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I. Primary cultured hepatocytes as a key *in vitro* model to improve preclinical drug development

SUMMARY

Over past decades, numerous *in vitro* model has been developed to investigate drug metabolism. In the order of complexity we found the isolated perfused liver, hepatocytes in co-culture with epithelial cells, hepatocytes in suspension and in primary culture and subcellular hepatic microsomal fractions. Because they can be easily prepared from both animals (pharmacological and toxicological species) and humans (whole livers as well as biopsies obtained during surgery) hepatocytes in primary culture provide the most powerful model to better elucidate drug behavior at an early stage of preclinical development such as :

1. the characterization of main biotransformation reactions.
2. the identification of phase I and phase II isozymes involved in such reactions
3. the evaluation of inter-species differences allowing the selection of a second toxicological animal species more closely related to man on the basis of metabolic profiles
4. the detection of the inducing and/or inhibitory effects of a drug on metabolic enzymes, the prediction of drug interactions
5. the estimation of inter-individual variability in biotransformation reactions.

The use of hepatocytes, and in particular those obstained from humans, at an early stage of drug development allows the obtention of more predictive preclinical data and a better knowledge of drug behavior in humans before the first administration of the drug in healthy volunteers.

INTRODUCTION

Sinec the liver is the main organ involved in the biotransformation of xenobiotics, determination of hepatic metabolic pathways of a new drug is of major inportance. For this purpose, various *in vitro* experimental models have been proposed and used, including liver homogenates and subcellular fractions of liver tissue, and freshly isolated and cultured hepatocytes of different species. Hepatocytes retain most of the metabolic capabilities of the intact liver, mainly because the functional relationship between the various metabolizing enzymes are preserved, in contrast with subcellular fractions. Therefore, hepatic tissue cultures provide an opportunity to study the pathways and extent of metabolism of new drugs.

During the development of a drug, information on species' difference in routes and rates of metabolism is essential for the interpretation of data obtained from preclinical safety and efficiency studies and for the extrapolation to man. At the early stage of development, a new compound can not ethically be administred to human subjects. *In vitro* methods using tissues or cells obtained from laboratory animals and man are the only possible approach for generationg comparative metabolism data.

SC-42867 and SC-51089 are two PGE_2 antagonists developed as potential non-narcotic analgesics for mild pain treatment. They both possess an 8-chlorodibenzoxapine moiety but differ from each other by the nature of the side chain connected to the nitrogen atom.

The present study was designed to compare the *in vivo* data in the rat with the *in vitro* metabolism of both compounds in rat hepatocyte cultures and also to investigate the *in vitro* metabolism of these drugs in human hepatocyte cultures in order to evaluate if findings observed in the rat can be extrapolated to man.

Table. Expermental use of isolated liver cells

	Freshly isolated cells in suspension	Primary cultures	Long-term cultures
Time available for experimentation	A few hours	One to three weeks	A few months
Resemblance to in vivo characteristics	Close to liver cells in vivo. membrane damage are possible	Close to liver cells in vivo, retain most of the liver specific function	loss of liver specific function with time, differentiation
Advantaged and limitation	easy to use can't use studies for longer time	good physiological condition loss of drug metabolizing enzymes	used over long period transformed or dediffentiated cells
Typical application	Metabolism studies uptake studies, protein synthesis	As freshly cells hormonal effect enzyme induction	results may be less reliable; transformation study

Fig 3 Proposed in vitro metabolic pathways of SC-51089 in cultured hepatocytes of rats and humans

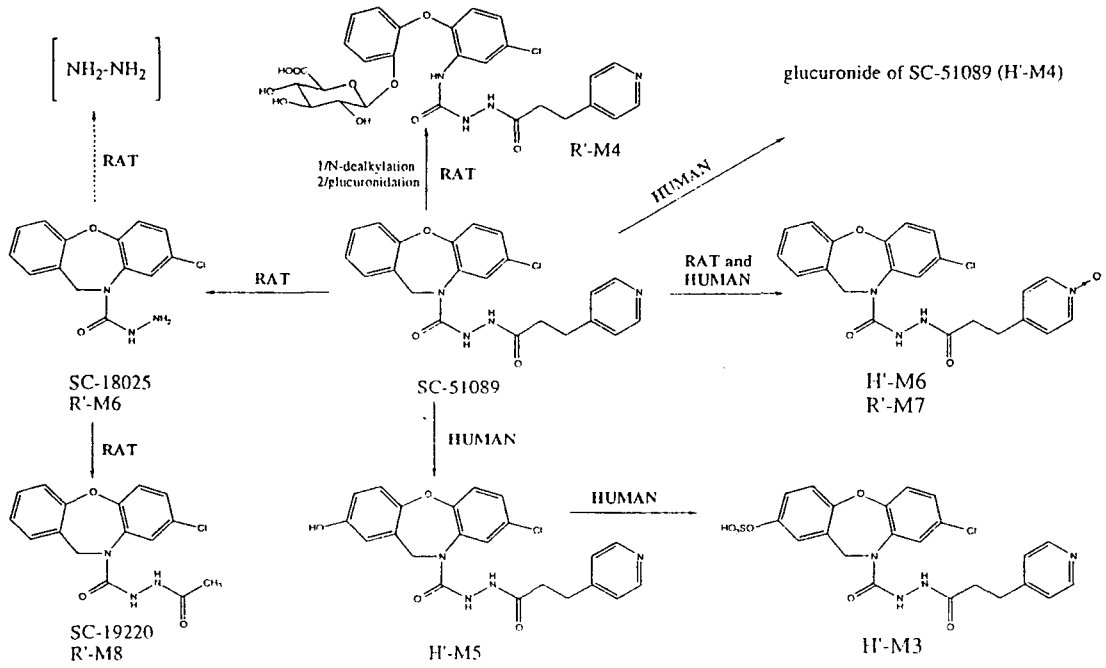
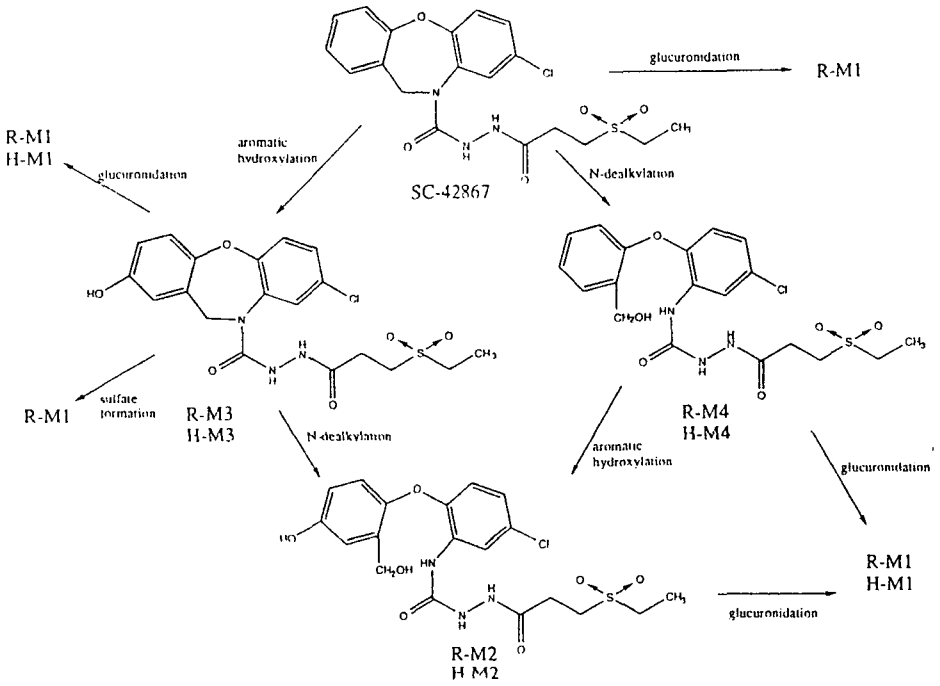


Fig 2 Proposed in vitro metabolic pathways of SC-42867 in cultured hepatocytes of rats and humans



II. Hypercin : Tyrosine Kinase Blockers as Antiproliferative Agents

SUMMARY

Intracellular signalling pathways mediating the effects of growth factors and oncogenes on cell growth and transformation present a challenging new class of target sites for anticancer drug development. Several drugs are already available that may act in this way, including drugs that act on protein serine/threonine kinase, protein tyrosine kinase and phopholipase C, as well as inhibitors of myo-inositol signalling. As our understanding of the signalling pathways involved in growth control increases, new sites for pharmacological intervention will become apparent

INTRODUCTION

Traditionally, anticancer drugs have been targeted to inhibit DNA synthesis and function. Although drug treatment has given limited success against some rapidly growing cancers, the majority of human cancers remain refractory and there is an urgent need for more effective anticancer drugs. A new apporach is to use the signalling pathways that mediate the effects of growth factors and oncogenes on cell proliferation as the molecular targets for anticancer drug development.

Changes in protein phophorylation constitutes a major mcchanism by which extracelluar signals are transduced in cellular responses leading to cell proliferation or differentiation.

Kinases are generally classified as Ser/Thr-soecific or Tyr-specific according to their ability to specifically phophorylated serine/threonine or tyrosine residues in target proteins. The protein-tyrosine kinase(PTK) activity was discovered a little more than a decade ago as the transforming activity of Rous sarcoma viral protein *pp60src* and has since been associated with other oncoproteins and with many growth factor receptors.

Aberrant phophorylation by mutations or overexpression of cellular kinases has been implicated in the molecular mechanisms underlying the progressive transformation of a normal cell to an cancer cell. The therapeutic use of specific PTK inhibitors could therefore represent a major breakthrough in the chemotherapy of neoplastic diseases. Furthermore, if specific, these compounds would also be convenient tools for studying the biochemical function of a particular kinase.

Since enhanced PTK activity often accompanies cell transformation, e.g. in the case of truncated, overexpressed or overstimulated EGF-receptors or related membrane receptors, there has been an intense search for PTK-inhibitors over the last decade. Several active compounds have been isolated from bio-source (e.g. erbstatin, herbimycin A, staurosporine, flavonoids), and many more have been chemically synthetized (e.g. hydroxycinnamamides, tyrphostins, thiazolidine-diones and multisubstrated analogues). Consequently, it was estabilished that compounds like herbimycin A, tyrphostins, thiazolidine-diones and sulfonylbenzoylnitrostyrenes all inhibit the growth of cultured cells by their specific interference with PTK activities.

Conclusively genaralization on the basis structure of a PTK-inhibitor are difficult to make since active compounds seemingly belong to several different chemical classes. However, the majority of synthetical routes have been based on the idea of mimicking tyrosine in an attempt to find nonphosphorylatable analogues that compete with the PTK substrates. Consequently, many inhibitors are substituted hydroxylated aomatic molecules.

The effect of hypercin on the PTK activity of EGF-receptor at submicromolar concentration was studied. The inhibition is irreversible but varies with the incubation time, the temperature, the membrane concentration and the incidence of light.

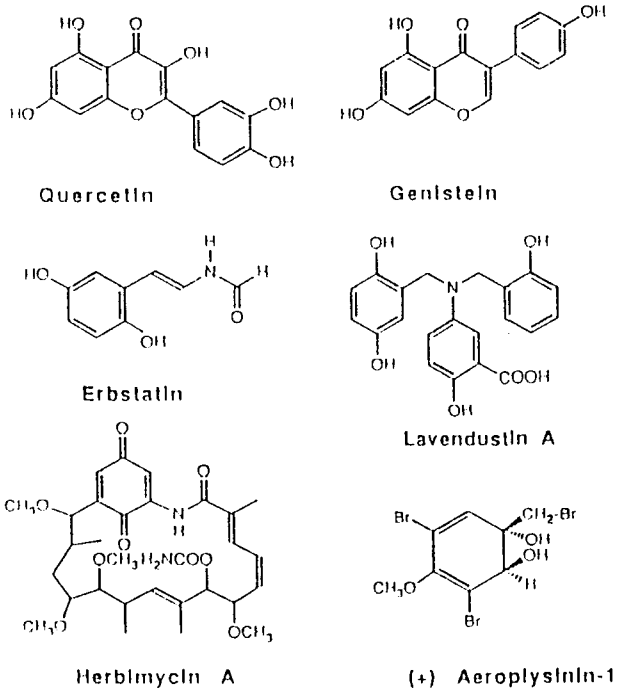


Figure 1. Some naturally occurring PTK inhibitors. The compounds depicted inhibit a number of PTKs, including growth factor receptors and cellular PTKs. Although no pattern of specificity has been found, no systematic attempt was made to determine the selectivity, if any, of these compounds.