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Original article

A semi-automated method for measuring the potential for protein covalent binding in drug discovery

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Abstract

Introduction: Covalent protein binding of metabolically reactive intermediates of drugs has been implicated in drug toxicity including the occurrence of idiosyncratic drug toxicity. Investigators therefore would prefer to avoid developing compounds that produce significant amounts of reactive metabolites. By incubating the radiolabeled drug of interest with liver microsomes it is possible to evaluate the propensity of a drug candidate to covalently bind to proteins. **Methods:** Here we present a semi-automated method in which a Brandel cell harvester is used to collect and wash proteins that have been incubated with radiolabeled drug. This method utilizes glass fiber filter paper to capture precipitated protein, rather than the more traditional exhaustive extraction/centrifugation approach. Using model compounds (including [^{14}C]diclofenac, [^{3}H]imipramine, [^{14}C]naphthalene, and [^{14}C]L-746530) we compare the covalent binding results obtained using this method to results generated using the traditional method and we performed cross-laboratory testing of assay reproducibility. **Results:** It was found that results from new method correlated highly with the traditional method (R^2=0.89). The cross-laboratory testing of the method showed an average interlaboratory coefficient of variation of only 18.4%. **Discussion**: This method provides comparable results to the more traditional centrifugation-based method with considerable time and labor savings.

Keywords: Covalent protein binding; Human; Idiosyncratic drug reactions; In vitro methods; Rat; Radiolabeled compounds; Reactive metabolites

1. Introduction

With the currently available models it is not possible to accurately predict the likelihood that a drug candidate will precipitate idiosyncratic toxicity. However, the occurrence of such toxicity, during or after clinical trials, is very costly, both in terms of human suffering and financial resources. Investigators therefore attempt to design drugs that have a reduced risk of idiosyncratic toxicity even in the absence of good predictive tools. Probably the most reliable way to design such drugs is to ensure that the potency is high so that the dose will be low. Generally, high dose drugs are associated with higher incidences of idiosyncratic reactions. It follows that very low dose drugs (<10 mg/day) appear to be void of high incidences of idiosyncratic drug reactions (Uetrecht, 2000). Often though, the potency required for such a low dose may be very difficult to achieve and/or high in vitro or preclinical potency may not translate into a low dose in human. Another possible way to minimize the risk of idiosyncratic drug toxicity is to design drugs that have a low propensity to produce metabolically reactive metabolites. It is thought that if such metabolites

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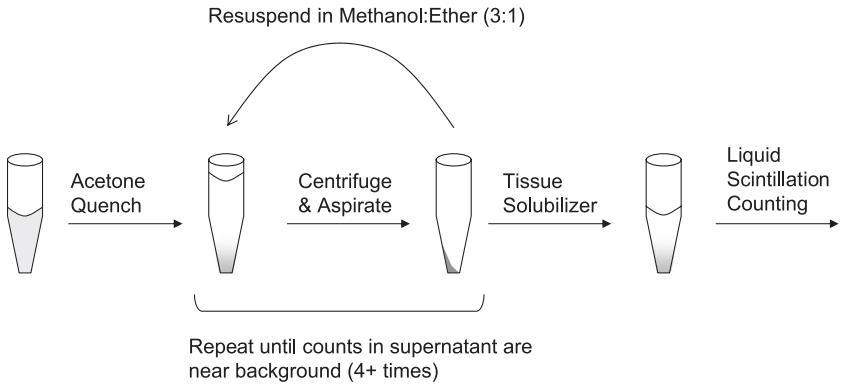

Fig. 1. Schematic of the traditional centrifugation-based method for measuring covalent protein binding.

covalently bind to biological molecules, an undesired immune response could result. There is growing evidence that many idiosyncratic drug reactions follow this mechanism (for a review see Park, Pirmohamed, & Kitteringham, 1998).

One way to assay the propensity of a drug candidate to produce reactive metabolites is to determine the amount of drug that covalently binds to proteins during metabolism. As the liver is the primary organ of drug metabolism, such studies usually focus on this organ. Using radiolabeled drug, incubations can be done in vitro with liver microsomes or with freshly isolated hepatocytes, or studies can be done in vivo by examining the liver of animals up to 24 h after dosing. An integral part of these approaches involves precipitating the liver protein and washing it thoroughly with an organic solvent mixture to remove all non-covalently bound material. Traditionally, this washing process tended to be very labor intensive and low throughput (Fig. 1) (Pohl & Branchflower, 1981). In this paper we present a filter-based and semi-automated method for washing that greatly reduces the labor and increases the throughput of the assay. This assay was largely developed to be used at multiple sites within Merck Research Laboratories. To this end we also present results of a cross-site standardization effort in which the covalent binding of [^{14}C]diclofenac, [^{3}H]imipramine, [^{14}C]naphthalene, [^{14}C]L-746530, and [^{3}H]MRL-A was tested in rat and human liver microsomes (see Fig. 2 for structures). A summary of the standardization results, along with a broader industry perspective on drug bioactivation, has recently been published in a review (Evans, Watt, Nicoll-Griffith, & Baillie, 2004).

2. Methods

2.1. Chemicals

Pico-Fluor™ 40 was purchased from Perkin-Elmer Life Sciences (Boston, MA). The radiolabeled compounds [^{3}H]imipramine and [^{14}C]naphthalene were also purchased

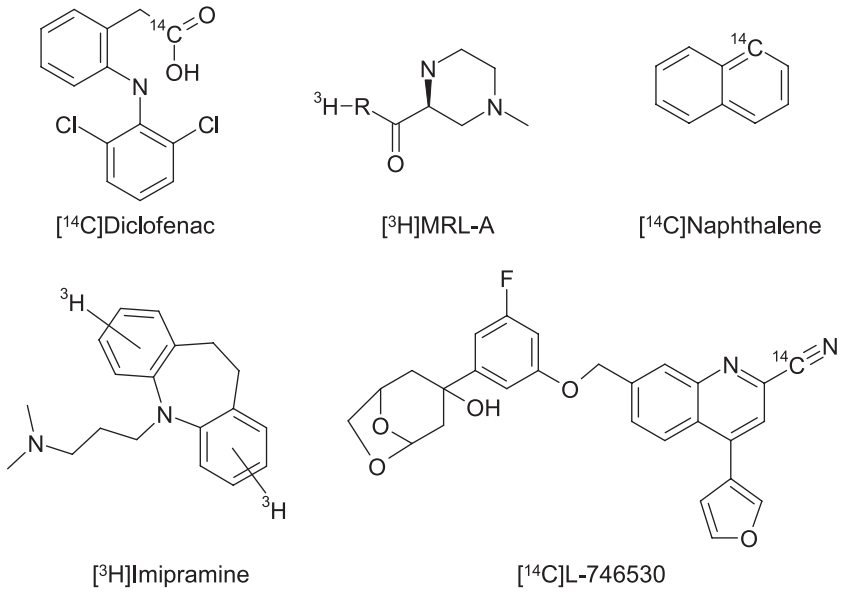

Fig. 2. Structures of radiolabeled compounds used.