Initial characterization of the metabolic stability of the selective and potent glutathione transferase P1-1 inhibitor 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) in liver microsomes and cytosol: an interspecies comparison

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The 7-nitro-2,1,3-benzoxadiazole derivative 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) is a promising investigational anticancer agent with strong and selective inhibitory activity towards human glutathione transferase P1-1 (glutathione *S*-transferase P1-1; GSTP1-1) (Ricci *et al*., 2005). This protein is a GSH-conjugating enzyme overexpressed in a variety of human tumors and capable of interacting with and inhibiting c-Jun N-terminal kinase (JNK), a kinase involved in the apoptotic response to various stimuli. NBDHEX disrupts the complex between GSTP1-1 and JNK, leading to tumor cell apoptosis, and displays significant antitumor activity in *in vivo* melanoma models at non toxic oral doses (Turella *et al*., 2005; Pellizzari Tregno *et al*., 2009).

This study was undertaken in order to get preliminary information about NBDHEX metabolic fate both in humans and in laboratory animal species, to identify the most appropriate animal model(s) for future preclinical pharmacokinetic and toxicological studies.

The metabolic stability of NBDHEX was assessed *in vitro* by reverse-phase (RP)-HPLC with visible detection or RP-LC-DAD/MS analysis upon incubation of the drug with rat, mouse or human liver microsomes in the presence or in the absence of UDP-glucuronic acid (UDPGA) or reduced nicotinamide adenine dinucleotide phosphate (NADPH). Further experiments, based on the use of rat, mouse and human cytosolic liver fractions, have been carried out to get preliminary information on the possible metabolism of the drug by sulfotransferases, which are 3'-phosphoadenosine-5'-phosphosulfate (PAPS)-dependent enzymes.

RP-HPLC traces of supernatants from incubation mixtures containing NBDHEX, and rat, mouse or human liver microsomes plus UDPGA were characterized by a single new visible (433 nm)-absorbing peak which was absent when UDPGA was omitted from the mixtures. Further LC-DAD/MS analysis in negative ion mode revealed that this metabolite peak had a UV-visible spectrum quite similar to that of NBDHEX, and a mass-to-charge ratio (m/z) of 472.10, indicating incorporation of a glucuronic acid moiety into NBDHEX. The rate of NBDHEX glucuronidation was in the rank order: rat >> mouse > human.

A disappearance of NBDHEX from the medium was also observed upon its incubation with rat, mouse or human liver microsomes in the presence of NADPH. Similar RP-HPLC chromatograms (detection at 433 nm) characterized by four new peaks, in addition to that of NBDHEX, were obtained for the three species, and no significant interspecies differences were observed in the rate of metabolism. Finally, NBDHEX was unstable in rat, mouse and human liver cytosol, both in the presence or in the absence of a PAPS-generating system.

Ongoing work is dedicated to (a) obtaining sufficient amounts of NBDHEX glucuronide for use as authentic standard in enzyme kinetic experiments, (b) evaluating of the possible role of cytochrome P450s in NADPH-dependent liver microsomal metabolism, and (c) identifying the factor(s) responsible for NBDHEX degradation in liver cytosol.

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