feature

Memo to the FDA and ICH: appeal for in vivo drug target identification and target pharmacokinetics

Recommendations for improved procedures and requirements

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This memorandum is addressed to members of regulatory agencies, as well as managers of pharmaceutical companies. Pharmacokineticists and toxicologists may consider this proposal, weigh its merits, and provide input for implementation. Experience from academic research and ADME experiments during drug development has prompted this appeal for improved drug target recognition. Similar demands have been made repeatedly in the literature. Such efforts are not new, but a renewed urgency has come from comparing results obtained with methods of different resolution and sensitivity, namely high-resolution receptor microscopic autoradiography compared and viewed in parallel to conventional low-resolution 'cut-and-count' radioassays and whole body autoradiography. Conflicting results reveal astounding deficiencies of current ADME approaches. False negatives and false positives of favored 'expedient' procedures allow drugs to reach the market with misleading and inaccurate information about the total drug effect.

For the development and approval of new drugs, identification of *in vivo* target tissues and related information on target pharmacokinetics and functions are neither required nor recommended by the US Food and Drug Administration (FDA) and the International Conference on Harmonization (ICH). As a consequence, important information ends up missing. One wonders why *in vivo* target identification is not required even though the need for target identification and recognition of related functions is evident and has been emphasized repeatedly over many years in numerous accounts by pharmacologists and toxicologists [1–10].

The low success rate for new drugs and the high cost of drug development are related to a lack of information on *in vivo* mechanisms of action and (over)reliance on data from 'expedient' *in vitro* tests and low-resolution *in vivo* (or *ex vivo*) procedures. Current problems in drug developments stem partly from permissive regulations that downplay consideration of *in vivo* tissue and cellular heterogeneities relevant to drug targeting and action.

"...For approval and intelligent use of drugs, is it not indispensable to know tissue and cellular sites of action? Efforts are stifled by demands for expediency and profit. But "we learn from history that expediency has rarely proved expedient." (B.H. Liddell Hart: in "The shortsightedness of expediency")"

Background: *high-resolution in vivo (ex vivo) methods.*

Drug actions cannot be understood unless information on *in vivo* targets is available. Statements in recent literature reflect the significance of target-related pharmacology:

"... what's most important is to develop the kind of assay that best recapitulates the function of the target in the cell. That's really the bottom line [3]."

"... target site drug levels may substantially differ from corresponding plasma levels. Suboptimal target site concentrations may have

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important clinical implications, as it is a potential explanation for therapeutic failure [4]."

"In spite of the importance of drug distribution as a key factor in determining pharmacologic response, research on drug distribution has historically received much less attention than that of absorption, metabolism, and excretion." "The scarcity of research on drug distribution is quite a surprise, since the concept of drug concentration at active site has been recognized for almost a half century [5]."

Reliance on data from *in vitro* procedures, low-resolution radioassays, whole body macroautoradiography, and low-resolution imaging is not sufficient. Additional high-resolution *in vivo* information is needed. Targets determined through *in vitro* tests may not be identical to targets *in vivo*, both qualitatively and quantitatively.

The current emphasis is on *in vitro* and molecular procedures with rapid data acquisition and robust statistics, using tissue fragmentation, cell lines, and computer models. This might make sense as a work-around if more precise and direct information was not available, but it is. Even so, the study of intact tissue under *in vivo* conditions with preserved systemic relationships and with high cellular resolution is not required and, therefore, usually omitted. High-resolution *in vivo* (*ex vivo*) histochemical approaches are avoided because they are considered not only unnecessary but also inexpedient, cumbersome, and costly. This attitude disregards the significance of cellular complexities and functional relationships.

'. . .one of the ultimate goals of pharmacology, [is] the localization and characterization of the highly specific receptor sites with which potent drugs react in order to bring about their characteristic effects.

The matter of localization, with which high resolution autoradiography is primarily concerned, is unquestionably of prime importance." George B. Koelle. . .."

Tissue heterogeneities: *need for high tissue and cellular resolution.*

Organs are complexly composed of heterogeneous tissues with specific topographical arrangements, diverse characteristics, and multiple functions. Therefore, organs should be viewed and studied not only whole, as representative chunks, or fragmented and homogenized as subcellular components but also as cellular systemic organizations. As has been stated in the literature: tissue homogenates mix up heterogeneous elements, which means, concentration at the sites of action cannot be distinguished from concentrations in other elements of the tissue that contribute to the overall tissue concentration but not to the actual drug effect.

Target tissues may consist of cell populations embedded in non-target tissues with important functional–topical relationships. Target tissues frequently are low-capacity high-specificity binding sites, undetectable in large tissue samples used in routine ADME approaches, which record mostly high-capacity low-specificity sites of drug binding and deposition [10–12].

In preclinical ADME studies, radioassays use whole organs or pieces of organs and disregard structural and functional heterogeneities. Whole body autoradiography, with 40 μm thick sections and tissue structures disrupted through ice crystal freezing artifacts, is also unsuited to resolving small cell populations. Drug imaging procedures, albeit important diagnostic tools to be further developed, likewise do not provide sufficient resolution and sensitivity, and in most cases cannot yield necessary details about target sites of drug action. It would be erroneous, therefore, to state and accept that any of these techniques reveal *the* tissue localization of a drug [13]. On the contrary, comparative studies with high-resolution (cellular) receptor autoradiography have made patently clear that none of the current ADME approaches is sufficiently suited for drug target detection [11,12]. Such comparisons reveal an alarming number of false negatives from radioassay and whole body autoradiography. Even when the results of radioassays and whole body autoradiography correspond to and support each other, the combined effect of equally deficient low-sensitivity approaches merely validates the same erroneous result and should not be invoked – as has been done – as proof of their utility for the tissue localization of drugs [13]. Twice wrong is not once right. Instead, tissue localization with these methods mostly yields information on 'sites of loss' [10] rather than on sites of action.

High-resolution methods: *need for in vivo* (*ex vivo*) *drug targeting.*

During the past decades, many efforts have been made to develop and apply microscopic methods for the cellular localization of drugs in intact tissues. Both autoradiography with radiolabeled compounds and immunocytochemistry with antibodies to receptors protein have been attempted [14]. Most of the procedures recommended in the literature did not achieve high-tissue resolution with simultaneous retention of non-covalently bound compounds at their original *in vivo* receptor sites and were instead compromised either through low resolution or through misleading translocation, loss or other artifacts.

At present, high-resolution receptor microscopic autoradiography is the method of choice [11,12]. Development of this method started at the University of Chicago in 1963 at a time when it was considered nearly impossible [7]. Other investigators resorted to easier low-resolution apposition methods, like whole body macroautoradiography or *in vitro* section incubation autoradiography [15]. But the tissue and cellular localization of drugs and other diffusible compounds through receptor microautoradiography has since been developed for routine use. Adequate equipment is available. The technique is neither overly cumbersome nor costly. It has been applied to studies of many compounds that have resulted in important discoveries and new concepts, thus testifying to its high diagnostic value [9,11,16].

The importance of resolution and sensitivity has become evident especially in studies and discoveries related to steroids, such as estrogens, progestins, androgens, adrenal glucocorticoids and mineralcorticoids, vitamin D, and thyroid hormone, retinoic acid, glucose, and 2-deoxyglucose (reviewed [15–17]). Despite these impressive and instrumental results, the potential for advancing drug development, including both time and money savings, potentially even life savings, has not been utilized.

In vitro versus in vivo: *need for high-resolution in vivo validation and correlation.*

Certain information from *in vitro* approaches (high throughput, cell lines, and so on) is important and expedient. However, reliance on *in vitro* data and their validation with alternate *in vitro* approaches may not be valid for all aspects. Unless *in vitro* data are supplemented and validated through suitable *in vivo* checks, extrapolation from one or more *in vitro* tests to *in vivo* conditions can be misleading. Validation through high-resolution *in vivo* experiments should be considered and applied for selected lead compounds in selected locations. Results from such *in vivo* studies can then inform the need for further *in vivo* analysis.

Bioavailability: *need for determination in plasma and in target tissues.*

Bioavailability of a drug in blood plasma is not identical to that in target tissues. The half-life ($t_{1/2}$) in plasma and in target tissues differs. Even among target tissues of the same drug, bioavailability, receptor binding, saturation, and $t_{1/2}$ can vary, as has been shown for vitamin D and its analog OCT [9]. This is likely to be the case for other compounds as well. While these differences can be considerable, they remain undetected with the low-resolution, low-sensitivity limitations of common radioassay and whole body macroautoradiography.

Tissue distribution determined with radioassay and whole body autoradiography reveals predominantly unspecific deposition that typically tracks blood levels, metabolism, or excretion, and reflects what are considered high-capacity low-specificity 'sites of loss' [10], rather than high-specificity low-capacity sites of action.

With routine radioassays, target cells usually cannot be separated from non-target tissues. Similarly, with whole body autoradiography, target cell populations frequently cannot be identified and distinguished from non-target tissues. Without separation, low-capacity high-specificity target sites may be overshadowed by high-capacity low-specificity deposition signals, or simply may remain unrecognized because of their own weak signals (especially with the conventional [^{14}C] label) and the low-tissue resolution.

Here is one example of vitamin D distribution in the brain. 'Quantitative immunoradiometric assay tissue levels of 1alpha,25(OH)$_2$ vitamin D receptor in the rat' scored negative for 'cerebrum' and 'cerebellum' [18]. Similarly, 'organ distribution of 1alpha,25(OH)$_2$ vitamin D$_3$ receptors in sucrose density gradient cytosol' was equally found negative in 'brain', 'heart' and 'thymus' [19], although all contain specific target cell populations [16]. Likewise, the vitamin D analog OCT (22-oxa-1,25-dihydroxyvitamin D$_3$) did not display any accumulation in the brain with radioassays as well as whole body autoradiography. According to these data, a blood–brain barrier for vitamin D and analog was postulated. By contrast, with receptor microscopic autoradiography maps of target neuron circuits in forebrain, midbrain, hindbrain, and spinal cord have been published and a basis for topographical and functional follow-up has been provided. As a consequence, related effects on cognition and neuro-endocrine processes are increasingly recognized and vitamin D-related drugs for the treatment of neurological–psychiatric disorders are being developed.

Comparative studies show that drug barrier determinations for brain and fetal tissues based on data from radioassay and whole body autoradiography alone remain wholly unsatisfactory.

Similarly, computer modeling of drug effects based on plasma bioavailability alone, without relevant target data, is deficient if not fallacious. Drug bioavailability should also be assessed at tissue and target levels. Assessment of tissue deposition of compounds should distinguish between (a) unspecific 'sites of loss' [15] and (b) specific sites of action and should seek to reveal the latter more than the former. While whole body autoradiography can provide a helpful synopsis on compartmental drug deposition and, if applied with sensitive sheet film, furnish a degree of detailed tissue distribution [20], superior to routine cut and burn radioassays, whole body autoradiography – without competitive suppression of receptor occupation and with its notorious low-resolution (Figure 1) – does not (cannot) produce the needed information. Accordingly, data from whole body autoradiography and, similarly, radioassays alone are inadequate for predicting drug effects.

The predominant use of [^{14}C]-labeled compounds is a contributing factor to shortcomings in preclinical ADME data acquisition. The [^{14}C]-label with its long half-life, the related low-specific activity of labeled compound, and the high energy of its beta particles, provides only low sensitivity and low-cellular resolution [11]. Accordingly, high-specificity low-capacity binding sites are likely to remain undetected, as demonstrated in Figure 1. By contrast, receptor microscopic autoradiography uses tritium-labeled compounds with high-specific activity.

Implementation of *in vivo* high-resolution requirements would result in:

- improving recognition of drug effects;
- minimizing false negatives and false positives;
- avoiding false leads;

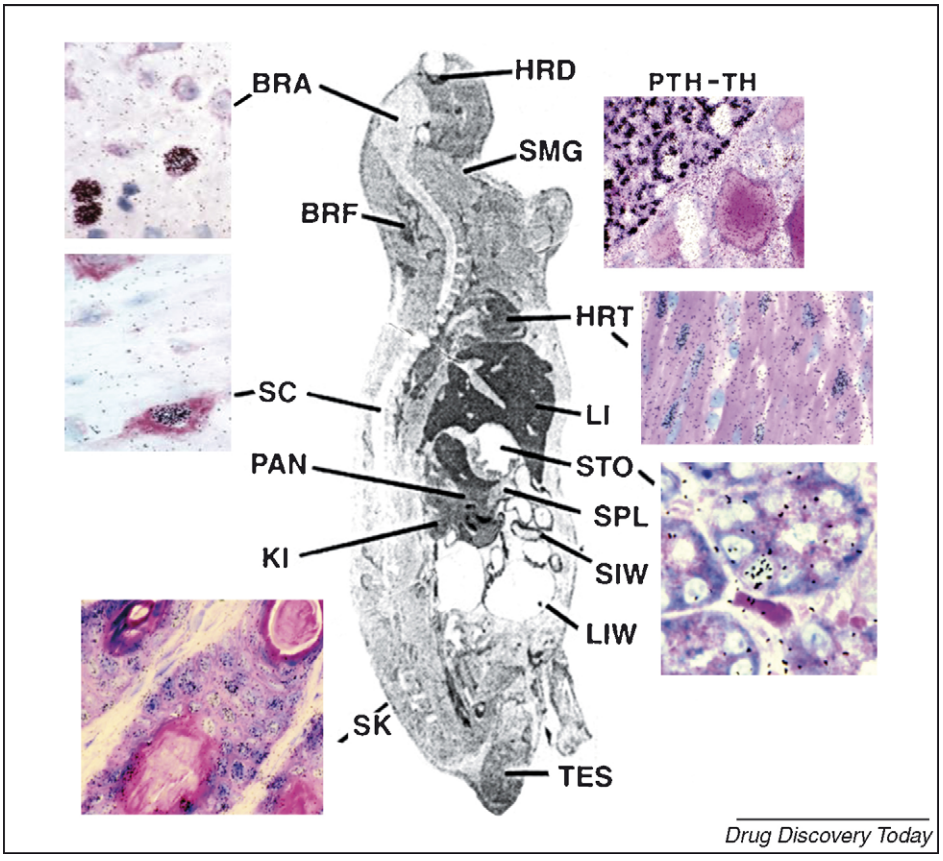

FIGURE 1

Example for comparison of Vitamin D distribution assessed by whole body autoradiography (rat, black and white center) with negative results for brain (BRA), spinal cord (SC), stomach (STO), and skin (SK), and by receptor microscopic autoradiography (side color images) with specific nuclear receptor binding demonstrated in distinct cell populations in brain, spinal cord, stomach endocrine G-cells; and skin keratinocytes, as well as parathyroid chief cells (PTH), heart atrial myocytes (HRT) and many others not shown (for detail see ref. [11]). The evidence provided in common ADME studies, based on data from whole body autoradiography and corresponding radioassays - both of which satisfy regulatory requirements, is prone to false negatives and clearly deficient.

TABLE 1

Vitamin D advanced drug development through high-resolution target discoveries

Drug targets	**Therapeutics**
	Applied or potential
Brain	Alzheimers
Specific regions—circuits	Seasonal affective disorder (SAD) Mental diseases
Spinal cord motor neurons	Muscle weakness Rickets, vitamin D deficiencies, and old age Multiple sclerosis
Skin keratinocytes	Wound healing
Hair sheaths	Psoriasis
Sebaceous glands	Hair growth
Sweat glands	Hypohydrosis
Heart atrial myocytes	Hypertension—Atrial Natriuretic Factor
Pituitary thyrotropes	TSH blood level increase, thyroid function
Salivary glands	Digestive functions
Striated ducts	
Granular convoluted tubules	
Myoepithelial cells	
Stomach	
Mucous neck cells	Gastric mucosa, renewal and repair
G-cells	Gastritis, gastric ulcer
Pyloric muscle	Digestive problems
Intestine	
Crypt cells	Cell proliferation, Tumor
Liver	
Ito cells	Storage of vitamin D and precursor
Pancreas	
Beta cells	Insulin secretion, Diabetes
Adrenal medulla cells	Stress, Fatigue
Female reproductive organs	Female fertility
Ovary germinal epithelium	Cell proliferation, Tumor
Oviduct epithelium	
Vagina epithelium	
Uterine glands epithelium	
Male reproductive organs	Male fertility
Prostate	Cell proliferation, Tumor
Testis Sertoli cells	
Epididymis certain epithelia	
Ductus deferens different cell types	
Thymus reticular cells	Immune system

- advancing recognition of specific sites of action and possible 'side effects';
- providing leads for valid biochemical, functional, and clinical follow up;
- advancing drug development through positive leads and improved diagnosis;
- saving of time and money;
- validating and complementing results from low-resolution imaging methods;
- providing correlative data for *in vitro* and other approaches;
- providing quantitative data on bioavailability in target tissues;
- providing essential data for computer modeling for better prediction.

Microdose therapies, hormesis: *need for high resolution in vivo target information.*

The importance of achieving safety and efficacy through low-dose therapies is being increasingly recognized [2,21,22]. This includes salubrious effects of 'toxic' substances. Determination of enantiodromic conversion, as shown for vitamin D [22], ranging from stimulatory (sanitary) to inhibitory (toxic) effects requires an appreciation of precise thresholds, assisted through methods with high resolution and high sensitivity. Current 'expedient' ADME procedures are lacking the necessary sensitivity [8]. *In vivo* information, such as from receptor microscopic autoradiography, is not just useful but essential.

Systems (holistic) pharmacology: *need for both detailed and comprehensive data.*

Comprehension of drug effects requires consideration of both focal and systemic aspects. Systems evaluation is meaningful only if sufficient relevant detail is provided. Detail for systems pharmacology requires both qualitative and quantitative information about specific distribution and binding to target tissues and cell populations related to dose, time, and experimental conditions of age, endocrine, and disease status [22]. Systems data based on radioassays, *in vitro* approaches, and nuclear imaging alone, or even in combination, are likely to be just insufficient and misleading. Although use of non-invasive imaging techniques would be ideal, the resolution and sensitivity of *in vivo* scanning methods are still limited.

With receptor microscopic autoradiography a target 'drug homunculus' [11,12] can be created for finger(body)printing of drug target distribution and action. Validated data from other methods can be included. Through such organized overviews, results from high-resolution approaches and their quantitative holistic evaluations can open new insights for drug action, as has become apparent for estradiol and especially vitamin D [23].

Table 1 is a partial listing of vitamin D nuclear targets discovered and characterized through the use of receptor microautoradiography (see also reference [16]) (in the case of Sertoli cells and pancreatic insulin effects, histochemical and biochemical data were reported independently in parallel accounts). Also important is the autoradiographic identification of tissues that do not display characteristics of genomic targets, such as skeletal striated muscle and intestinal smooth muscle cells.

Conclusions: *advanced drug development, improved prediction, reduced cost.*

Delays and pitfalls can be minimized during the development and approval of drugs if more

attention is given to the identification of *in vivo* target sites and related action, with consideration of target pharmacokinetics.

Clinical pharmacokineticists with their elegant but still low-resolution magnetic resonance spectroscopy, single photon emission computer tomography, tissue microdialysis, and other similar techniques, would benefit from correlating and complementing their data with high-resolution receptor microscopic autoradiography results.

In vivo drug target elucidation has been neglected and even bypassed. Available methods can be readily applied and new ones may be developed. Macro-autoradiography and micro-autoradiography may be combined to complement each other better to serve the distribution aspects (and related functions) in preclinical ADME studies.

Microscopic target tissue characterization is neither very costly nor complicated, nor is it time consuming, as some have been arguing. Ultimately, it is time and money saving. *In vivo* target pharmacokinetics should be an essential part of pharmaceutical drug development and prediction.

Members of the FDA and ICH, and other regulatory agencies and scientific bodies – and consequently also leading members of pharmaceutical companies – may consider the importance of *in vivo* target identification and characterization for improved drug development and safety predictions. Pharmaceutical companies would benefit, and medical therapies would be advanced. Should pharmaceutical companies, however, fail to recognize their own incentives to adopt more precise *in vivo* procedures, implementation may be facilitated through related regulatory recommendations and/or requirements. The evidence calls for it.

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