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ORIGINAL PAPER

Reductive metabolism of nabumetone by human liver microsomal and cytosolic fractions: exploratory prediction using inhibitors and substrates as marker probes

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Abstract The metabolic reduction of nabumetone was examined by inhibition and correlation studies using human liver microsomes and cytosol. This reduction was observed in both fractions, with the V_{max} values for reduction activity being approximately fourfold higher, and the V_{max}/K_m values approximately three-fold higher, in the microsomes than in the cytosol. The reduction of nabumetone was inhibited by 18β-glycyrrhetinic acid, an 11β-hydroxysteroid dehydrogenase (11β-HSD) inhibitor, in the microsomal fraction. The reduction activity was also inhibited by quercetin and menadione [carbonyl reductase (CBR) inhibitors], and by phenolphthalein and medroxyprogesterone acetate [potent inhibitors of aldo–keto reductase (AKR) 1C1, 1C2 and 1C4] in the cytosol. A good correlation ($r^2 = 0.93$) was observed between the reduction of nabumetone and of cortisone, as a marker of 11β-HSD activity, in the microsomal fractions. There was also an excellent relationship between reduction of nabumetone and of the AKR1C substrates, acetohexamide, and ethacrynic acid ($r^2 = 0.92$ and 0.93, respectively), in the cytosol fractions. However, a poor correlation was observed between the formation of 4-(6-methoxy-2-naphthyl)-butan-2-ol (MNBO) from nabumetone and CBR activity (with 4-benzoyl pyridine reduction as a CBR substrate) in the cytosol fractions ($r^2 = 0.24$). These findings indicate that nabumetone may be metabolized by 11β-HSD in human liver microsomes, and primarily by AKR1C4 in human liver cytosol, although multiple enzymes in the AKR1C subfamily may be involved. It cannot be completely denied that CBR is involved to some extent in the formation of MNBO from nabumetone in the cytosol fraction.

Keywords Nabumetone · Carbonyl reductase · Aldo–keto reductase · 11β-Hydroxysteroid dehydrogenase · In vitro metabolism

1 Introduction

Nabumetone is a non-steroidal anti-inflammatory drug which has proven to be effective in the treatment of rheumatoid arthritis and osteoarthritis (Friedel et al. 1993; Davies 1997; Friedel and Todd 1988). Nabumetone is a pro-drug that undergoes extensive first-pass metabolism to 6-methoxy-2-naphthylacetic acid (6-MNA), the major circulating metabolite, which is a potent inhibitor of prostaglandin synthesis (Mangan et al. 1987). Two main metabolic pathways of nabumetone appear to be operating: reduction of the ketone to an alcohol and oxidative cleavage of the side chain to yield acetic acid derivatives. All animals studied, including humans, use both pathways (Haddock et al. 1984). Tsuchiya et al. (1988) investigated the route of conversion and confirmed the existence of two metabolic pathways from nabumetone to 6-MNA; one is via 4-(6-methoxy-2-naphthyl)-butan-2-ol (MNBO), a reductive metabolite of nabumetone, to 6-MNA (60 %) and

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the other results in 6-MNA (40 %) in rats. In our previous study, cytochrome P450 (CYP) 2C9 was identified as the major isoform involved in the oxidation of 6-MNA, which is a pharmacologically active metabolite of nabumetone, by human liver microsomes (Matsumoto et al. 2011a). Recent studies have suggested that metabolism of nabumetone to 6-MNA is primarily mediated by CYP1A2 (Turpeinen et al. 2009). Carbonyl reduction is also the most common phase I metabolic reaction in the majority of xenobiotic aldehydes and ketones. Carbonyl reduction of aldehyde and ketone moieties may be catalyzed by alcohol dehydrogenases, aldo–keto reductases (AKRs), short-chain dehydrogenases/reductases (SDRs) including carbonyl reductase (CBR) and 11β-hydroxysteroid dehydrogenase (11β-HSD), and quinone reductases (Matsunaga et al. 2006). In contrast to CYPs, however, the absence of commercially available recombinant systems and the lack of specific inhibitors mean that further discrimination of individual isoforms has not yet been achieved. Therefore, individual isoforms involved in the metabolic reductions of most carbonyl compounds in human liver have not yet been well characterized.

Incubation of nabumetone with human liver subcellular fractions led to the observation of both the preferential contribution of microsomal and cytosolic enzymes, and of the pronounced inhibition of ketone reduction by potent inhibitors including quercetin, menadione, ethacrynic acid, and 18β-glycyrrhetinic acid (Matsumoto et al. 2011b). Comprehensive screening based on inhibition and correlation studies for evaluating involvement of individual isoforms offers a quick and easy-to-use approach. Recently, Skarydova et al. (2013) investigated the metabolic reduction of nabumetone, mediated mainly by AKRs and CBR, through incubation with eight recombinant carbonyl reducing enzymes which they had prepared. However, a new chemical entity usually has little information on the predominant metabolic process during the drug discovery stage. A combination of inhibitory activities, and the correlation with commercially available substrates based on the formation rates of metabolites, appears to be a useful approach for estimating the contribution of individual enzymes. In addition, this approach might eliminate more time consuming, cumbersome and complicated approaches. Therefore, we attempted to compare the predictive performance of our approach with the results of Skarydova et al. (2013), because their method using recombinant systems is likely to be accurate.

The primary objective of the present study was to estimate enzymes possibly involved in the metabolic reduction of nabumetone (Fig. 1) through inhibition and correlation studies using human liver microsomes and cytosol, and to compare these results with the recombinant system reported by Skarydova et al. (2013).

2 Materials and methods

2.1 Chemicals

Nabumetone (4-(6-methoxy-2-naphthyl)-2-butanone) and *rac*-MNBO (4-(6-methoxy-2-naphthyl)-butan-2-ol) were supplied by Sanwa Kagaku Kenkyusho (Mie, Japan). (+)- and (−)-MNBO were obtained by chromatographic separation (CHIRALPAK AS-H column, 10 × 250 mm, 5 μm particle size, Daicel, Tokyo, Japan) of *rac*-MNBO. (+)-MNBO was converted to the diastereomeric MTPA esters with (*R*)- and (*S*)-MTPACl. ^{1}H NMR spectra of the diastereomers were measured and each signals was assigned by COSY. The absolute configuration of (+)-MNBO was determined to be *S* according to the modified Mosher's method (Ohtani et al. 1991). Naproxen was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form) and NADP (β-nicotinamide adenine dinucleotide phosphate) were purchased from Alexis (San Diego, CA). Glucose 6-phosphate was purchased from Oriental Yeast (Tokyo, Japan). Glucose 6-phosphate dehydrogenase was purchased from MP Biomedicals (Aurora, OH). Hydroxyhexamide was synthesized from acetohexamide using sodium borohydride. All other chemicals were of the highest purity available.

2.2 Enzyme preparations

Individual human liver microsomes (HG3, HG74, HG95, HH13, HH47, HK37 and HG103) and individual human liver cytosolic fractions (HH18, HG42, HG43, HK34, HG64, HH31, HH47 and HH35) were purchased from BD Gentest Co. (Woburn, MA). Pooled human liver microsomes and cytosol were also purchased from BD Gentest Co. The pooled human liver microsomes and cytosol were obtained from 20 to 10 donors, respectively.

2.3 Metabolism of nabumetone in human liver microsomes and cytosol

Metabolism of nabumetone in human liver microsomes and cytosol were evaluated in the presence of NADPH. A typical incubation mixture (0.5 ml of total volume) contained 50 mM phosphate buffer (pH 7.4), an NADPH-generating system (0.5 mM $NADP^{+}$, 5 mM glucose 6-phosphate, 5 mM $MgCl_2$, 1 U/ml glucose 6-phosphate dehydrogenase) containing 0.5 mM NADPH, nabumetone, and human liver microsomes or cytosol (0.1 mg/ml). The reaction was initiated by adding 250 μl of the NADPH-generating system after a 3 min pre-incubation of the microsomes at 37 °C. The reaction mixtures were incubated for 20 min, and

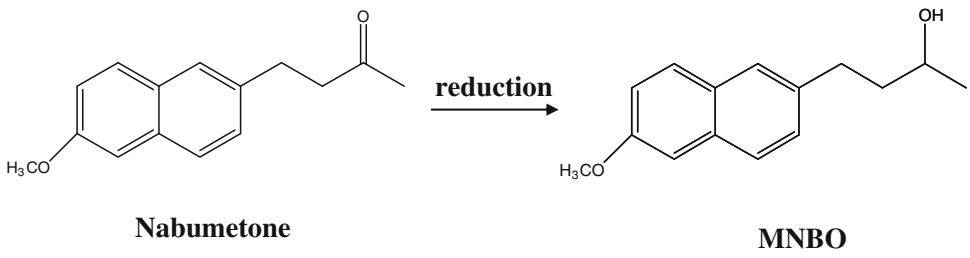

Fig. 1 Metabolic reduction of nabumetone

reactions were terminated by the addition of 100 µl of 10 % trichloroacetic acid and 500 µl of acetonitrile containing 10 µM of naproxen as an internal standard. After removal of the protein by centrifugation at 10,000×*g* for 5 min, a 500 µl portion of the supernatant was applied to cartridges. Solid phase extraction was performed using Bond Elut Certify II cartridges (200 mg, Varian, Harbor City, CA). Each cartridge was first conditioned with 6 ml of 5 % methanol, at a flow rate of 2 ml/min. After application of the samples at 2 ml/min, the cartridges were washed with 4 ml of water. The cartridges were eluted with 6 ml of a freshly prepared mixture of solvents (hexane and ethyl acetate, 1:1 v/v). The purified extract eluted from the solid-phase extraction was transferred to a test tube, and the solvents from the eluate were evaporated to dryness on a heating block at 40 °C. The residue was dissolved in 5 ml of mobile phase, and 20 µl was injected into the high-performance liquid chromatograph (HPLC) column. The HPLC system was a Shimadzu LC-10A$_{VP}$ equipped with a RF-10A$_{XL}$ fluorescence detector (Kyoto, Japan). The samples were injected into the HPLC system and separated on a YMC-Pack ODS-A column (4.6 × 150 mm, 5 µm particle size, YMC, Kyoto, Japan). The temperature of the column oven was set at 30 °C. The mobile phase consisted of acetonitrile: 20 mM K_2HPO_4 (pH 3.0) (50:50, v/v), and the flow rate was 1.0 ml/min. The peaks were monitored at an excitation wavelength of 280 nm and an emission wavelength of 350 nm (Mikami et al. 2000; Kobylinska et al. 2003).

Separation of enantiomers was performed using a CHIRALPAK AS-RH column (4.6 × 150 mm, 5 µm particle size, Daicel, Tokyo, Japan). The temperature of the column oven was set at 40 °C. The mobile phase consisted of water:acetonitrile (55:45, v/v), and the flow rate was 1.0 ml/min. The UV detection was set at 320 nm.

2.4 Inhibition of MNBO formation from nabumetone by chemical inhibitors

Inhibition studies were performed by incubating pooled human liver microsomes and cytosol with chemical inhibitors. The following chemical inhibitors and substrates were used: *n*-benzylimidazole (1 mM) for CYP, methimazole (1 mM) for flavin-containing monooxygenase (FMO) (Grothusen et al. 1996), $TlCl_3$ (64 µM) for NADPH-cytochrome P450 reductase, dicumarol (0.1 mM) for NAD(P)H:quinone oxidoreductase, barbituric acid (0.1 mM) for aldehyde dehydrogenase, pyrazol (0.1 mM) for alcohol dehydrogenase (Tani et al. 2005), quercetin (0.1 mM) for CBR, menadione (0.5 mM) for CBR and 11β-HSD, 18β-glycyrrhetinic acid (200 µM) for 11β-HSD (Maser et al. 2000; Atalla and Maser 2001; Lee et al. 2008; Diederich et al. 2000; Maser et al. 2003), ethacrynic acid (1 mM) for CBR and AKRs (Maser et al. 2000; Barski et al. 2008), phenolphthalein (20 µM) and flufenamic acid (20 µM) for AKR1C1, 1C2, 1C3 and 1C4, medroxyprogesterone acetate (20 µM) for AKR1C1, 1C2 and 1C4 and 5β-cholanic acid-3α,7α-diol (50 µM) for AKR1C2 (Hara et al. 1990; Deyashiki et al. 1992; Atalla et al. 2000; Higaki et al. 2003; Steckelbroeck et al. 2006; Blech et al. 2010; Tong et al. 2010). Nabumetone (50 µM) was incubated with NADPH or NADH for 20 min at 37 °C.

2.5 Correlation between 11β-HSD activity and MNBO formation from nabumetone in microsomes

MNBO formation rates were correlated with 11β-HSD activities in seven human liver microsome fractions. Nabumetone (50 µM) was incubated for 20 min at 37 °C. The activity of 11β-HSD was determined with the substrate cortisone. A typical incubation mixture (200 µl total volume) contained 50 mM phosphate buffer (pH 7.4), NADPH (4 mM), cortisone (100 µM), and human liver microsomes (0.4 mg/ml). The reaction was initiated by adding NADPH solution after a 3 min pre-incubation of human liver microsomes at 37 °C. The reaction mixtures were incubated for 60 min, and reactions were terminated by the addition of 300 µl of ice-cold acetonitrile and 100 µl of 50 µM 6α-methylprednisolone as an internal standard. The reduction product (cortisol) was determined by HPLC according to the method of Piwowarska et al. (2009) with slight modification. The samples were injected into the HPLC system and separated on an YMC-Pack ODS-A column (4.6 × 150 mm, 5 µm particle size, YMC, Kyoto, Japan). The temperature of the column oven was set at

40 °C. The mobile phase consisted of water/acetonitrile (70:30, v/v), and the flow rate was 1.0 ml/min. The UV detection was set at 252 nm.

2.6 Correlation between CBR and/or AKR1C activities and MNBO formation from nabumetone in cytosol

MNBO formation rates were correlated with CBR and/or AKR1C activities (Matsunaga et al. 2006; Nakayama et al. 1985) in liver cytosol derived from 6–8 human. Nabumetone (50 μM) was incubated for 20 min at 37 °C. The activities of ethacrynic acid (AKR1C1 and 1C4), metyrapone (CBR1 and AKR1C4), loxoprofen (CBR1, AKR1C1, 1C2 and 1C4) and 4-benzoylpyridine (CBR) reductase were determined according to the method of Ohara et al. (1995) and acetohexamide (AKR1C1, 1C2 and 1C4) reductase were determined according to the method of Imamura and Shimada (2004).

2.7 Data analysis

The results are presented as the mean of estimates obtained in triplicate experiments. The apparent Michaelis–Menten parameters for the formation of MNBO from nabumetone were estimated for Eadie–Hofstee plots.

Analyses for correlations and statistical significances were carried out using EXCEL statistics software (Esumi Co. Ltd., Tokyo, Japan).

3 Results

3.1 Kinetic analyses of MNBO formation from nabumetone by human liver microsomes and cytosol

The kinetics of the NADPH-dependent metabolism of 10–300 μM nabumetone to the metabolite MNBO was examined using pooled human liver microsomes and cytosol. Michaelis–Menten kinetics for MNBO activity was observed in human liver microsomes and cytosol (Fig. 2), and the results of the kinetic analysis are shown in Table 1. The V_{max} value for MNBO formation in the human liver microsomes was approximately fourfold higher than that in the human liver cytosol, and the V_{max}/K_m values in human liver microsomes were approximately threefold higher than those in human liver cytosol. Both subcellular fractions produce both enantiomers. The formation ratios of (*S*)-/(*R*)-MNBO were 74/26 and 67/33 in microsomes and cytosol, respectively. The reduction of nabumetone was observed on incubating with either

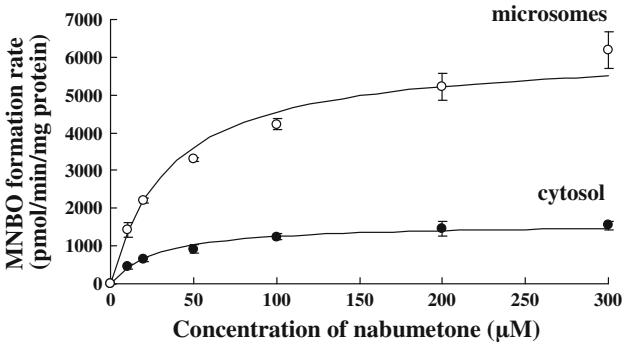

Fig. 2 Michaelis–Menten plots for MNBO formation from nabumetone in human microsomes and cytosol. Each point represents the mean ± SD of three measurements

NADPH or NADH as the cofactor in the cytosol fraction (data not shown).

3.2 Inhibition of MNBO formation from nabumetone by chemical inhibitors

MNBO formation from nabumetone in human liver microsomes and cytosol is shown in Figs. 3 and 4, respectively. The reductive formation to MNBO from nabumetone in the microsomes was not inhibited by *n*-benzylimidazole (P450), methimazole (FMO), $TlCl_3$ (NADPH-cytochrome P450 reductase) or dicumarol (NAD(P)H:quinone oxidoreductase), whereas it was inhibited by 18β-glycyrrhetinic acid and menadione (11β-HSD) (Fig. 3). The mean levels of inhibition elicited by 18β-glycyrrhetinic acid and menadione were 78.5 and 52.3 %, respectively, compared with the control value. MNBO formation from nabumetone in the human liver cytosol was not inhibited by barbituric acid (aldehyde dehydrogenase), pyrazol (alcohol dehydrogenase) or dicumarol, whereas it was inhibited by quercetin and menadione (CBR), ethacrynic acid (CBR and AKRs) and phenolphthalein, flufenamic acid and medroxyprogesterone acetate (AKR1C) (Fig. 4). The mean values of inhibition elicited by the various inhibitors (compared with the control value) were: quercetin 64.0 %, menadione 77.4 %, ethacrynic acid 78.5 %, phenolphthalein 47.1 %, flufenamic acid 43.8 % and medroxyprogesterone acetate 56.2 %.

3.3 Correlation between 11β-HSD activity and MNBO formation from nabumetone

The correlation between 11β-HSD activity and MMBO formation from nabumetone was assessed in liver

Table 1 Michaelis–Menten kinetic parameters of MNBO formation from nabumetone in human liver microsomes and cytosol

Subcellular fraction	K_m (μM)	V_{max} (pmol/min/mg protein)	V_{max}/K_m (μl/min/mg protein)	(*S*)-/(*R*)-MNBO
Microsomes	35.0 ± 5.7	6,136.7 ± 306.1	177.5 ± 20.6	74/26
Cytosol	27.1 ± 2.0	1,592.8 ± 105.3	59.1 ± 7.4	67/32

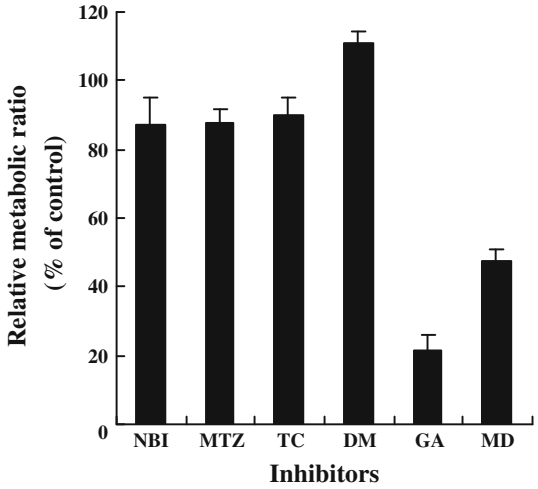

Fig. 3 Effect of enzyme inhibitors on MNBO formation from nabumetone in human microsomes. Each bar represents the mean ± SD ($n = 3$). NBI: 1 mM *n*-benzylimidazole (P450), MTZ: 1 mM methimazole (FMO), TC: 64 μM $TlCl_3$ (NADPH-cytochrome P450 reductase), DM: 0.1 mM dicumarol (NAD(P)H:quinone oxidoreductase), GA: 200 μM 18β-glycyrrhetinic acid (11β-HSD), MD: 0.5 mM menadione (11β-HSD)

microsomes from seven human samples. A well-known reaction, cortisol formation from cortisone, was used for assaying the 11β-HSD activity in the microsomal fractions. Figure 5 shows a good correlation between 11β-HSD activity and MNBO formation from nabumetone ($r^2 = 0.93$, $P < 0.0005$).

3.4 Correlation between CBR and/or AKR1C activities and MNBO formation from nabumetone

Correlation between CBR activity and MNBO formation from nabumetone were compared in the liver cytosol from 6–8 human samples. Enzymatic reduction of acetohexamide, ethacrynic acid, metyrapone and loxoprofen reductase was used for assaying the CBR and/or AKR1C activities in cytosolic fractions. Figure 6 shows a good correlation between the reduction of acetohexamide ($r^2 = 0.92$, $P < 0.0005$), ethacrynic acid ($r^2 = 0.93$, $P < 0.005$), metyrapone ($r^2 = 0.93$, $P < 0.005$) and loxoprofen ($r^2 = 0.86$, $P < 0.01$) and MNBO formation from nabumetone. In contrast, a poor correlation was observed between CBR activity with 4-benzoyl pyridine reduction as a substrate and MNBO formation from nabumetone ($r^2 = 0.24$).

4 Discussion

Nabumetone is established as a non-acidic, non-steroidal anti-inflammatory drug. It is an example of a pro-drug, because nabumetone itself exists only for a short time in

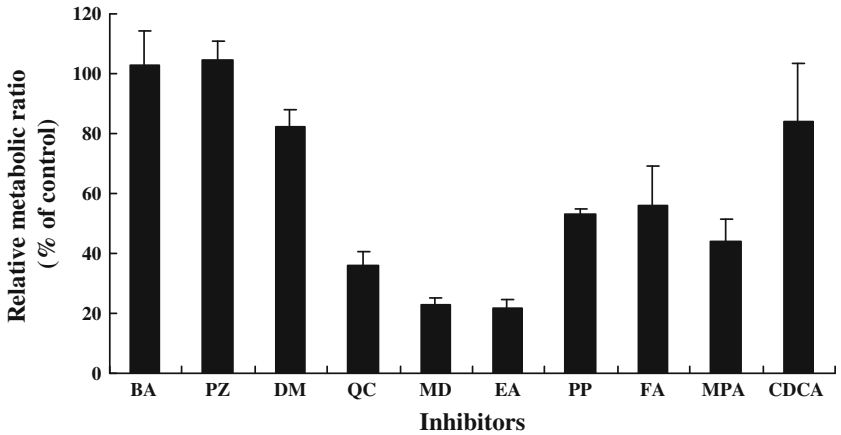

Fig. 4 Effect of enzyme inhibitors on MNBO formation from nabumetone in human cytosol. Each bar represents the mean ± SD ($n = 3$). BA: 0.1 mM barbituric acid (aldehyde dehydrogenase), PZ: 0.1 mM pyrazol (alcohol dehydrogenase), DM: 0.1 mM dicumarol (NAD(P)H:quinone oxidoreductase), QC: 0.1 mM quercetin (CBR), MD: 0.5 mM menadione (CBR), EA: 1 mM ethacrynic acid (CBR and AKRs), PP: 20 μM phenolphthalein (AKR1C1, 1C2, 1C3 and 1C4), FA: 20 μM flufenamic acid (AKR1C1, 1C2, 1C3 and 1C4), MPA: 20 μM medroxyprogesterone acetate (AKR1C1, 1C2 and 1C4), CDCA: 50 μM 5β-cholanic acid-3α,7α-diol (AKR1C2)

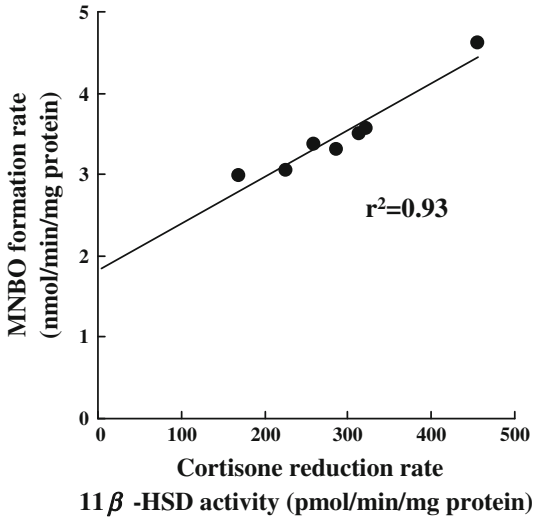

Fig. 5 Correlation between 11β-HSD activity and MNBO formation from nabumetone by liver microsomes obtained from seven human samples

the plasma, while its active metabolite, 6-MNA (which exerts an anti-inflammatory effect) remains in the plasma for a long period of time (Kendall et al. 1989). The existence of metabolic pathway from an intermediate compound, MNBO, to 6-MNA has been confirmed in rats (Tsuchiya et al. 1988). MNBO has no anti-inflammatory properties and is a weak inhibitor of cyclooxygenase (Nobilis et al. 2003).

In this study, we investigated the in vitro formation of MNBO in human liver subcellular fractions and characterized the enzymes involved in the carbonyl reduction of nabumetone. To identify the human liver enzymes involved in the metabolic clearance of nabumetone, enzyme inhibition studies were performed using an in vitro metabolism system deemed to reflect in vivo metabolism. The effects of enzyme inhibitors on metabolic clearance of nabumetone were assessed in terms of MNBO formation.

The kinetic parameters in human liver microsomes showed that the capacity for carbonyl reduction was approximately three times greater than that in human liver cytosol. In vitro metabolism of nabumetone in human liver microsomes with NADPH was inhibited by 18β-glycyrrhetinic acid and menadione (inhibitors of 11β-HSD), but not by *n*-benzylimidazole (an inhibitor of CYP), methimazole (an inhibitor of FMO), $TlCl_3$ (an inhibitor of NADPH-cytochrome P450 reductase), or dicumarol (an inhibitor of NAD(P)H:quinine oxidoreductase). Nabumetone was reduced to the two MNBO enantiomers by human liver microsomes and cytosol. The use of chemical inhibitors in inhibition studies inhibited (*S*)- and (*R*)-MNBO at approximately the same ratio (data not shown).

The results of the in vitro metabolism studies presented here have several implications. These could be inferred from the sequential demonstration that (1) nabumetone was metabolized by human liver microsomes; (2) NADPH was the favored cofactor; (3) the 11β-HSD substrate menadione was highly efficient in inhibiting this metabolism; (4) the 11β-HSD inhibitor 18β-glycyrrhetinic acid decreased nabumetone metabolism in a dose-dependent fashion (data not shown); (5) good correlation was observed between MNBO formation rate and cortisone reduction rate ($r^2 = 0.93$). It is clear that nabumetone is a substrate for 11β-HSD. 11β-HSD has been shown to be capable of catalyzing the reductive metabolism of a variety of xenobiotic compounds (Matsunaga et al. 2006).

Nabumetone reduction was assessed using selective chemical inhibitors of AKR isoforms and CBR/SDR. The reduction of nabumetone was most effectively inhibited by ethacrynic acid, which inhibits CBR and AKRs. Formation of MNBO was also inhibited (by up to 52.9 % of control) by phenolphthalein, which inhibits AKR1C isoforms with greater selectivity for AKR1C4 than for AKR1C1, AKR1C2 or AKR1C3 (Higaki et al. 2003). Medroxyprogesterone acetate, which inhibits AKR1C1, AKR1C2 and AKR1C4, and AKR1C4 with high specificity (Hara et al. 1990), inhibited the formation of MNBO by up to 43.8 % of control. Fufenamic acid, a selective inhibitor of AKR1C1, AKR1C2 and AKR1C3 and a weak inhibitor of AKR1C4 (Deyashiki et al. 1992), also inhibited the formation of MNBO. The AKR1C2-selective inhibitor 5β-cholanic acid-3α,7α-diol (Deyashiki et al. 1992) did not appear to significantly affect the reduction of nabumetone. Significant correlations were observed between the nabumetone reductive activity and reduction of acetohexamide ($r^2 = 0.92$), a marker for the AKR1C subfamily, or ethacrynic acid ($r^2 = 0.93$), a marker for AKR1C1 and 1C4, in cytosol. Some residual activity done by different enzymes not related to nabumetone reduction may be existed because the regression lines did not go through the origin in Fig. 6. The lack of an inhibitory effect of barbituric acid (an inhibitor of aldehyde dehydrogenase), dicumarol and pyrazol (alcohol dehydrogenase and NAD(P)H:quinone oxidoreductase) on the reduction of nabumetone confirmed that in humans, the AKR1C subfamily are the primary AKRs capable of reducing xenobiotic ketones. In addition, MNBO was detected when nabumetone was incubated with human liver cytosol in the presence of either NADPH or NADH as the cofactor. The rate of nabumetone reduction of the human liver cytosol with NADH as a cofactor was nearly 1.1 times higher than with NADPH. The dehydrogenation of (*S*)-1-indanol catalyzed by AKR1C4 proceeded at similar rates when either NADPH or NADH were used as the cofactor (Hara et al. 1990). The efficient reduction of nabumetone with NADH as a cofactor was observed, which might suggest involvement of AKR1C4. Together,

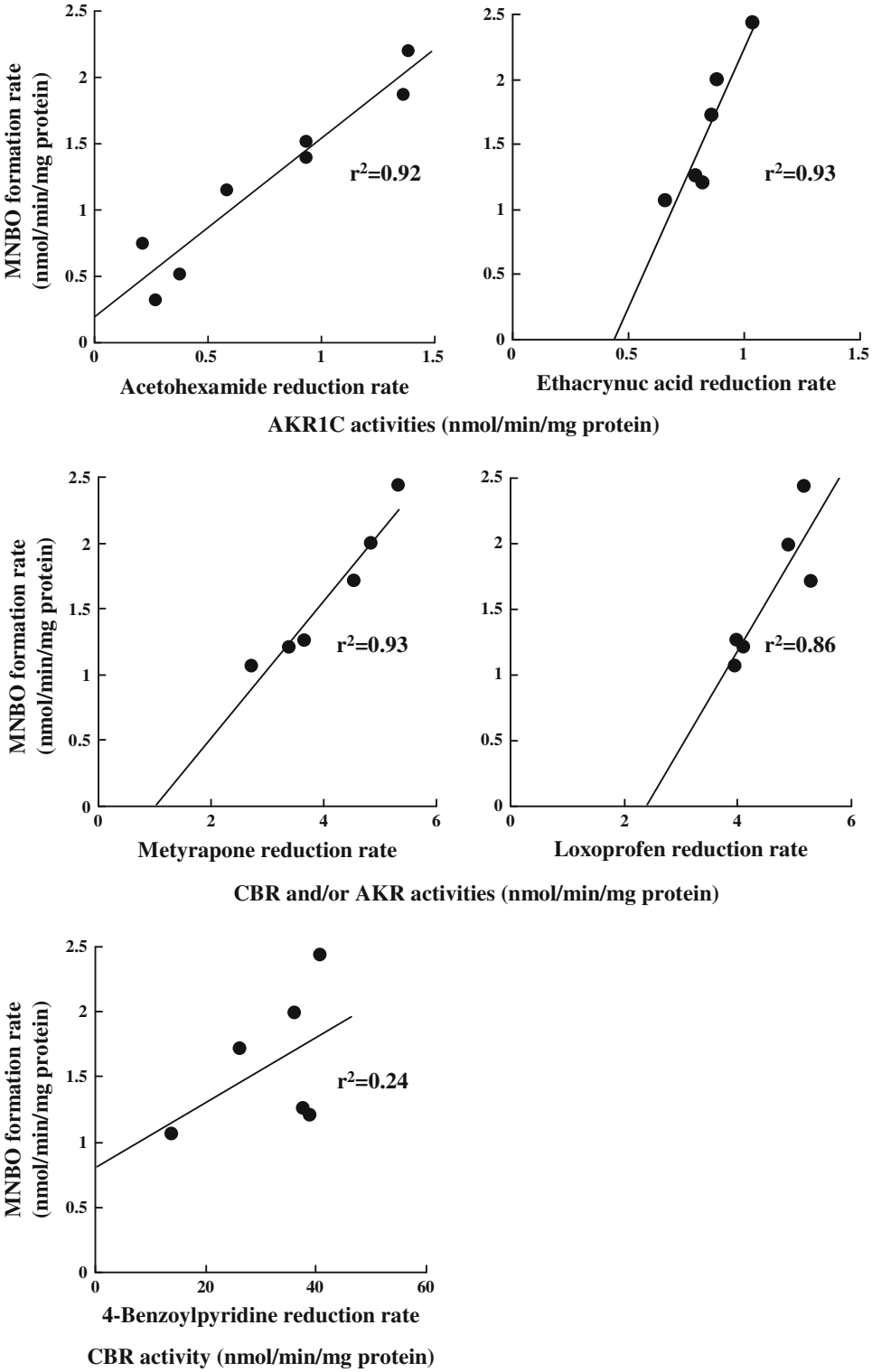

Fig. 6 Correlation between CBR and/or AKR1C activities and MNBO formation from nabumetone by liver cytosol obtained from 6–8 human samples

these results indicate that multiple isoforms in the AKR1C subfamily are responsible for nabumetone reduction, with the predominant contribution probably from AKR1C4, which accounts for the largest amount of enzyme activity in human liver (Penning et al. 2000; Steckelbroeck et al. 2006).

In correlation studies, however, poor correlation was observed in the cytosol between the formation of MNBO from nabumetone and CBR activity with 4-benzoyl pyridine reduction as a substrate ($r^2 = 0.24$). However, some contribution of CBR could not be excluded. Quercetin and menadione, inhibitors of CBR, caused a significant

decrease in the reduction of nabumetone. When metyrapone, which has better selectivity of CBR and AKR1C4, was used as a substrate, a significant correlation was revealed ($r^2 = 0.93$). It is known that CBR is highly expressed in human liver, and its expression is an order of magnitude higher than that of AKR1C isoforms (Steckelbroeck et al. 2006). These observations suggest that CBR could be involved to some extent in the metabolism of nabumetone to MNBO. The absence of commercially available AKR and CBR isoforms and the lack of specific inhibitors did not allow further discrimination of the individual isoforms. Therefore, the ability of other enzymes to convert nabumetone to MNBO could not be completely excluded.

Published results of Skarydova et al. (2013) dealt with the reductive biotransformation of nabumetone to MNBO by recombinant forms of carbonyl reducing enzymes. They described seven important carbonyl reducing enzymes (CBR1, AKR1C1-4, AKR1B1 and AKR1B10) which participate in the biotransformation of nabumetone in the cytosol. Among these enzymes, AKR1C4 showed the highest intrinsic clearance. Our results using inhibition and correlation studies in human liver cytosol indicate that nabumetone may be metabolized primarily by AKR1C4, and supported the results of the study by Skarydova et al. using recombinant enzymes.

5 Conclusions

In conclusion, the present investigation provides evidence that nabumetone is metabolized by 11β-HSD in human liver microsomes. AKR1C4 appears to play a major role in the carbonyl reduction of nabumetone, although multiple enzymes in the AKR1C subfamily may be involved in the reduction. In addition, CBR appears to be responsible for the formation of MNBO from nabumetone. These results are in accordance with those of Skarydova et al. (2013). We suggest that a combination of inhibition and correlation studies is a useful approach for estimating the contribution of individual enzymes.

Conflict of interest None.

References

Atalla A, Maser E (2001) Characterization of enzymes participating in carbonyl reduction of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in human placenta. Chem Biol Interact 130–132:737–748

Atalla A, Breyer-Pfaff U, Maser E (2000) Purification and characterization of oxidoreductases-catalyzing carbonyl reduction of the tobacco-specific nitrosamine 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) in human liver cytosol. Xenobiotica 30:755–769

Barski OA, Tipparaju SM, Bhatnagar A (2008) The aldo–keto reductase superfamily and its role in drug metabolism and detoxification. Drug Metab Rev 40:553–624

Blech S, Ludwig-Schwellinger E, Gräfe-Mody EU, Withopf B, Wagner K (2010) The metabolism and disposition of the oral dipeptidyl peptidase-4 inhibitor, linagliptin, in humans. Drug Metab Dispos 38:667–678

Davies NM (1997) Clinical pharmacokinetics of nabumetone. The dawn of selective cyclo-oxygenase-2 inhibition? Clin Pharmacokinet 33:404–416

Deyashiki Y, Taniguchi H, Amano T, Nakayama T, Hara A, Sawada H (1992) Structural and functional comparison of two human liver dihydrodiol dehydrogenases associated with 3α-hydroxysteroid dehydrogenase activity. Biochem J 282:741–746

Diederich S, Grossmann C, Hanke B, Quinkler M, Herrmann M, Bähr V, Oelkers W (2000) In the search for specific inhibitors of human 11β-hydroxysteroid-dehydrogenases (11β-HSDs): chenodeoxycholic acid selectively inhibits 11β-HSD-I. Eur J Endocrinol 142:200–207

Friedel HA, Todd PA (1988) Nabumetone. A preliminary review of pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in rheumatic diseases. Drugs 35:504–524

Friedel HA, Langtry HD, Buckley MM (1993) Nabumetone. A reappraisal of its pharmacology and therapeutic use in rheumatic diseases. Drugs 45:131–156

Grothusen A, Hardt J, Bräutigam L, Lang D, Böcker R (1996) A convenient method to discriminate between cytochrome P450 enzymes and flavin-containing monooxygenases in human liver microsomes. Arch Toxicol 71:64–71

Haddock RE, Jeffery DJ, Lloyd JA, Thawley AR (1984) Metabolism of nabumetone (BRL 14777) by various species including man. Xenobiotica 14:327–337

Hara A, Taniguchi H, Nakayama T, Sawada H (1990) Purification and properties of multiple forms of dihydrodiol dehydrogenase from human liver. J Biochem 108:250–254

Higaki Y, Usami N, Shintani S, Ishikura S, El-Kabbani O, Hara A (2003) Selective and potent inhibitors of human 20α-hydroxysteroid dehydrogenase (AKR1C1) that metabolizes neurosteroids derived from progesterone. Chem Biol Interact 143–144:503–513

Imamura Y, Shimada H (2004) Strain- and sex-related differences of carbonyl reductase activities in kidney microsomes and cytosol of rats. J Appl Toxicol 24:437–441

Kendall MJ, Chellingsworth MC, Jubb R, Thawley AR, Undre NA, Kill DC (1989) A pharmacokinetic study of the active metabolite of nabumetone in young healthy subjects and older arthritis patients. Eur J Clin Pharmacol 36:299–305

Kobylinska K, Barlinska M, Kobylinska M (2003) Analysis of nabumetone in human plasma by HPLC. Application to single dose pharmacokinetic studies. J Pharm Biomed Anal 32:323–328

Lee SK, Kim JH, Seo YM, Kim HC, Kang MJ, Jeong HG, Lee ES, Jeong TC (2008) In vitro characterization of the enzymes involved in the metabolism of 1-furan-2-yl-3-pyridin-2-yl-propenone, an anti-inflammatory propenone compound. Arch Pharm Res 31:764–770

Mangan FR, Flack JD, Jackson D (1987) Preclinical overview of nabumetone. Pharmacology, bioavailability, metabolism, and toxicology. Am J Med 83:6–10

Maser E, Stinner B, Atalla A (2000) Carbonyl reduction of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by cytosolic enzymes in human liver and lung. Cancer Lett 148:134–144

Maser E, Friebertshäuser J, Völker B (2003) Purification, characterization and NNK carbonyl reductase activities of 11β-hydroxysteroid dehydrogenase type 1 from human liver: enzyme

cooperativity and significance in the detoxification of a tobacco-derived carcinogen. Chem Biol Interact 143–144:435–448

Matsumoto K, Nemoto E, Hasegawa T, Akimoto M, Sugibayashi K (2011a) In vitro characterization of the cytochrome P450 isoforms involved in the metabolism of 6-methoxy-2-napthyl-acetic acid, an active metabolite of the prodrug nabumetone. Biol Pharm Bull 34:734–739

Matsumoto K, Hasegawa T, Akimoto M, Sugibayashi K (2011b) Reductive metabolism of nabumetone, an anti-inflammatory drug. Abstracts vol. IV, The 131rd annual meeting of the pharmaceutical society of Japan, Shizuoka, 28–31 March 2011, p 219

Matsunaga T, Shintani S, Hara A (2006) Multiplicity of mammalian reductases for xenobiotic carbonyl compounds. Drug Mtab Pharmacokinet 21:1–18

Mikami E, Goto T, Ohno T, Matsumoto H, Nishida M (2000) Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphthylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography. J Pharm Biomed Anal 23:917–925

Nakayama T, Hara A, Yashiro K, Sawada H (1985) Reductase for carbonyl compounds in human liver. Biochem Pharmacol 34:107–117

Nobilis M, Kopecký J, Kvetina J, Svoboda Z, Pour M, Kunes J, Holcapek M, Kolárová L (2003) Comparative biotransformation and disposition studies of nabumetone in humans and minipigs using highperformance liquid chromatography with ultraviolet, fluorescence and mass spectrometric detection. J Pharm Biomed Anal 32:641–656

Ohara H, Miyabe Y, Deyashiki Y, Matsuura K, Hara A (1995) Reduction of drug ketones by dihydrodiol dehydrogenases, carbonyl reductase and aldehyde reductase of human liver. Biochem Pharmacol 50:221–227

Ohtani I, Kusumi T, Kashman Y, Kakisawa H (1991) High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. J Am Chem Soc 113:4092–4096

Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, Moore M, Palackal N, Ratnam K (2000) Human 3α-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo–keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochem J 351:67–77

Piwowarska J, Wrzosek M, Radziwoń-Zaleska M, Ryszewska-Pokraśniewicz B, Skalski M, Matsumoto H, Biernacka-Bazyluk A, Szelenberger W, Pachecka J (2009) Serum cortisol concentration in patients with major depression after treatment with clomipramine. Pharmacol Rep 61:604–611

Skarydova L, Nobilis M, Wsól V (2013) Role of carbonyl reducing enzymes in the phase I biotransformation of the non-steroidal anti-inflammatory drug nabumetone in vitro. Xenobiotica 43:346–354

Steckelbroeck S, Oyesanmi B, Jin Y, Lee SH, Kloosterboer HJ, Penning TM (2006) Tibolone metabolism in human liver is catalyzed by 3α/3β-hydroxysteroid dehydrogenase activities of the four isoforms of the aldo–keto reductase (AKR)1C subfamily. J Pharmacol Exp Ther 316:1300–1309

Tani N, Yabuki M, Komuro S, Kanamaru H (2005) Characterization of the enzymes involved in the in vitro metabolism of amrubicin hydrochloride. Xenobiotica 35:1121–1133

Tong Z, Chandrasekaran A, Li H, Rotshteyn Y, Erve JC, Demaio W, Talaat R, Hultin T, Scatina J (2010) In vitro metabolism and identification of human enzymes involved in the metabolism of methylnaltrexone. Drug Metab Dispos 38:801–807

Tsuchiya S, Ishibashi K, Asano H, Hirano K, Noguchi H (1988) Pharmacokinetics of nabumetone and its metabolic pathways in rats. Xenobio Metab Dispos 3:67–74

Turpeinen M, Hofmann U, Klein K, Mürdter T, Schwab M, Zanger UM (2009) A predominate role of CYP1A2 for the metabolism of nabumetone to the active metabolite, 6-methoxy-2-naphthyl-acetic acid, in human liver microsomes. Drug Metab Dispos 37:1017–1024