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Strategies for using *in vitro* screens in drug metabolism

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In vitro **assays are increasingly being used in drug metabolism studies to screen novel chemicals. Their advantages are twofold: first, they allow testing early in the drug discovery phase, providing important information on chemical characteristics; second, human cells or cell constituents can be utilized, increasing the relevance to man. However, the process of isolation, transformation or storage of these cell systems may alter their phenotype (and, in the case of tumour-derived cell lines, genotype as well). A review of the systems currently employed shows that, whereas all systems have their own caveats, it is possible to find an appropriate system for any particular question that is asked.**

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▼ With the advent of combinatorial chemistry, the number of new chemical entities (NCEs) that can be produced in a short space of time has rapidly escalated, with drug discovery teams being faced with libraries of tens of thousands of compounds to screen [1]. Although this has produced a wealth of possible new therapeutic compounds, it has also raised an important question: how does one whittle these tens of thousands of NCEs down to the few that will be progressed along the discovery/development pipeline [2]?

One approach to solving this problem has been the use of *in vitro* screens to identify early on the characteristics of an NCE, particularly with respect to its drug metabolism. Such information is crucial to the decision-making process of which compounds to progress with and which to discard. As well as screening large libraries of NCEs, such an approach can also be used to screen smaller sets of structurally related compounds, allowing determination of the chemical structure that is the strongest possible lead candidate [3]. Obviously, correct decision-making at this point is critical: failure to identify a potentially useful compound, or the erroneous progression of a chemical with inappropriate properties, wastes huge amounts of time and money, and ultimately retards the release of beneficial therapeutics into the marketplace.

A survey of the literature reveals a wealth of different *in vitro* screens. How then does one decide which screen is the most applicable to a given scenario? The first important step is to define the question(s) to be asked. These might include testing for therapeutic effect, alteration of cellular proliferation via perturbing apoptosis/DNA synthesis, or characterization of the metabolic fate and effects of the chemical. This review will concentrate on the advantages and disadvantages of the various *in vitro* systems with respect to the last question: drug metabolism.

Enzyme inductions, reporter genes or genomic endpoints?

Even within the field of drug metabolism, multiple questions can be asked about an NCE. First, the metabolic fate of an NCE must be described, i.e. which metabolites are formed and in what relative amounts. The function and/or fate of these metabolites might also need to be studied to gain full insight into the potential biological consequences of exposure to the NCE. Second, the potential for the NCE to alter the drug-metabolizing enzyme (DME) profile of the cell must be examined. Alteration in the DME profile (through induction and/or inhibition of specific DMEs) will lead to altered pharmacokinetics of the NCE and of any co-administered compounds. Study of these effects is important for the prediction of potential adverse effects and drug–drug interactions that might occur once the NCE is in general use.

In order to decide which *in vitro* screen(s) to employ, perhaps the first decision is what endpoint is going to be measured to assess these questions. Whereas each of the systems

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described below comes with a long list of pros and cons, perhaps the most significant are the relevance of the endpoint measured and the ease of its extrapolation to the *in vivo* situation. Two levels will be discussed here: transcriptional activation (either genomic or reporter gene-based) and enzyme activity. As will be seen, each of these endpoints has a trade-off between ease of use and ease of extrapolation.

Measurement of transcriptional activation provides a sensitive system that can easily be formatted for high-throughput technologies, allowing the rapid screening of large chemical libraries. However, changes in transcriptional activation do not always relate directly to changes in enzyme activity, owing to both differing translation efficiencies of mRNA transcripts and to the role of post-translational modifications. For example, whereas regulation of levels of the cytochrome P450 isoform CYP3A4 is mainly carried out at the transcriptional level [4], inflammatory nitric oxide synthase responses are regulated at both the transcriptional level [5] and the post-translational level [6], and apoptosis is regulated mainly via post-translational events [7]. Hence, a transcript-based analysis might provide important information on the first two processes, but will miss potential perturbations of the latter.

An alternative to measuring transcript levels is to measure enzyme activity levels, usually through the formation of metabolites by DMEs. Such measurements might represent a more biologically relevant endpoint, with data being generated from the ultimate, biologically active, molecule. However, the specificity of probe substrates used in these assays has been questioned, and often the data will be indicative of the role of an enzyme (sub)family (e.g. CYP3A) but not of which isoform (i.e. CYP3A4 or CYP3A5) [8]. More-specific probe substrates are becoming available for closely related CYP (sub)families [9], but at present the most complete approach appears to be the use of 'cocktails' of probe substrates from which individual enzyme inputs can be derived [10]; a comprehensive list of probe substrates for CYP enzymes can be found in the recent review by Brandon [11]. This inability to distinguish between closely related enzymes might not always be a major issue, as the substrate specificities of the various isoforms for a (sub)family are often relatively close, and an 'overall impression' of the potential for metabolism is sufficient.

Thus, it can be seen that the choice of endpoint could have a major impact on the results from an *in vitro* screen. Whereas enzyme activity-based endpoints provide the most proximate information on potential biological effects, they might lack the sensitivity and/or selectivity of the more biologically removed transcriptional measurement. Such drivers have resulted in the use of these two measurements at distinctly different stages in the drug discovery pipeline. During early NCE screening or for mechanistic studies, the increased sensitivity and applicability to high throughput of transcriptome-based screens means that they are often preferable. However, as a compound progresses further along the development pipeline, proteome-based assays will be increasingly used. This reflects both the concentration on a smaller number of compounds and the need to gain more biologically relevant data. Another driver for this temporal distinction is the fact that regulatory bodies currently require proteome-based measurements within any submission, but not transcriptome-based measurements. Obviously, as NCEs progress further down the drug discovery and development pipeline, only assays that can support a submission package will be employed, thus favouring the proteome-based assays in later stage development.

Enzyme preparations

Measurement at the enzyme level provides information on the proximate biological molecule, and this is of direct use in any submission to a regulatory body. Simple enzyme preparations, specifically S9 fractions, microsomes and supersomes, represent systems targeted at examining the potential metabolic fate of a compound within the body.

Human liver S9 fraction

Human liver S9 fraction is a preparation containing both the microsomal and cytosolic fractions of the cell. This system offers the most complete representation of DMEs, as it incorporates both the majority of phase I (mainly microsomal) and phase II (often cytosolic) enzymes, allowing a relatively complete metabolite profile to be achieved. As with any enzyme-based system, it is necessary to ensure that there are sufficient cofactors, reducing equivalents, and so on. to allow any reaction to proceed without limitation. In the case of phase I enzymes, the major requirement is an NADPH-regenerating system, and this must be added to S9 fractions, microsomes and supersomes. However, in the case of the S9 fraction, it is necessary to consider any cofactors required for full functioning of the phase II enzymes as well. Owing to the wide range of phase II enzymes, this list can be quite lengthy, from single cofactors such as 3′-phosphoadenosine 5′-phosphosulphate or UDP-glucuronic acid, used in sulphation and glucuronidation reactions, respectively, to complete acetyl-CoA-regenerating systems, which are required for acetylation reactions. It is possible to use S9 fractions without addition of all these phase II cofactors and indeed this is usually the case, but if such an approach is adopted then this will impact on the observed metabolic profile of a compound, as cofactor limitation

might well become a factor. Such artificial limiting of enzyme reactions would move the model away from the *in vivo* situation, thus reducing the predictive power of the system. A second limiting factor of S9 fractions is that they generally contain lower enzyme activities (on a per unit basis) than either microsome or supersome preparations, thus reducing the overall sensitivity of the system.

Because of these limitations, S9 fractions are often used in combination with microsomes and supersomes, providing additional information on the phase II metabolic profiles of an NCE. For example, S9 fractions are routinely used in mutagenicity testing, where their addition to the Ames test [12] means that both the NCE and its metabolites can be tested, providing more information on the potential mutagenicity of the compound [13].

Human liver microsomes

Microsome preparations are probably the most widely used *in vitro* system for assessing the metabolic profile of an NCE. Such preparations can be simply prepared using differential centrifugation [14], and are widely, and cheaply, available from commercial sources; they can thus be used to screen large numbers of compounds with relative ease. In addition, microsomes can be prepared or purchased from single individuals, pools of subjects or single-sex pools, allowing the examination of population variation [15,16], the 'average' response [17] and age-specific [18] and sex-specific [19] effects as required. As with the S9 fraction, appropriate cofactors (e.g. an NADPH-regenerating system) must be added to the system to ensure that supply of cofactor is not limiting.

Whereas microsomes are probably the most widely used *in vitro* test system, they are by no means perfect, and data gained from them are subject to several caveats. The major caveat, from which all other caveats stem, is that microsomes do not represent the whole cell profile of metabolic enzymes. Unlike the S9 fraction, microsomes contain only the endoplasmic reticulum-localized enzymes, which, in terms of DMEs, equates to CYPs, flavin monooxygenases (FMOs) and glucuronosyltransferases (UGTs). Hence, metabolites produced via other enzymes will not be detected with this system. Because of this bias, it is not possible to quantify accurately the complete metabolic fate of a chemical.

Supersomes

The final, and in many ways most focused, 'enzyme-only' system is supersomes. This baculovirus-based system allows functional expression of recombinant proteins in insect cells [20]. As the metabolic enzymes endogenously expressed by insect cells are markedly different from those found in humans, microsomes prepared from this expression system (termed supersomes) effectively represent a functional assay for the single human DME that is expressed. One advantage of supersomes over human liver microsomes is that, in addition to the enzyme under study, components for the NADPH-regenerating system are also engineered into the cells, negating the need to add these cofactors separately.

As with human liver microsomes, only membrane-bound DMEs (CYPs, FMOs and UGTs) can be studied with this system, and the majority of these are now available commercially [21]. Of course, one important question is, do enzymes expressed in insect cells possess the same characteristics as their naturally expressed liver counterparts? Comparison of the metabolic activity of supersomes and microsomes using probe substrates suggests that baculovirus-expressed enzymes are indeed a reasonably accurate model of the *in vivo* situation with respect to K_m values, although V_{max} values are not always as reproducible [22]. The expression of a single enzyme for study is a double-edged sword: on one hand, it excludes the examination of the contribution of other enzymes to the metabolism of a compound, but, on the other, it allows the roles of individual enzymes in a metabolic profile to be studied. Hence, supersome-based systems are often used to examine the findings of whole microsome preparations, confirming and characterizing the involvement of single enzymes in the metabolism of an NCE [17,23].

Summary

It might appear from the above that enzyme preparations, in all their guises, represent an ideal *in vitro* test system for NCEs. However, two major considerations applying to all three of these systems must be accounted for when deciding on their applicability. First, the lower sensitivity of the endpoints and the sometimes overlapping abilities of enzymes to metabolize 'specific' probe substrates mean that answers may not be as definitive as desired. Second, the use of 'pure' enzyme systems reduces the ability to measure the impact of pre-translational modifications on reactions. The DME profile of a cell, and therefore its activity profile, will depend greatly on the pre-translational forces exerted on the system, both at the time of sample preparation and during the experiment. Enzyme preparations taken from individuals exposed to compounds that cause increases in enzyme activity via pre-translational mechanisms (e.g. the action of rifampicin on the rate of CYP3A4 gene expression) will be markedly different from those of a naïve individual. It is therefore important to obtain comprehensive information on the exposure of donors to such chemicals before production of enzyme preparations,

particularly to aid the explanation of outlier values. In addition, once made, enzyme preparations are incapable of modelling pre-translational effects caused by the NCE under test. Such transcriptome changes might have a large impact on the DME profile in a cell, meaning that quantification of a metabolite profile based on enzyme-only systems might not accurately reflect the situation *in vivo*.

Despite these general caveats, proteome and metabolome measurements are currently required by all regulatory bodies as part of the submission package for any new compound and hence are routinely used for screening NCEs. Such screening provides important information on the route and overall profile of a compound's metabolism.

Whole cell systems

As most of the caveats associated with the use of 'enzyme-only' systems stem from the lack of a complete DME profile, an obvious extension is the use of whole cells. Utilization of such systems has a host of potential benefits, perhaps the most important being that the study of reactions occurring in an intact liver cell should provide the most reliable data for extrapolation to the human situation. Indeed, intact liver cells are being increasingly used to study the metabolic profiles of compounds [24,25], particularly with the development of high-resolution NMR technologies together with the software required to analyse the complex chemical profiles generated by such studies [26].

Enzyme activity and/or metabolic fate measurements can be made with all the systems so far described, including whole cell systems. However, measurements of transcriptome endpoints are wholly the domain of whole cell systems. Such measurements can be made at one of two levels. First, measurement of gene expression occurring from the genomic copy of a gene can be made. Second, reporter genes can be used to provide an easily measurable endpoint (e.g. fluorescence or luminescence), driven from a plasmid construct containing the promoter and/or enhancer of the gene of interest.

As with the enzyme-only-based systems, there are several different sources of whole cells that can be used in such assays, and several different ways of culturing them. As regards liver cell systems, primary cells represent hepatocytes that are derived from healthy tissue; transformed cell lines are either derived from liver tumours or are artificially transformed, thereby losing many of their growth-inhibitory characteristics.

Primary cell cultures

Primary human hepatocytes might be thought of as the 'gold standard' for an *in vitro* liver cell system, representing the closest approximation to hepatocytes *in vivo*. Indeed, primary human hepatocytes are being increasingly used to study drug-transformation reactions, in experiments ranging from mechanistic studies [27] through to low- and even high-throughput induction screens [28,29].

One major issue with the use of primary human hepatocytes has been the supply stream and quality control of samples. Primary human hepatocytes are, by necessity, produced from many different donors, and this can be both a boon and a cause for concern. The advantage of such variability is that, as for microsomes, the possibility of studying individual, single-sex or pooled hepatocytes exists. The disadvantage of such variability is that experimental robustness will be more sorely tested, as the increased variability will result in a greater standard error for the majority of experiments, potentially reducing their predictive power. Such variability might be technical (i.e. viability of one preparation compared to another), pharmaceutical (i.e. pre-exposure of individuals to DME-inducing compounds before harvest) or biological (i.e. genetic polymorphisms in DMEs). With human liver microsomes, experimental robustness can be examined using cryopreserved microsomes, with several experiments being carried out from a single batch of microsomes. However, successful cryopreservation of primary human hepatocytes has been a constant battle for many years. Cryopreservation has been successfully carried out in several model species [30–33], but application of this technology to human hepatocytes has met with mixed success. Recent advances in cryopreservation have solved many of the initial problems and, although issues of reliability and quality still exist, cryopreserved human hepatocytes now represent a viable option for many studies [34–36]. Indeed, cryopreserved human hepatocytes have been shown to better predict metabolism *in vivo* than primary rat hepatocytes, although still not as accurately as freshly isolated human hepatocytes [37].

As stated earlier, primary human hepatocytes are often said to represent the closest possible model to the *in vivo* situation. This is certainly true to a large extent, with transcriptome and proteome changes being observed in primary hepatocytes following exposure to compounds similar to those seen *in vivo* [27,28,38,39]. However, it is apparent that some differentiated features are lost during hepatocyte culture, with CYP expression in particular diminishing [40]. Such changes in phenotype might represent a limitation of primary hepatocytes, with the DME profile not accurately modelling that seen *in vivo*. Many of these phenotypic changes can be reduced, at least to some degree, through the use of specialized matrices [41], co-culture with epithelial cells [42,43] or continuous superfusion

[44]. The use of co-cultures with epithelial cells in particular represents an important approach, as *in vivo* the liver is composed of both hepatocytes and several nonparenchymal cells such as Kupffer and Ito cells. Increasing evidence suggests that nonparenchymal cells influence the whole liver response to compounds [43,45]; hence inclusion of these in a culture system should increase the accuracy of the model.

An interesting recent development is the use of transdifferentiated pancreatic cells as an alternative to primary hepatocytes [46]. Pancreatic cells can be stimulated to undergo transdifferentiation, producing cells that present several 'liver-specific' phenotypic markers (albumin, glutamine synthetase, and so on.) [46]. As such, they might represent an alternative source of hepatocyte-like cells, although the exact utility of such a system is still under review.

Whereas the use of specialized culture conditions has largely answered this important caveat, it is still vital that any model system be fully characterized and validated before it is used in any screening and mechanistic studies, so that any differences from the *in vivo* situation are fully understood. Potential validation approaches will be discussed later in the article.

As their supply and quality become more constant, primary human hepatocytes are increasingly being used for metabolic studies in preference to enzyme preparations [34–37]. Obviously, the use of a whole cell system should provide the most *in vivo*-like response, increasing the accuracy of extrapolation of results to *in vivo*. In addition, as primary hepatocytes represent a complete system, the need to add cofactors, as one must with enzyme preparations, is negated, removing another possible source of error. In addition to their use in metabolic studies, whole cell systems have the benefit that transcriptome changes can also be measured. Primary hepatocytes lend themselves to the measurement of both activation of genomic transcription [28,39,47] and reporter gene assays [34] for the measurement of compound-mediated effects on gene expression. In general, however, the latter is often measured in transformed cell lines, due to their ease of use and ability to withstand the disruptive processes involved in transfection.

In summary, primary human hepatocytes, when cultured under the correct conditions, represent a good model for studying handling of compounds by the liver. The use of a whole cell model allows data to be gained on both transcriptome- and proteome-based effects, and this should allow the accurate extrapolation of data to the human system. However, the high cost of cryopreserved hepatocytes, batch-to-batch variability and phenotypic changes from the *in vivo* situation are potential limitations to their utility.

Transformed cell lines

A potential solution to the supply and variability issues associated with primary human hepatocyte preparations is the use of transformed cell lines, derived from either tumour tissue (e.g. Huh7 [48]) or artificially transformed primary cells (e.g. NKNT-3 [49]). A search of the American Tissue Culture Collection's online catalogue (http://www.atcc.org/) for 'human hepato*' produced 14 possible cell lines, with several more existing in private depositories. As previously discussed, it is well documented that isolation of liver cells alters phenotype, and in particular DME profile. Add to this the potential complication caused by using either naturally or artificially transformed cells, and it becomes clear that one must be very careful in deciding which cell line is the most appropriate and accurate model to use. Literature citations could provide an initial indicator of useful models, suggesting which lines have found favour with the general scientific population. A search for each cell line in PubMed reveals that HepG2 cells are cited in over 5000 references, a large number of which are concerned with drug metabolism [50–53]. By comparison, the second most cited cell line, Hep3B, has generated only 555 citations to date. PLC/PRF/5, HuH7 and SK-Hep-1 have also received a significant number of citations (434, 258 and 134, respectively), whereas the remaining identified cell lines show poor usage, with fewer than ten citations each. Based on this weight of evidence, it would appear that HepG2 cells are a good starting point for the development of an *in vitro* assay system. It should be noted that such a search is obviously biased towards more well-established cell lines (HepG2 cells were isolated in 1979), and it is important to evaluate 'new and improved' cell lines when they appear in the marketplace.

Hepatoma cell lines are, by definition, 'stably transformed', and therefore it should be expected that experiments carried out with them would show little or no variation. Although this is true to a large extent, it is becoming increasingly apparent that cell lines do alter phenotype over time, often resulting in markedly different functionality [54,55]. In addition, the same cell line grown under different medium conditions can exhibit different characteristics [43,56]. It is hence important that, if cell lines are to be employed for screening compounds, they are fully validated in-house for the assay to be used, with defined growth medium and limitations on the 'age' of cells used.

Whereas primary hepatocytes are equally applicable to transcriptome- and proteome-based measurements,

Table 1. Comparison of *in vitro* test systems used to examine drug metabolism characteristics of new chemical entities

	Proteome/metabolome		Transcriptome: genomic		Transcriptome: reporter gene	
	Pros	**Cons**	**Pros**	**Cons**	**Pros**	**Cons**
S9 fraction	Easy to use Cheap Phases I and II present Whole metabolic profile observed ✓✓	Addition of co-factors (complex mixture) Lower enzyme activity than microsomes/ supersomes Induction not modelled	Not applicable		Not applicable	
Microsomes	Easy to use Cheap 'Population' pools ✓✓✓	Addition of co-factors (simple mixture) CYPs, FMOs and UGTs only Partial metabolic profile Induction not modelled	Not applicable		Not applicable	
Supersomes	Easy to use Moderately cheap No addition of co-factors Single enzyme only ✓✓	CYPs, FMOs and UGTs only Single enzyme only Accuracy of kinetics? Induction not modelled	Not applicable		Not applicable	
Transformed cell lines	Ease of culture Cheap Phases I and II present Whole metabolic profile observed Induction modelled ✓	Alteration of phenotype More complicated than enzyme-only systems	Ease of culture Cheap ✓	Alteration of phenotype/genotype Poor or no expression of key receptors Poor or no response often seen	Ease of culture Cheap Robustness to transfection ✓✓✓	Alteration of phenotype Poor or no expression of key receptors
Primary hepatocytes	Phases I and II present Whole metabolic profile Best extrapolation to *in vivo* 'Population' pools Induction modelled ✓✓✓	Expensive More complicated than enzyme-only systems Batch variability Quality control	Best extrapolation to *in vivo* 'Population' pools Good response often seen ✓✓✓	Expensive More complicated than transformed cells Batch variability Quality control	Best extrapolation to *in vivo* 'Population' pools ✓	Expensive More complicated than transformed cells Batch variability Quality control

Description of ticks: one tick signifies that there are major caveats to applicability and better systems exist; two ticks signify that the best system is applicable but several caveats exist; three ticks signify that the best system is highly suitable for the desired application.
Abbreviations: CYP, cytochrome P450; FMO, flavin monooxygenase; UGT, glucuronosyltransferase.

transformed cell lines do have important caveats in their use for transcriptome-based measurements. It is well established that cell lines exhibit significant alterations in higher chromatin order [57,58], a consequence of their transformation, and this may potentially impact upon genomic measurements. Only limited reports exist of the measurement of genomic activations in transformed cell lines [39,59], and the changes observed are often modest when compared to primary hepatocytes. By comparison, transformed cell lines are used extensively for reporter gene assays [28,53,60]. Alterations in chromosome structure will not alter responses in a nonchromosomal reporter gene, although lowered expression of necessary nuclear receptors has become an acknowledged problem [61].

In summary, if experimental design is robust and care is taken to fully validate systems before use, hepatoma cell lines are applicable for NCE screening, particularly as their low cost and ease of culture lend them well to high-throughput systems [53,62,63].

Validation of in vitro *systems*

As all the *in vitro* systems described have known limitations in their accuracy for modelling the *in vivo* situation, it is important that any assay using them is fully validated in-house before its full use. Reliance on the validation of others is not optimal, as laboratory-to-laboratory variations in the way techniques are carried out and variations within a cell line might make such comparisons invalid.

The choice of validation strategy is key, with many possible variations. Perhaps the simplest system, and one applicable to all the systems thus far described, is to use a 'training set' of ten or more compounds whose characteristics *in vivo* are well known. For example, in the case of a reporter gene-based induction assay, chemicals known either to induce or to have no effect on the expression of the target gene *in vivo* should be examined. Ideally, all chemicals in the training set should be correctly 'called' inducers/noninducers; however, very often this is not the case and here the training set has an additional benefit. Through study of incorrectly called compounds it is possible to gain an idea of the false positive and/or negative rate for a system as well as information on the type of chemicals that are incorrectly called. For example, lack of a drug transporter in HepG2 cells might limit access of pravastatin to the cell interior, providing a false negative in reporter gene studies [53]; other compounds transported by the same drug transporter may also be incorrectly called in such a system.

With the advent of high-throughput transcriptome and proteome analysis it is now also becoming possible to compare the genomes, transcriptomes and even proteomes of cell lines to their *in vivo* counterparts. Multivariate analysis of such profiles will provide important information on the similarities and/or differences between model systems and their relationship to the *in vivo* situation, in terms of the levels of mRNA transcripts/proteins present, how these change with time and/or culture conditions, and also the prevalence of genetic polymorphisms. These data will allow utility of any cell line in modelling an *in vivo* situation to be predicted.

Summary

The use of *in vitro* test systems is becoming increasingly important, particularly in view of the desire to reduce time spent in the drug discovery and development phases. As can be seen from this review, there is a large number of available systems that are capable of measuring multiple endpoints. It is thus important that the researcher first decide what questions must be answered by an assay before deciding on the most appropriate system. Transcriptome-based systems lend themselves to high-throughput analysis of large sets of NCEs, but their lack of impact with regulatory bodies reduces their utility in compound submission. By contrast, protein-based measurements might have lower sensitivity and/or selectivity, but they are required by regulatory bodies and hence will be used in later stage analysis of a smaller number of candidate compounds. These different markers can also be assessed in several different systems. Whole cell systems might represent a more biologically accurate model, but their additional complexity might not always be required, and in such cases simpler, easier to control systems such as enzyme preparations are probably more appropriate to use. An overview of the pros and cons for the systems described, plus their applicability to different assays, is given in Table 1.

The choice of an individual system will therefore be a compromise between two factors: endpoint and model complexity: Early in the drug discovery process, primary hepatocytes or reporter gene systems will be of high utility, but as compounds progress down the discovery and development pipeline these systems might be replaced by microsome/supersome-based systems, which are ideal for protein-based measurements.

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Erratum

In the 15th February 2004 issue of *Drug Discovery Today* (Vol. 9, No. 4; 165–172), in the article entitled *Biological basis for the benefit of nutraceutical supplementation in arthritis* by Clare L. Curtis, John L. Harwood, Colin M. Dent and Bruce Caterson, Figure 3 on p. 168, should have been as follows (the arrows in the bottom box face down, not up as in the previously published figure):

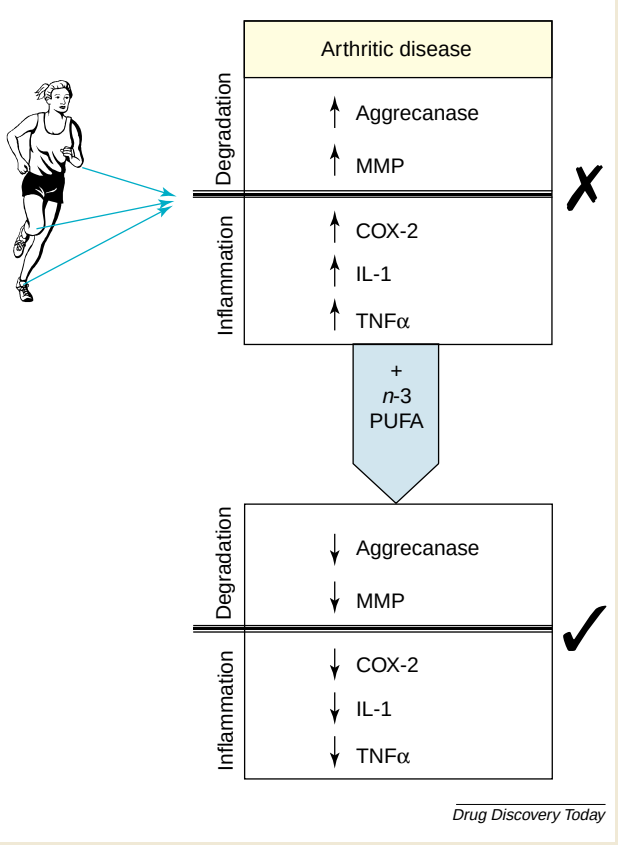

Figure 3. Cartoon summary depicting the effects of *n*-3 (omega-3) polyunsaturated fatty acid (PUFA) supplementation to cartilage explant cultures *in vitro*. In arthritic disease (top half of diagram), there is up-regulation of the enzymes that degrade cartilage (aggrecanases ADAMTS-4 and -5, and MMP-3 and -13) and also the expression of mediators of inflammation (COX-2, IL-1 and TNF-α). After exposure to *n*-3 PUFAs (lower half of diagram), both the expression and activity of degradative enzymes and factors that propagate inflammation are reduced, resulting in a slowing of the progression of arthritic disease. MMP, matrix metalloproteinase; COX-2, cyclooxygenase-2; IL-1, interleukin 1; TNF-α, tumour necrosis factor-α.

The editorial team would like to apologize for any confusion that this might have caused.

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