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The dose makes the medicine

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Dose and time considerations in the development and use of a drug are important for assessing actions and side effects, as well as predictions of safety and toxicity. This article deals with epistemological aspects of dose selection by probing into the linguistic and cultural roots for the *measure* of *medicine mediated* by the *medical* doctor. Because toxicity is related to dose, historic and recent views suggest that less can be more. At low, medium and high dose levels, effects can differ not only quantitatively but also qualitatively. Dose-related target activation and recognition of enantiodromic thresholds between beneficial and toxic effects require elucidation of underlying events. Such studies, including hormesis and microdosing, call for extended ADME procedures with high-resolution methods in addition to the current low-resolution approaches. Improved information of drug logistics and target pharmacokinetics enables effective drug selection, dose determination and prediction. It also allows considerations of systems biology [i.e. integral (gestalt) pharmacology] exemplified by the drug homunculus, as in the case of vitamin D, that might lead to new paradigms and drug design.

The measure

Publications about the adverse effects of pharmaceuticals occasionally provide statistics and conclusions without sufficient considerations of dose, thus leaving the impression that any treatment with that compound is perilous. For example, in recent years, a discussion raged in the media about the negative effects of estradiol and, in particular, estrogen-replacement therapy and its relationship to cancer. In numerous articles, the side-effects of estrogen treatment were highlighted in a generalized fashion and, although consideration was given to the duration of treatment, the relationships to dose were frequently left out. And yet, considerations of dose and time in pharmacology and toxicology are paramount [1]. The effects of high doses can be different from those of low doses and those of very high doses, and extrapolations from the results of limited dose studies can be misleading. Different dose ranges need to be evaluated separately.

Relationships between quantity and location are also relevant. To act, drugs have to reach the right places, as expressed in the rule *corpora non agunt nisi in loco* [substances do no act unless in the (right) place] [2,3], with the amounts modifying the degree and kind of action, *sola dosis facit venenum* (only the dose makes the poison; Paracelsus, 1538) [4].

The importance of dose – the amount of anything – is apparent in everything biological, and in all other matters. Wise people have espoused the need for the right dose throughout history, in philosophy, religion, medicine and pharmacology. Selecting the right amount is inherent in eternal wisdom. In the origin of our language, it is the recognition and implementation of measure (German, *Maß*) with the Indo-Germanic root **me**(d), meaning to wander, to measure (by defining an area or related path), and used in **me**dium, **mi**ddle, **me**dicine, **me**al, **me**ditation, **me**diation, **moo**n, **mu**st and **mo**re. All of these words stem from me(d), indicating the right amount of doing, dosing and timing [5]. Accordingly, it is essential to recognize what is beneficial to life and what can be harmful, what is too little and what is too much, what is the right and **me**dium dose for the individuum. The **me**dical doctor with his knowledge and experience defines the **me**asure and the **me**dicine, taken in relation to the **me**al

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(German, **mahl**), once (German, *ein***mal**) or **ma**ny times (German, **me**hr**ma**ls).

The search for the **me**asure, the happy **me**dium, the **mi**ddle way, is the hallmark of the wise and religious, in behavior and work, eating and drinking, waking and sleeping, in health and disease.

Drug logistics

To understand drug actions, the locations and identities of specific targets need to be known. Questions of drug logistics that require consideration and answers include whether the target is reached, what amount is available and acting at the target over time, and how receptor-binding, metabolism and excretion occur. Assessment of drug logistics is an important part of preclinical ADME studies and of clinical imaging.

Maintenance of life is fundamentally related to the right movements to the right places, justifying the formulation of the rule that 'everything in life is logistics' [6]. Information on *in vivo* drug logistics, delivery, deposition and excretion can be aided through the use of tagged radiolabeled compounds. Isotope labeling is still the most widely used and expedient way to monitor the *in vivo* path of a molecule, provided it can be assured that the label does not change its chemical properties. Changes of binding properties to receptors have been noted when steroid hormones were labeled with radioiodine in specific positions of the molecule or when conjugation with fluorescein was used [3].

Deficient target identification, lack of validation of results from *in vitro* methods, radioassays with dissected organs, and low-resolution whole-body scanning procedures leave gaps and present a deficient picture, as made evident in comparative studies [7]. Automated high-throughput screening without sufficient *in vivo* validation of targets can lead to meaningless, if not misleading, answers [8]. 'Sound target validation is mandatory' [9].

Assessment of the *in vivo* path of delivery, of specific receptor sites of action, of tissues of metabolism and routes of excretion requires methods with high sensitivity and high resolution. Receptor microscopic autoradiography has been developed to that purpose and documented to provide needed information [3].

Ancient wisdoms

Ne quid nimis – meden agan – ariston metron – in medio virtus – aurea mediocritas – minima maxima sunt

The importance of the right dose in all things has been recognized at various times – related adages and admonitions can be traced to early history. The famous *ne quid nimis* (nothing that is too much) is ascribed to the Roman playwright Terentius (~190–159 BC); it has been popularized through his play *Andria*. This adage apparently is of Greek origin, where it is inscribed as *meden agan* (μηδεν αγαν, meaning 'nothing too much') on the Apollo Temple in Delphi, and variably credited to Solon of Athens (~638–558 BC), Theogonis of Megara (~540–470 BC) and, as *ariston metron* [best (is the right) measure], to Cleobulus of Lindos (633–564 BC) – all are advocating moderation and warning against excess. The same concept is expressed in the Latin adages *in medio virtus* [the virtue (is) in the middle] and *minima maxima sunt* (the minimal is the maximal), as well as in philosophical and religious admonitions throughout history, like *temperantia* (temperance, moderation), *aurea mediocritas* (the golden middle, Horaz), *sophrosyne*, and harmonia (moderation and harmony, Hesiod), thus avoiding fatalities of *hybris* (hubris). Absence of moderation could lead to the divine retribution of Nemesis, the goddess of retributive justice. Hesiod, a Boeotian farmer and writer during the 8th century BC, made this remarkable statement [10]: 'Fools! They know not how much more the half is than the whole.'

Paracelsus

'Allein die dosis macht das ein ding kein gift ist'/Sola dosis facit venenum

Similar wisdom in the context of pharmacology came from the Swiss–German physician Theophrastus of Hohenheim (Paracelsus) whose use of poisons, such as opium, to treat the sick resulted in accusations against him by academics in Basel. In 1538, in his defense, he made the fundamental statement that the distinction between poisonous and nonpoisonous substances is not real and that a beneficial nonpoisonous substance could become poisonous, and vice versa. In his words [4] (Figure 1):

'*Was ist das nit gifft ist? alle ding sind gift/und nichts ohn gifft/Allein die dosis macht das ein ding kein gift ist.*' (What is not poison? All things are poison and nothing [is] without poison. Only the dose makes a thing not to be poison.)

This axiom has become one of the foundations of toxicology with continuing impact on its concepts [11]. Paracelsus also states in his *Defensiones* [4]: '*Merket auf diesen Punkt, darum geht es*: *Es ist nicht zu viel, noch zu wenig*. *Wer das Mittel*(*maß*) *trifft, nimmt kein Gift zu sich*.' (Consider this point, this is what matters: not too much, not too little. Who hits the right measure is not endangered with poison.)

Sengai

Sajikagen

The notion of right dose and its significance for life and death have arisen in different cultures. The similarity between Paracelsus and Sengai is noteworthy.

Sengai (1750–1838), the Japanese Zen monk and calligrapher, painted a spoon with the comment: *ikasou to korosou to – sajikagen* [whether for life, whether for death – (it depends on) the right spoon-measure] (Figure 2). This is well expressed in Sengai's drawing at the Idemitsu Museum, Tokyo, and in the Chinese characters for *sajikagen: saji*, spoon; *kagen*, dose, measure, the right measure (*ka*, increase; *gen*, decrease).

Dosis minima

The maxim of the smallest possible dose – *dosis minima* – for therapy is a tenet in homeopathy. However, it is linked to the principle of *similia similibus* that the identical substance that causes symptoms of a disease in large doses can be used as a remedy for a similar kind of disease when given in small doses.

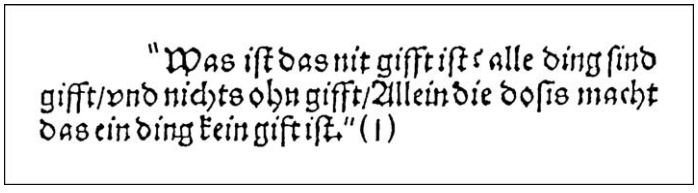

FIGURE 1

What is not poison? All things are poison and nothing [is] without poison. Only the dose makes a thing not to be poison. By the Swiss–German physician, Theophrastus of Hohenheim (Paracelsus).

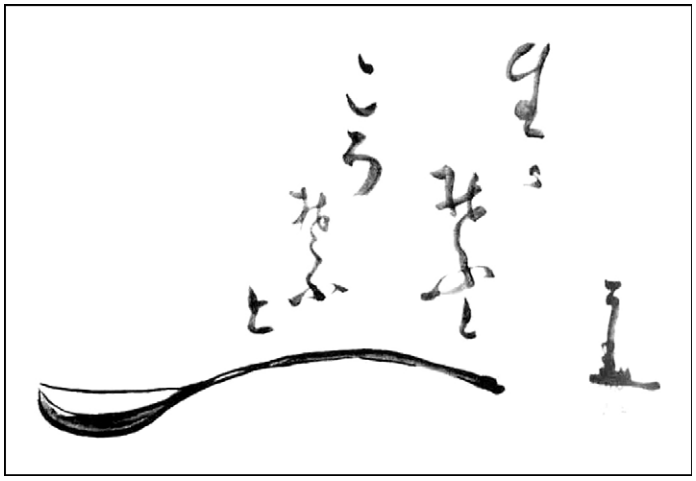

FIGURE 2

Ikasou to korosou to – sajikagen. Whether for life, whether for death – (it depends on) the right spoon-measure. By Sengai, the Japanese Zen monk and calligrapher. Reproduced, with permission, from Idemitsu Museum, Tokyo.

The advocated low-dose treatment appears ideologized in concepts and practices. By decreasing the concentration of an active ingredient in a solvent or solid by dilution and succussion, the augmentation of effects, called potentiation, is claimed. As explanations of the action of infinitesimal doses, the 'law of least quantity' and 'transmission of action' are invoked. Through shaking and related alteration, powers and qualities of the drug are believed to be progressively transferred to the diluting medium (alcohol, water, or lactose for solids). Thus remedial actions of the original agent are thought to be imparted and preserved in dilutions offered as therapeutics by the apothecary.

Scientific proofs-of-concept of homeopathic dynamization through the process of serial dilutions is lacking and controversial as a mixture of empirical truths and presumptions. However, the principle of *dosis minima* is of historic interest, because it appears conceptually akin to the contemporary efforts toward microdosing for optimally safe and effective treatment.

Arndt–Schulz Rule

Hormesis – enantiodromy – pharmacon

Substances considered to be toxic, inhibitory or lethal, and therefore designated toxins, were recognized as stimulatory and beneficial at low doses by Rudolf Arndt und Hugo Schulz [12,13]. According to their observations, some toxic pharmaca, or poisons, when applied in highly diluted form enhance life processes, and moderately strong doses are still favorable, whereas strong concentrations inhibit these processes and can even terminate them. There are exceptions – many paralyzing substances are said to have no stimulatory effects at low doses. However, the rule formulated by Arndt and Schulz more than 100 years ago [the term hormesis (Greek, όρμαω, *hormao* – arouse) was introduced later to designate events pertaining to that rule] applies to a wide range of substances and has been increasingly recognized as applicable in pharmacology and toxicology [14,15].

Hormetic effects of drugs are of considerable interest and relevant studies are needed. Low-dose studies require methods of high sensitivity and high resolution. Current ADME procedures for routine drug development might satisfy regulatory requirements, but do not provide adequate information to predict toxicity [16] and recognize low-dose effects and underlying low capacity but high specificity targets.

There are investigators who consider the Arndt–Schulz rule to be a biogenetic ground rule applicable to all biological systems. This principle of nature deserves careful consideration in pharmacology and toxicology. It includes encouragement of studies of mechanisms specific for low-dose effects, reversal and high-dose effects. It also includes determining thresholds and related events for recognizing and characterizing hormetic events. A threshold might not be a fixed determinant and can vary among target tissues, based on endocrine status and other changing systemic and organ conditions. Evidence from high-resolution studies with receptor microautoradiography indicates quantitative differences for estradiol and vitamin D in receptor-binding in different target-cell populations [7], suggesting target-specific responses.

As an example, autoradiographic studies with ^{3}H-estradiol revealed a saturation of nuclear uptake of 0.3–0.9 μg per 100 g body weight in pituitary cells, and of 2.7 μg per 100 g body weight in uterus and vagina cells [17], suggesting different functional thresholds in different target cell populations. After topical application of tritium-labeled vitamin D, autoradiograms of skin close to the application site displayed high levels of radioactivity in the cytoplasm of epidermal cells, but low levels in their nuclei, whereas levels of radioactivity measured further from the application site were low in cytoplasm but high in nuclei [18]. In epidermal cells, nuclear concentration is characteristic after systemic administration [19]. These observations together suggest differences in subcellular distribution and binding depending on the concentration of labeled compound that reaches the target. It also suggests a correlation between subcellular binding and function. Skin keratinocytes, recognized as target cells for vitamin D, are stimulated during wound-healing with low doses vitamin D, whereas excessive cell proliferation in psoriasis can be antagonized with high doses [20,21]. In addition, high doses of vitamin D are now considered growth inhibitory for various tumors. Vitamin D appears to be a strong example for hormetic effects as predicted by the Arndt–Schulz rule.

The transition of vitamin D from stimulatory and beneficial to inhibitory and toxic action can be regarded as *enantiodromia* (Greek, εναντίωζ, *enantios* – contrary, δρομοζ, dromos – course) – enantiodromy is the conversion of a linear process into its opposite. This simultaneous representation of positive and negative effects of a drug (a pharmacon) is reflected in its Greek root *pharmakon* (φ?ρμᾶκον), which means both remedy and poison.

Heraclit (~540–480 BC) observed that contrary properties can be co-instant in an object [22]. One opposite can succeed another or opposites can coexist simultaneously. A succession of opposed states occurs in cases of change, like wakefulness and sleep, and love and hate. Although the observed event might appear static, it is in fact dynamic, as expressed in Heraclit's concept of change (*panta rhei*). Everything is in flux, change and permanence coexist. A pair of contraries can coexist in a pattern of Heraclit's 'unity of opposites', aiming toward equilibrium and harmony. Some analytical minds may resist such epistemologic cogitations and hol-

istic comparisons, but an integrated view can be helped by philosophical aspects in seeking to understand underlying events in transitions from the beneficial and curative to the toxic.

Changes of dose and drug effects

Recommendations for a therapeutic dose need to consider variations related to age, and endocrine and disease states, as well as to arrangements of ADME experiments.

Drug effects with a selected and initially determined dose are not stable and can be modified under chronobiological and other factors. Changes in quantity and quality of drug effects can be expected and can be related to receptor desensitization, down-regulation or tachyphylaxis – conditions that are not well understood. Some of these changes can be subtle, at times remaining unrecognized and accounting for therapeutic failures. Analyzing, understanding, and dealing effectively with these processes require sensitive methods, such as receptor microscopic autoradiography.

Although considerations of low dose, moderation and the dictum *ne quid nimis* reflect general principles, doctors must remain mindful that special circumstances of disease and nature can lead to overshooting or undershooting responses that demand commensurate therapeutic actions that digress from the desired middle path of treatment.

Distinguishing between a safe dose and a toxic dose for any chemical depends on our ability to identify thresholds for all of the adverse effects that can be produced by it [15]. However, dose and time are independent variables in exposure and each must be considered [1].

Modern microdosing

Observations in clinical medicine recognize the effectiveness of low dose treatments. Its soundness is reflected in results from modern studies of hormesis as a scientific verification of the empirical Arndt–Schulz rule [23]. The maxim of *dosis minima*, albeit originating from concepts of homeopathy, means treatment with the least possible therapeutic dose and is not entirely different from the modern recommendation of *microdosing* [24,25]. It is aimed at minimizing undesirable side effects and increasing safety, while maintaining and possibly increasing efficacy. Such aspects, among others, are also contained in Linus Pauling's orthomolecular principle.

Microdosing can be applied empirically. Its purposeful development and understanding requires sensitive methods similar to those needed for studies of hormesis. Such procedures are likely to be considered non-expedient by marketing-driven mentalities. Current routine approaches that produce strong and quick statistics with beautiful graphs, serving expedient application for drug approval, are not conducive to providing the needed detail of target pharmacokinetics and target hierarchies necessary for recognition and advanced prediction of low-dose drug effects. 'Screening programs that are based on non-validated or poorly validated targets' [9] lead to meaningless and misleading answers about potency and toxicity [9,16].

Systems biology – the case of vitamin D

Going beyond ADMET studies in drug research and development, an integrative interpretation of information about drug dose, time and related kinetics is useful, albeit sufficient and sound detail must be provided.

A systems approach [26] can include relationships between *in silico*, *in vitro*, high-throughput and biochemical data, together with information on *in vivo* high-resolution target recognition and related characterization, as well as associated clinical effects. *In vivo* expression profiles of targets in diseased compared with healthy states can be developed as clinical guides using, for example, animal models [9]. This extended application of receptor microautoradiography and related processing of sophisticated data could be an important avenue for promoting innovation in drug development [27].

An example of systems biology in pharmacology is the proposed drug homunculus [3,7], which seeks to integrate and survey multiple qualitative and quantitative data. Such a drug or target homunculus, like a finger(body)print of a drug, facilitates recognition and display of potential therapeutic effects, side effects, and toxic effects, as well as comparisons between agonists, analogs and antagonists. Computerized links can be provided to quantitative, functional and even clinical information.

Consider a drug homunculus for the polyfunctional vitamin D (Figure 3). Red dots and lines in the drug homunculus indicate target tissues for $1,25(OH)_2$ vitamin D_3. The actions of most of the target tissues are unrelated to systemic calcium regulation and are instead related to the regulation of endocrine and exocrine secretion, cell proliferation and cell differentiation. Since 1979 [19] – decades before biochemical evidence appeared – more than 50 target tissues have been identified and characterized by receptor microautoradiography that resulted in a change of concept about the main biological action of vitamin D, beyond calcium homeostasis, as a seasonal polyfunctional regulator of major biological actions [28].

Looking at the vitamin D target homunculus, it can be easily seen that many, if not all, target tissues of the vitamin D system will be activated in patients treated with a vitamin D-related compound – whether taken against osteoporosis, tumor growth or any other single condition – unless a specific analog can be demonstrated to bind and act differently. The degree of target activation depends on the dose and time of treatment and the hierarchy of receptor binding. Such a 'target hologram' of drug-receptor binding is not static. It relates to specific conditions, akin to principles of holomovement, the adaptive flux in cybernetic systems. More work is required to understand such fundamentals of hormone and drug action. Qualitative and quantitative *in vivo* data need to be gathered using high resolution and high sensitivity approaches, such as receptor microscopic autoradiography; thus establishing what could be called integral, or *gestalt*, pharmacology in distinction to common focal pharmacology.

By learning more about specific target sites and their respective quantitative interactions with an agent, a certain relationship, or harmony, is likely to become recognizable. Optimal functional relationships between targets can characterize health. Disrupted harmonies and chaotic conditions can prevail in disease, especially in cancer. Such a view would be consistent with basic principles of existence.

In this respect, an awareness of proper dosage is crucial to the development of future vitamin D therapies. Physiologic dosing of vitamin D does not cause hypercalcemia – hypercalcemia is related

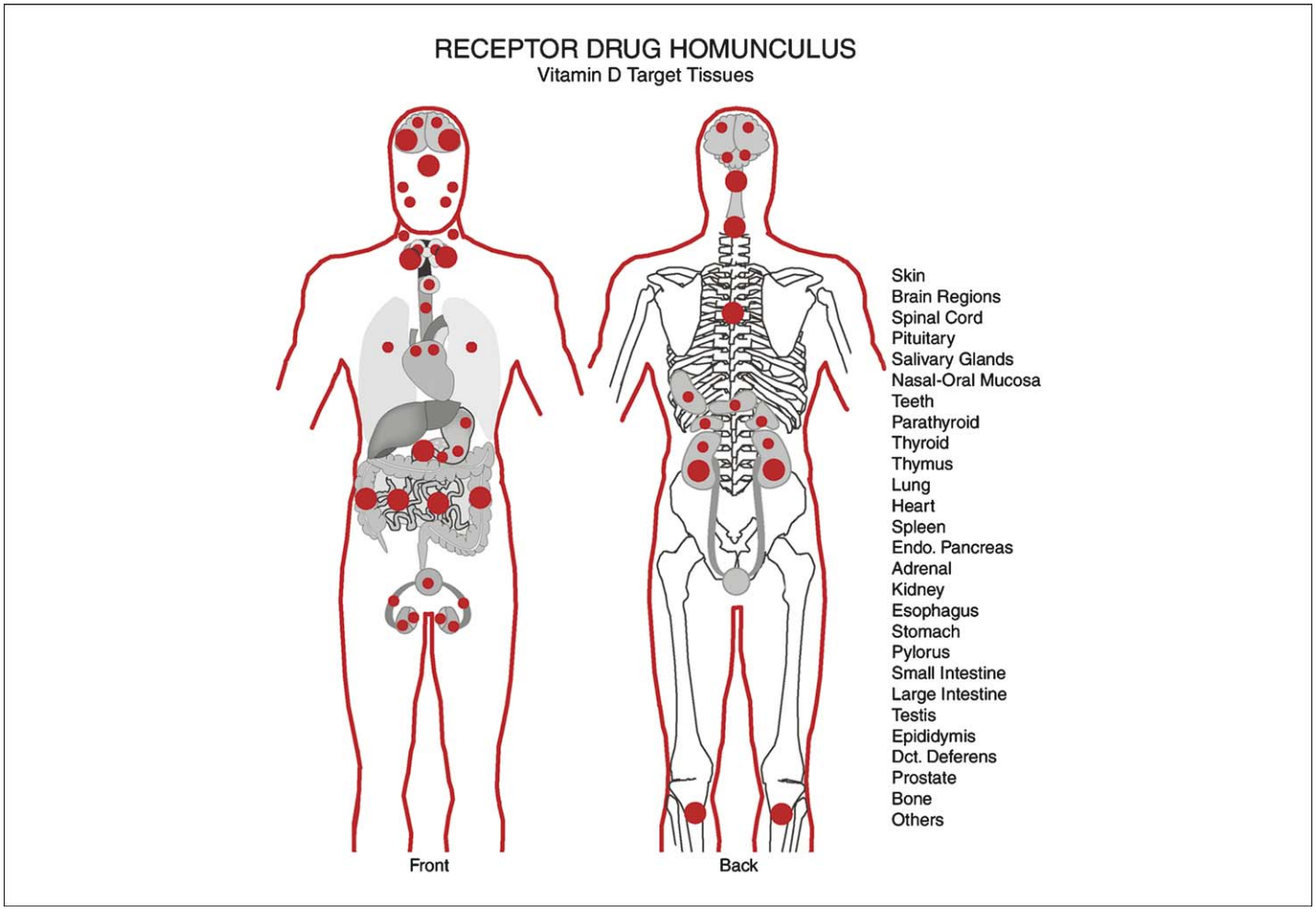

FIGURE 3

Receptor drug homunculus for vitamin D target tissues. Red dots and lines in the drug homunculus indicate target tissues for 1,25$(OH)_2$ vitamin D_3. Reproduced, with permission, from Ref. [3].

to overdosing. Considering the many target tissues that are unrelated to systemic calcium regulation, most therapeutic effects of vitamin D occur independently of the high-dose systemic calcium effects. Because of the biased focus on calcium, the many other effects tend to remain unnoticed and hidden. Future research needs to give more consideration to dose-effect relationships by monitoring target functions independently of systemic calcium regulation.

New therapeutic applications of vitamin D can be established for cardiovascular, neurological, endocrine, immune, gastrointestinal, reproductive and other diseases, including posttraumatic and gerontological deficiencies, in which the polyfunctional effects of the hormone not only come to bear, but can also be controlled and maximized for optimal health. Development of related analogs could ensue. This will eventually lead to better recognition and separation of high-dose calcium-related effects from low- and medium-dose preventive and beneficial effects.

Conclusions

The **me**dical doctor, with the help of pharmaceuticals, **me**diates the right **me**asure of **me**dicine. How much is the right dose? Sometimes, perhaps quite often, less is more. Sometimes less is curative and more is toxic. Toxic effects result from high-dose-dependent time-related therapies that can be innocuous and favorable with low doses. It depends on many variables.

Assessment of safe amounts of a drug can be aided effectively through preclinical studies aimed at determining the range of the *dosis efficax* and the threshold to the *dosis toxicans*. It is apparent that low, intermediate and high doses of the same drug can have different effects, as expressed in the Arndt–Schulz rule. Diagnostic criteria for recognizing thresholds need to be sought.

Identification of *in vivo* targets based on dose-related binding and functional follow-up requires sensitive high-resolution approaches in preclinical drug research and development. In the construction of an integrated portfolio of technological capabilities [29], detailed information is essential for the assessment of *in vivo* targets, related target pharmacokinetics and prediction modeling. There is no successful drug discovery without reasonable biology [30].

Expanded ADME studies that include tissue and cellular high-resolution approaches can provide information for:

- Identifying and characterizing *in vivo* target tissues;
- Recognizing target hierarchy of drug binding;
- Enabling dose-related target selection for therapies;
- Revealing serendipitous discovery of targets [28];
- Fingerprinting drugs for records, comparisons, advertisement;
- Comparing a drug with analogs, competitors, combinations;

- Guiding biochemical, functional and clinical follow-up;
- Selecting suitable biomarkers and assessing their *in vivo* tissue patterns;
- Validating *in vitro* procedures (*in silico*, high-throughput, cell lines, etc.);
- Validating low-resolution *in vivo* scanning procedures (PET-scan, NMR, etc.);
- Improving drug development, prediction and safety.

Measuring the blood levels and whole-organ (or organ chunk) distribution of a labeled compound and metabolites is not enough. In most cases, this does not reveal target-specific binding and related effects. Bioavailability in blood differs from bioavailability in targets, and extrapolations from blood to targets can be fallacious.

Information on target pharmacology and kinetics is essential. How can we answer challenging questions about hormone replacement therapy [31] – whether bioidentical estradiol and progesterone act differently from Premarin® or synthetic analogs like Prempro™ and Provera® – unless dose and binding characteristics and related functions of the target cell populations of these compounds are identified? And what do we know about the pharmacology of digitoxin, its estrogenic, antitumor and central-nervous effects? What are the *in vivo* target organs for cardiac glycosides? In the heart alone, with its different cell populations, what are the main targets, and can membrane-receptor-binding and/or nuclear uptake be demonstrated?

The answers could be surprising. Once target cell populations have been identified, their functions can be studied. Such was the case with estradiol at a time when biochemists used 'the heart' as a non-target muscle to compare it with 'the uterus' – until atrial cardiomyocytes were identified as estradiol target cells [32] involved in the production and secretion of the heart hormone atrial natriuretic factor.

Receptor microscopic autoradiography is one approach that provides the necessary resolution and sensitivity. With this technique, information can be gained that is difficult or impossible to obtain otherwise. No matter how robust the statistics, pharmacokinetic data without information on related target-cell biology remain incomplete and can be misleading. By contrast, more sophisticated ADME procedures will result in better elucidation of mechanisms of action, prediction of therapeutic, side and toxic effects, and in a reduction of current high failure rates during drug development [29]. Partial reorientation of current pharmacokinetic approaches towards appreciation of tissue heterogeneities, involving a change in attitude and perception toward cell biology and 'histopharmacology', is required.

High-resolution microscopic *in vivo* tissue studies could be applied selectively to representative compounds and new chemical entities, either in parallel with or in addition to the current 'expedient' but less sensitive low-resolution methods. The process of introducing a new drug could last several years. Short-term time-expedient and long-term target (detail)-expedient methods can be performed simultaneously to satisfy regulatory requirements and further clarify mechanisms of action, promote new drug development, and optimize prediction, effectiveness and safety.

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