing observable toxic effects, but not death or severe suffering, and respecting the maximum limit of 1000 mg/kg/day*". These studies are conducted in at least two mammalian species (rodent and non-rodent) and must have a duration equal to or longer than that of the planned clinical trials in humans, as proposed by the FDA (2010). The information provided by repeated-dose toxicity studies is crucial for assessing whether the planned clinical trials will not expose research participants to foreseeable and avoidable risks.

These studies allow determination of the **NOAEL** (No-Observed Adverse Effect Level), defined as the highest dose level at which, under the study conditions, there is no biologically significant increase in the severity or frequency of adverse effects in the treated animal group compared with the control group. This dose is fundamental for selecting the initial dose to be administered to volunteers in a Phase 1 clinical trial (FDA, 2010) and is usually a small fraction (1/10 to 1/100) of the NOAEL observed in the most sensitive animal species used in the study (FDA, 2013).

References

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