INHIBITION OF HALOPERIDOL REDUCTION BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN HUMAN LIVER CYTOSOL

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SUMMARY

A thorough knowledge of drug-drug interactions is crucial as the practice of multiple drug therapy escalates. *In vitro* studies using human liver enzymes are a valuable and non-invasive tool for predicting potential drug interactions *in vivo*.

1. A simple radio-TLC method was developed to monitor the formation of reduced haloperidol from haloperidol in human liver cytosol.

2. Indomethacin, known to be a potent inhibitor of 3α-hydroxysteroid dehydrogenase, was chosen as a reference for the evaluation of several arylpropionic acid derived non-steroidal anti-inflammatory drugs, ketoprofen, tiaprofenic acid, fenbufen, ibuprofen, d-naproxen and l-naproxen. The IC_{50} ranged from 0.4-6.0 mM with indomethacin the most potent inhibitor of haloperidol carbonyl reductase.

3. The carbonyl reduction of haloperidol was inhibited significantly by these most commonly used non-steroidal anti-inflammatory drugs and the degree of inhibition reflected their pharmacological potency.

4. Sephadex G-100 fractionation of human liver cytosol yielded a fraction with haloperidol reductase activity at a molecular weight of about 32,000.

KEY WORDS

haloperidol, carbonyl reductase, NSAIDs

INTRODUCTION

Haloperidol is one of the most widely used drugs in the treatment of acute and chronic psychosis, abnormal movement disorders and in the management of confused states in the elderly and chronically hospitalized patients. Non-steroidal anti-inflammatory drugs (NSAIDs), some of which are OTC drugs, are frequently prescribed as an analgesic or an anti-inflammatory in the treatment of arthritis, rheumatism and other inflammatory disorders. As measured by prescription rates, the consumption of NSAIDs is rising steadily particularly in the elderly /1/. These drugs have been responsible for 21-25% of all adverse drug reactions reported in the U.K. and U.S.A.

However, studies of drug interaction between haloperidol and NSAIDs are scarce. Our literature search revealed only one small study /2/ and a case report which found clinical deterioration in patients given indomethacin with small doses of haloperidol /3/. In this instance, drowsiness and confusion were so severe that haloperidol therapy had to be discontinued. Haloperidol is among the most potent of antipsychotic drugs in causing extrapyramidal side effects, namely acute dystonic reactions, akathesia, drug-induced Parkinsonism and tardive dyskinesia. Drug-drug interaction, which may alter antipsychotic efficacy and compound the side effects of haloperidol treatment, is likely if haloperidol is combined with NSAIDs.

The metabolism of haloperidol in humans involves several pathways including carbonyl reduction of benzylic ketone to an alcohol /4/. This reduced haloperidol (RHAL) is a major metabolite of haloperidol biotransformation in humans. RHAL possesses some antipsychotic activity and is reoxidized to haloperidol *in vivo* /5/ and in human liver microsomes /6/. The reduction of haloperidol to RHAL has also been demonstrated in guinea-pig kidney /7/, human and guinea-pig liver /8/ and human erythrocytes /9/.

The enzyme involved in the reduction of haloperidol has the characteristics of carbonyl reductases. It is monomeric, pyridine nucleotide-dependent, cytosolic, of low molecular weight and inhibited by known substrates of carbonyl reductases such as menadione, daunorubicin and ethacrynic acid /8/. The carbonyl reductase is classified in the aldo-keto reductases superfamily which is widely distributed in humans and animals /10/.

Though functionally similar and thus classified with the aldo-keto reductases, carbonyl reductases appear structurally distinct and may in

fact be related to short chain alcohol dehydrogenases and hydroxysteroid dehydrogenase /11/. Wermuth /12/ purified an NADPH-dependent carbonyl reductase that exhibited 9-ketoreductase activity toward prostaglandins and 3-ketoreductase activity toward 3-ketosteroids. An association between carbonyl reductases and 3α-hydroxysteroid dehydrogenase (3α-HSD) is unfolding as the role of aldo-keto reductases in steroid metabolism becomes more evident.

3α-HSD is also a cytosolic, monomeric, pyridine nucleotide-dependent enzyme which catalyzes the oxidoreduction of various steroids. According to the study by Penning *et al.* /13/, the purified enzyme is effectively inhibited by NSAIDs and the degree of inhibition reflects the pharmacological potency of the NSAIDs at concentrations relevant to therapeutic doses /14/. Indomethacin was the most potent inhibitor of 3α-HSD.

This encouraging progress prompted us to further examine the effect of NSAIDs on haloperidol carbonyl reductase. Indomethacin was included as a reference and to facilitate comparison with Penning's work. The remaining NSAIDs are arylpropionic acid derivatives and were chosen because they represent the most commonly used of that group. In the present study we describe a radio-TLC method to monitor the formation of RHAL in human liver cytosol in the presence of these drugs.

METHODS

Materials

^{3}H-Haloperidol(15 Ci/mmol) was purchased from DuPont Canada Inc. (Markham, Ontario), and haloperidol and reduced haloperidol were donated by Janssen Pharmaceutical (Beers, Belgium). Tiaprofenic acid was donated by Roussel Canada Inc. (Montreal, Qc). Indomethacin, ketoprofen, fenbufen, ibuprofen, (-)l-naproxen, (+)d-naproxen and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). Silica gel 60 F_{254} TLC plates were obtained from Merck (Darmstadt, Germany), Sephadex G-100 was from Pharmacia LKB (Uppsala, Sweden), and all other chemicals used in this study were reagent grade and obtained from local suppliers.

Human liver preparations

Liver tissue from 2 kidney donors (designated K10 and K21) was obtained from the Human Liver Bank set up at the Department of Pharmacology, University of Toronto. Detailed description of sex, age, cause of death and drug history has been documented previously /15/. The cytosolic fractions were prepared according to the method previously reported /8/. The supernatant of the 9,000 *g* fraction prepared in 1.15% KCl was further centrifuged at 100,000 *g* for 60 min to obtain the cytosolic fraction used as the enzyme source in this study. Protein concentration was determined using B.C.A. method (Pierce Chemical) with bovine serum albumin as the standard.

Incubation conditions for *in vitro* haloperidol metabolism

Preliminary studies to establish optimal incubation conditions for reduction of haloperidol by human liver cytosol were described previously /8/. The incubation mixtures (in duplicate) contained a fixed amount of ^{3}H-haloperidol (100,000 dpm) and varying concentration of unlabelled haloperidol (0.05-0.8 mM), 0.2 ml 1.15% KCl, 2 mM NADPH, 0.2 ml cytosol and the balance pH 7.4 phosphate buffer for a final volume of 1 ml. The reaction was started by the addition of cytosol and incubated for 15 min at 37°C in a gyrating water-bath. The enzyme reaction was terminated by transferring tubes to an ice bath and extracting with 2 x 1.5 ml chloroform. The organic phase was evaporated to dryness under a stream of nitrogen. The residues were dissolved in 2 x 30 µl of methanol, applied to silica gel TLC plate prespotted with authentic haloperidol and RHAL, developed in a solvent system consisting of chloroform-methanol-acetic acid (80-15-5 by volume). The TLC bands were visualized under UV light and areas corresponding to haloperidol and its reduced metabolite were scraped into scintillation vials. After adding 0.5 ml methanol and 9 ml scintillation cocktail, the radioactivity was measured by a Beckman Liquid Scintillation Counter. Control incubations were performed in the absence of either cytosol or haloperidol. All assay and kinetic values are expressed as the means of duplicate experiments of two livers. Enzyme activity was determined by expressing the dpm in the RHAL region as a percentage of total radioactivity recovered from both bands on the TLC plate.

The kinetic parameters (K_m and V_{max}) for RHAL formation in human liver cytosol were determined by the method of least square, non-linear regression using the Enzfitter (Elsevier, Biosoft) curve-fitting PC program.

Inhibition studies

A fixed concentration of haloperidol (0.05 mM) and varying concentrations of NSAIDs (0.05-10 mM) were selected for inhibition studies. The incubations were carried out under the conditions described above. The inhibitors were dissolved in methanol and an aliquot added to the incubation medium. The final concentration of methanol was less than 4% and preliminary experiments indicated that under these conditions the solvent did not affect haloperidol reduction. Inhibition was expressed as the amount of RHAL formed in the inhibition mixture as a percentage of the RHAL formed in control incubation. IC_{50} values, the concentration of NSAIDs producing a 50% inhibition, were determined graphically. For the two NSAIDs with strong inhibition, further kinetic studies were performed to estimate apparent K_i values and determine the nature of inhibition by the method of Dixon and Cornish-Bowden.

Protein separation by gel filtration

Human liver cytosol (3 ml) was applied to a Sephadex G-100 column (2.8x100 cm) pre-equilibrated with 20 mM phosphate buffer pH 7.4 containing 0.5 mM EDTA and 2 mM dithiothreitol according to Kishimoto *et al.* /16/. The column was then eluted with the same buffer. The flow rate of the column was approximately 1 ml of buffer per min. The effluent was collected in glass tubes in 2 ml fractions and stored at 4°C. Protein concentration, reductase activity at a haloperidol concentration of 0.1 mM and inhibitor effect (indomethacin 0.5 mM) were determined as described above.

RESULTS

The concentrations of haloperidol and RHAL in biological samples in clinical studies have been measured by a variety of analytical techniques. Haloperidol levels determined by radioimmunoassays /17,

18/ were reported to be higher than those measured by HPLC and GC, probably due to cross-reactivity of antibody with haloperidol metabolites. Various HPLC assays, sensitive and reliable for the measurement of haloperidol and RHAL, exist; however, they often involve several extraction steps /9,19/. The GC method previously developed in this laboratory using a megabore column system and electron capture detector was sensitive and reliable /20/, but had a number of drawbacks. In an inhibition study, the limitations of an indirect measure such as the conventional spectrophotometric method of monitoring the oxidation of NADPH are evident.

Our radio-TLC method for simultaneous determination of haloperidol and RHAL is based on the extraction of the incubation mixture with chloroform and analysis of the extract by radio-TLC using a solvent system of chloroform, methanol and acetic acid. The recovery of radioactivity from the extracted incubation mixture is essentially complete when compared with a control sample similarly spiked but not extracted. The TLC analysis of the extract gives good separation of the two compounds. The bands are uniform and well resolved (Fig.1) with Rf values of 0.71 and 0.42 for haloperidol and RHAL, respectively. The method is simple, sensitive and allows a large number of samples to be analyzed simultaneously. The extraction procedure and radio-TLC analysis give good reproducible recoveries of radioactivity (>90%) as haloperidol and RHAL. It should be noted that the conversion of RHAL back to haloperidol, oxidative N-dealkylation and glucuronide conjugation of haloperidol occur only in the microsomal fraction of the liver. The reduction of haloperidol by human liver cytosol as determined by the radio-TLC method followed Michaelis-Menten kinetics (Fig. 2). The carbonyl reductase activity is expressed as nmol/min/mg protein and calculated as the percentage of total dpm recovered as RHAL. Kinetic parameters for reduced haloperidol formation in human liver are obtained using least square, non-linear regression method and K_m and V_{max} were 0.42 mM and 2.49 nmol/min/mg protein, respectively.

We examined the inhibitory effects of an indoleacetic acid (indomethacin) and arylpropionic acid analogues (ketoprofen, tiaprofenic acid, fenbufen, ibuprofen, l-naproxen and d-naproxen) on the activity of haloperidol reductase in human liver cytosol (Table 1). Among all the NSAIDs tested, the most potent inhibition was exhibited by indomethacin (IC_{50} 0.4 mM), ketoprofen (IC_{50} 0.9 mM) and tiaprofenic acid (IC_{50} 1.0 mM). The inhibition by ibuprofen and naproxen was

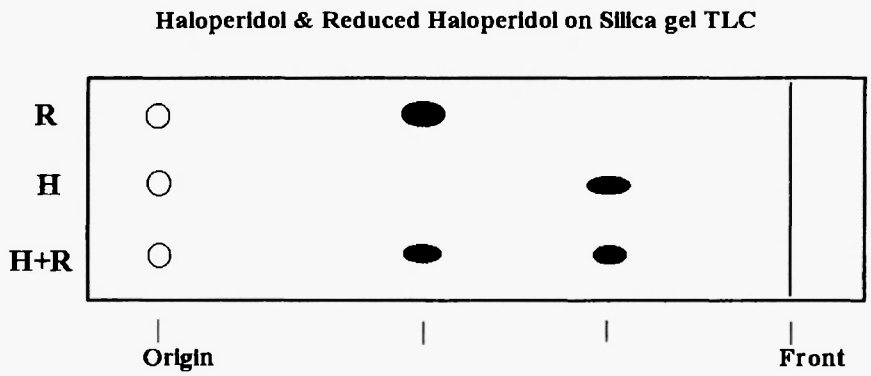

Fig. 1: TLC separation of a chloroform extract from human liver cytosol after incubation of haloperidol. The silica gel 60 F_{254} TLC plate under UV light showed haloperidol and RHAL as dark bands. Rf values were 0.71 and 0.42 for haloperidol and RHAL, respectively.

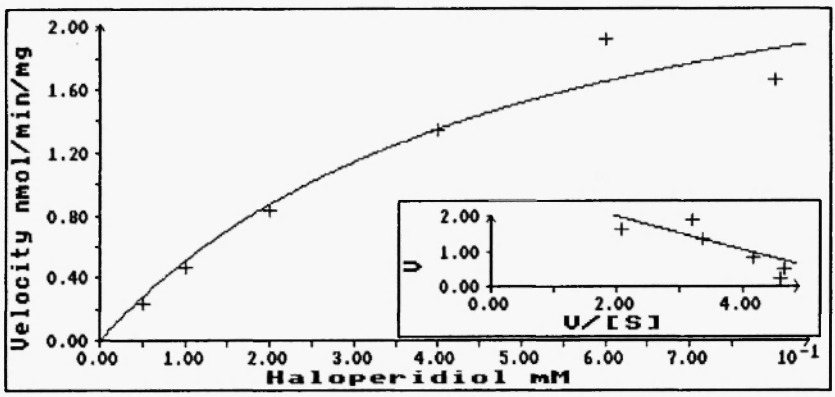

Fig. 2: Michaelis-Menten kinetics of haloperidol carbonyl reduction by human liver cytosol (K21). The inset shows Eadie-Hoffstee transformation.

weaker than by other NSAIDs. One pair of enantiomers, d-naproxen and l-naproxen, both inhibited haloperidol reductase activity, by 23% and 13%, respectively. Figure 3 shows the effect of various NSAIDs on the reduction of haloperidol by human liver cytosol. Applying the method of Penning and Talalay /13/ we observed a significant correlation ($r = 0.86$, $p < 0.05$) between the log of IC_{50} and the daily recommended dose required for anti-inflammatory effect (Fig. 4).

TABLE 1

Inhibition of haloperidol carbonyl reductase by NSAIDs

Drug	Daily dose (recommended) mg	mmol	IC 50 Values (μM) Carbonyl reductase (Haloperidol)	(log IC 50)	3α-HSD *** (9,10-PhQ)
Indomethacin	150 *	0.42	400	(2.6)	0.74
Ketoprofen	150 *	0.59	900	(2.9)	- -
Tiaprofenic acid	600 *	2.3	1000	(3.0)	- -
Fenbufen	900 **	3.5	1500	(3.2)	- -
Naproxen	750 *	3.2	6000	(3.8)	130
Ibuprofen	1200 *	5.8	5000	(3.7)	104

Haloperidol carbonyl reductase activity was measured in human liver cytosol.
* Values from Compendium of Pharmaceuticals and Specialities (Canada, 1994)
** Value from Martindale, The Extra Pharmacopoeia, 30th Ed. (1993)
*** Cited from Penning and Talalay, 1983 /13/. PhQ: Phenanthrenequinone

Since indomethacin and ketoprofen had the most potent inhibitory effect on the reduction of haloperidol, more comprehensive kinetic studies were undertaken with two fixed concentrations of haloperidol and varying concentrations of these compounds to estimate the apparent K_i and type of inhibition. The analysis of the data by the method of Dixon gave K_i values of 0.6 mM and 1.3 mM for indomethacin and ketoprofen, respectively. Cornish-Bowden plots revealed that indomethacin was a mixed-type inhibitor and ketoprofen was a non-competitive inhibitor of haloperidol reductase. The Dixon and

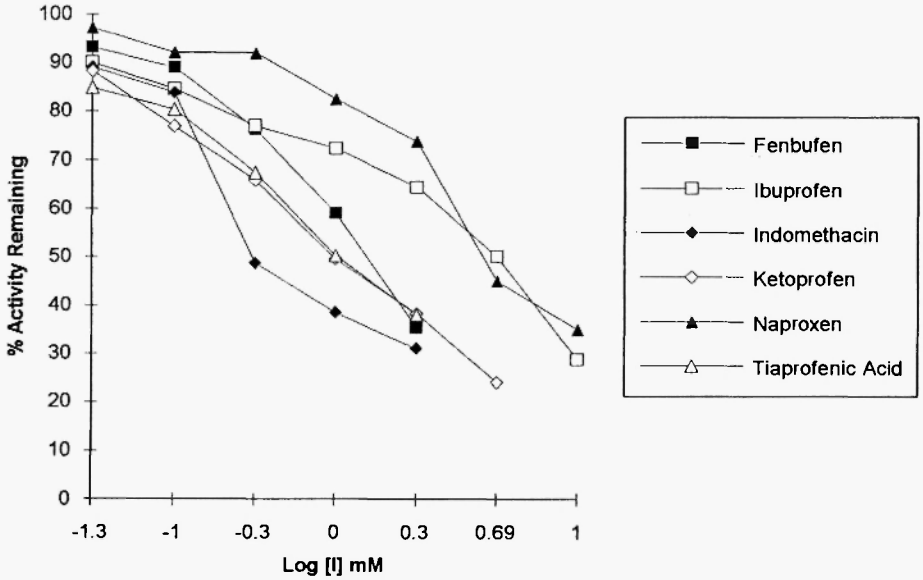

Fig. 3: Inhibitory effect of various anti-inflammatory drugs on the inhibition of haloperidol carbonyl reductase in human liver cytosol. IC_{50} values ranged from 0.4 to 6 mM, with indomethacin having the lowest and naproxen the highest IC_{50}.

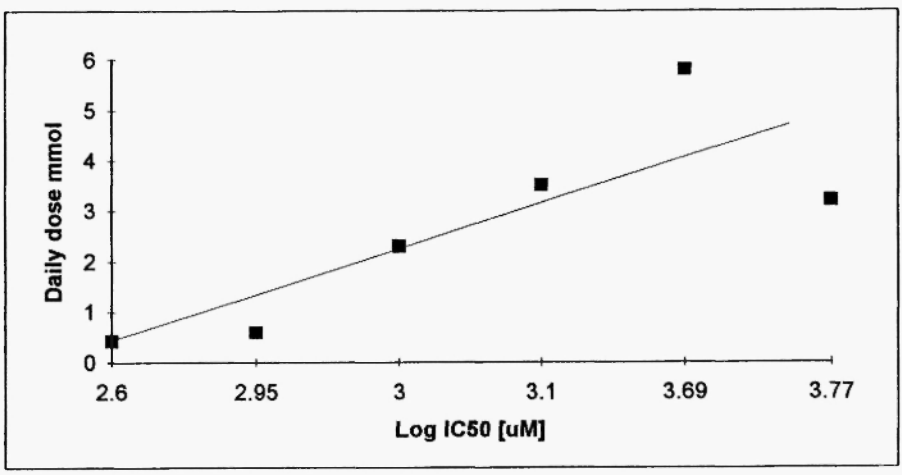

Fig. 4: Correlation between the log IC_{50} values and the daily human doses (recommended) for non-steroidal anti-inflammatory drugs. The line was drawn by linear regression and the correlation coefficient was 0.86 ($p<0.05$).

Cornish-Bowden plots for the inhibition of haloperidol reductase in human liver cytosol by indomethacin and ketoprofen are shown in Figures 5 and 6, respectively.

Protein separation

An attempt was made to separate, by Sephadex G-100, the protein responsible for haloperidol reduction in human liver cytosol. The fractions collected were measured for protein concentration, enzyme

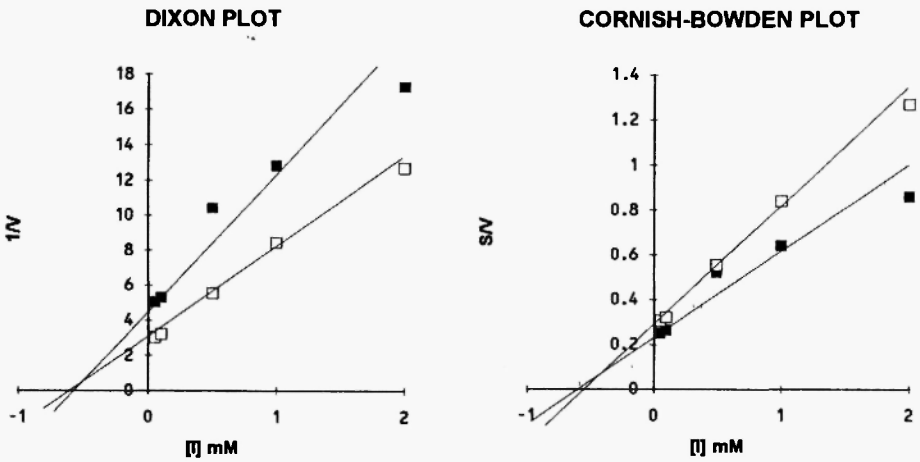

Fig. 5. Kinetic inhibition of haloperidol reduction by indomethacin. A: Dixon plot. B: Cornish-Bowden plot revealing mixed-type inhibition.

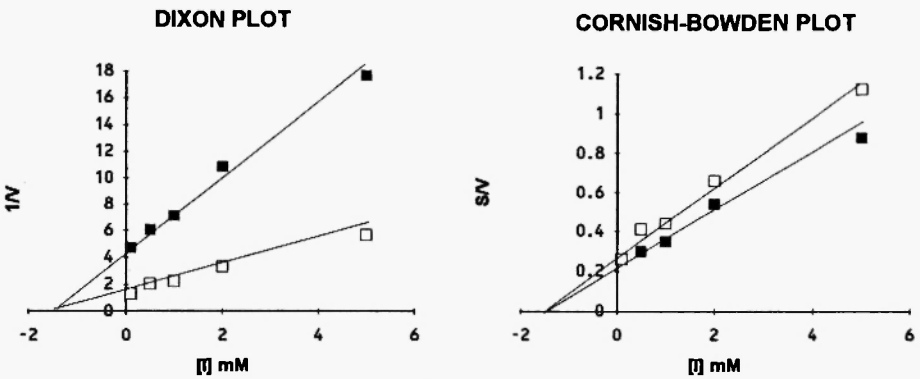

Fig. 6. Kinetic inhibition of haloperidol carbonyl reduction by ketoprofen. A: Dixon plot. B: Cornish-Bowden plot revealing non-competitive type inhibition.

activity and inhibitor effect. Carbonyl reductase activity was eluted as two peaks with molecular weight (M.W.) of 70,000 (fractions 21-37) and 32,000 (fractions 40-60). The haloperidol reductase activity was detected only in the second peak with M.W. of 32,000 and the activity of this peak was significantly decreased (60%) in the presence of indomethacin (0.5 mM). Figure 7 shows the elution profile of haloperidol reductase from Sephadex G-100 column chromatography. This fractionation of the cytosol enhanced haloperidol reductase activity 8.5-fold from 0.34 to 2.91 nmol/min/mg protein.

DISCUSSION

As anticipated, the results of this study show similarities in the enzymatic properties between haloperidol carbonyl reductase and 3α-HSD in the following points: the broad substrate specificity, pyridine nucleotide cofactor-dependence, inhibition by NSAIDs and a molecular weight (M.W.) of about 32,000. Penning and Talalay /13/, in their attempt to relate their findings to anti-inflammatory potency, correlated the log of IC_{50} values for the inhibition of 3α-HSD with maximal daily

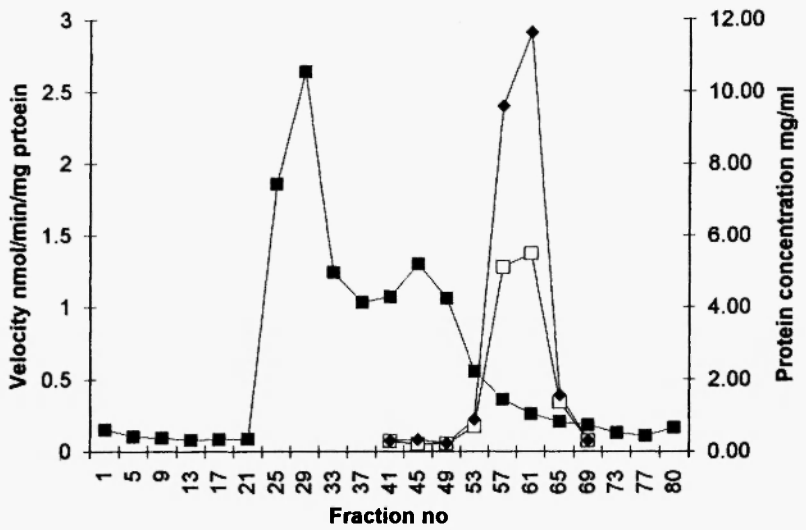

Fig. 7: Protein fractionation of human liver cytosol on Sephadex G-100. Selected fractions were examined for protein concentration (■) and haloperidol reductase activity (◆), and indomethacin effect (□).

doses recommended for anti-inflammatory therapy and showed that the pharmacological potency of NSAIDs can be predicted from the degree of inhibition of rat liver 3α-HSD. In our study, all NSAIDs tested inhibited the reduction of haloperidol by human liver cytosol and in direct proportion with their reported effectiveness in inhibiting cyclooxygenase. Indomethacin was the most potent inhibitor; ibuprofen and naproxen were weaker inhibitors of haloperidol reductase. We observed a correlation between the log of IC_{50} values and recommended daily anti-inflammatory doses for the six compounds examined.

Analysis of the inhibition data by Dixon plot and the Cornish-Bowden plot revealed mixed-type inhibition for indomethacin and non-competitive inhibition for ketoprofen which indicate that at least two sites are involved. Askonas and Penning /21/ reported that a free acid group was essential for this inhibition and the substitution of the carboxyl group with an alternative electronegative group yielded a compound without inhibitory effect. All NSAIDs that we examined for inhibition of haloperidol carbonyl reductase were carboxylic acid derivatives, the most commonly used anti-inflammatory agents.

Although several enzyme systems are inhibited by NSAIDs, the inhibition of cyclooxgenase is the primary site of action of these compounds. Our results indicate that haloperidol carbonyl reductase may be an additional target for these agents.

The IC_{50} and K_i values for the inhibition of haloperidol reduction in human liver cytosol by these compounds, though appropriate to our observed K_m and V_{max}, were of higher magnitude (mM) than those reported (Table 1) for the inhibition of 3α-HSD (μM) /13/. The difference may be explained by the fact that we used crude preparations of human liver cytosol and performed the incubation at physiological pH 7.4, whereas they used purified rat liver 3α-HSD at a non-physiological but optimal pH of 6.0 and showed that the inhibitory potency of anti-inflammatory carboxylic acids is pH dependent and falls sharply as the pH of the incubation medium is increased from 6.0 to 9.0.

It is, of course, difficult to translate the ***in vitro*** to ***in vivo*** situation to fully predict the impact of an altered rate of haloperidol carbonyl reduction. Evaluation of drug-drug interaction ***in vitro*** at a therapeutic plasma concentration may not reflect the true ***in vivo*** drug concentration at the enzyme site. In fact, in one postmortem toxicological analysis of a patient who died from natural causes, the blood concentration of haloperidol was 0.6 mg/l, and distribution of haloperidol and RHAL

was: bile, 0.4 and 0.5 mg/l; kidney, 0.7 and 1.3 mg/kg; liver, 5.0 and 13 mg/kg; and urine, 0.4 and 2.3 mg/l /22/.

Many drugs undergo extensive first pass metabolism and/or biliary excretion. In fact, enzymes will be subjected to much higher drug concentrations during first pass through the liver after oral drug administration. Large interindividual variability exists in the disposition of haloperidol such that plasma concentrations may vary by ten-fold between patients receiving the same dose /23/. The concentration of haloperidol in blood is dependent on a number of factors including the relative velocities of concurrent metabolic pathways, protein binding, body tissue composition and renal excretion. Conversion to RHAL may vary when haloperidol is coadministered with drugs which have higher affinity for or inhibit the carbonyl reductase.

In addition, NSAIDs are substrates of UDP-glucuronosyl transferases and may compete with haloperidol and RHAL glucuronidation which account for 50-60% of biotransformation of haloperidol /24/. This may increase the amount of haloperidol available to the oxidative N-dealkylation pathway leading to the formation of toxic metabolites and the possible development of some of the extrapyramidal side effects. Indomethacin and other prostaglandin synthesis inhibitors reduce renal blood flow and excretion of sodium and water /25/. They also can decrease renal elimination of other drugs, particularly in patients on high doses, chronic administration or with impaired renal function.

Haloperidol is commonly prescribed for the management of psychosis in the elderly and chronically hospitalized patients. The elderly are also frequently users of NSAID drugs on a chronic basis in conjunction with other medications. Since the incidence of severe, adverse effects increases with age and number of drugs taken, the elderly are particularly at risk. On average, the elderly have decreased body water content, increased body fat, decreased albumin concentration, decreased hepatic metabolism, and decreased renal function, all of which can potentially influence the disposition of drugs and ultimately the pharmacological response and toxicity. Haloperidol is a lipophilic drug, has a long elimination half-life and may accumulate in the increased adipose tissue and put the patients at increased risk of developing extrapyramidal side effects. Serum albumin concentrations decline in old age so that drugs like haloperidol, which is highly bound to albumin, may have an elevated 'toxic', free fraction. Diminished renal function in the elderly and the effect of NSAID drugs on renal

blood flow can significantly alter the elimination of haloperidol and those metabolites which are excreted renally.

From a clinical viewpoint, the inhibition of the carbonyl reduction of haloperidol may have significant consequences in the pharmacology and toxicology of haloperidol. Our findings suggest that if an anti-inflammatory drug has to be combined with haloperidol, ibuprofen and naproxen would be safer on the basis of their IC_{50} values. However, complementary clinical studies should be conducted to corroborate these *in vitro* results. Finally, this study is the first demonstrating NSAID inhibition of haloperidol carbonyl reduction in human liver cytosol and will provide useful information for a better understanding of haloperidol carbonyl reductase as a drug metabolizing enzyme.

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