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INHIBITORY EFFECT OF DRUGS WITH A KETONE GROUP ON REDUCTION OF ACETOHEXAMIDE CATALYZED BY CARBONYL REDUCTASE FROM RABBIT KIDNEY

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The reduction of acetohexamide catalyzed by carbonyl reductase from rabbit kidney was inhibited by befunolol, moperone, levobunolol, daunorubicin and loxoprofen, which have a ketone group within their chemical structures and are substrates for the enzyme. A significant correlation was observed between the common logarithm of V_{max}/K_m values of the enzyme for befunolol, moperone, levobunolol and daunorubicin and the percentage inhibition of the enzyme, confirming that these drugs are competitive substrates of the enzyme with respect to acetohexamide. However, the plot for loxoprofen, a nonsteroidal anti-inflammatory drug with a ketone group, was apparently distant from the regression line obtained. Although nonsteroidal anti-inflammatory drugs with a ketone group such as suprofen and fenbufen were not reduced by the enzyme, they strongly inhibited the reduction of acetohexamide catalyzed by the enzyme.

Keywords: Carbonyl reductase; acetohexamide reduction; inhibition; drugs with a ketone group; competitive substrates; nonsteroidal anti-inflammatory drugs.

INTRODUCTION

Carbonyl reductase (EC 1.1.1.184) is an important enzyme that catalyzes NADPH-dependent reduction of drugs having a ketone group within their chemical structures.[1–5] The enzyme is widely distributed in various tissues throughout the mammalian species.[6] The kidney, especially, as well as the liver, exhibits a high

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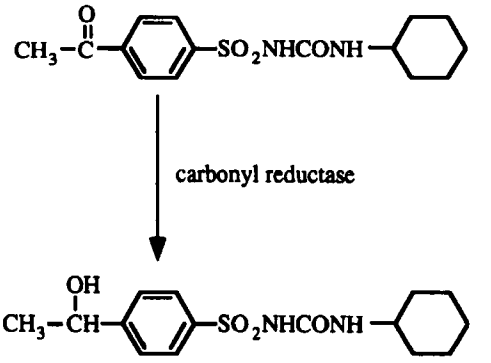


FIGURE 1 Reduction of acetohexamide catalyzed by carbonyl reductase from rabbit kidney.

carbonyl reductase activity.[6] We have recently purified a carbonyl reductase from the cytosolic fraction of rabbit kidney, by using acetohexamide, an oral antidiabetic drug, as a substrate.[7] The purified enzyme efficiently reduced many drugs with a ketone group such as daunorubicin and haloperidol including acetohexamide (Figure 1).[7] However, the enzyme had little or no ability to reduce nonsteroidal anti-inflammatory drugs with a ketone group, e.g., ketoprofen and fenbufen.[7] In the present study, we examined the inhibitory effect of various drugs with a ketone group on the reduction of acetohexamide catalyzed by carbonyl reductase from rabbit kidney.

MATERIALS AND METHODS

Materials

Carbonyl reductase was purified from the cytosolic fraction of rabbit kidney by using acetohexamide as a substrate.[7] Acetohexamide was supplied by Shionogi Co. (Osaka, Japan). Befunolol (Kaken Pharmaceutical Co., Tokyo, Japan), levobunolol (Warner-Lambert, Ann Arbor, USA), daunorubicin (Meiji Seika, Tokyo, Japan), loxoprofen (Sankyo Co., Tokyo, Japan), ketoprofen (Hisamitsu Pharmaceutical Co., Saga, Japan), suprofen (Nippon Chemiphar, Tokyo, Japan) and fenbufen (Nippon Lederle, Tokyo, Japan) were obtained from the manufacturers. Moperone was donated by Dr. T. Aimoto (Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan). NADPH was purchased from Oriental Yeast Co. (Tokyo, Japan). All other chemicals were of reagent grade.

Synthesis of Hydroxyhexamide and 4-Acetylpyridine Analogues

Hydroxyhexamide was synthesized from acetohexamide according to the method of Girgis-Takla and Chroneos.[8] 4-Acetylpyridine analogues were synthesized as described previously.[7] The structures of these compounds were confirmed by elemental analysis and spectroscopic methods.

Assay of Enzyme Activity

Enzyme activity was assayed according to the method of Takagishi *et al.*[9] The reaction mixture in a final volume of 1.4 ml consisted of 0.1 M sodium potassium phosphate buffer (pH 6.0), 0.25 mM NADPH, 1.0 mM acetohexamide and the enzyme. Drugs with a ketone group and 4-acetylpyridine analogues used as inhibitors were added to the reaction mixture at final concentrations of 1.0 and 0.2 mM, respectively. The mixture was incubated at 30°C for 10 min and the reaction was stopped by adding 0.5 ml of 1.0 N HCl. The mixture was extracted with 5.0 ml of benzene-ethyl acetate (1:1, v/v) containing fenbufen as the internal standard. The organic layer (4.0 ml) was removed and evaporated *in vacuo*. The residue was dissolved in 0.3 ml of acetonitrile and subjected to high-performance liquid chromatography to measure hydroxyhexamide produced in the reaction mixture. Protein concentration was determined by the method of Lowry *et al.*[10] using bovine serum albumin as standard. The percent inhibition was derived by taking the enzyme activity in the absence of inhibitors (control) as 100 percent activity.

RESULTS

Inhibition by Drugs with a Ketone Group

The inhibitory effects of various drugs with a ketone group on carbonyl reductase from rabbit kidney were examined. Befunolol, moperone, levobunolol, daunorubicin and loxoprofen, which have a ketone group within their chemical structures and are substrates for the enzyme,[7] were used as inhibitors (Figure 2). The reduction of acetohexamide catalyzed by the enzyme was found to be inhibited by these ketone drugs at a concentration of 1.0 mM. Figure 3 shows the relationship between the common logarithm of V_{max}/K_m values of the enzyme for these drugs and the percentage inhibition of the enzyme by these drugs. A significant regression line was obtained from the plots for befunolol, moperone, levobunolol and daunorubicin ($r = 0.980$, $P < 0.05$). However, the plot for loxoprofen, a nonsteroidal anti-inflammatory drug, was apparently distant from the regression line.

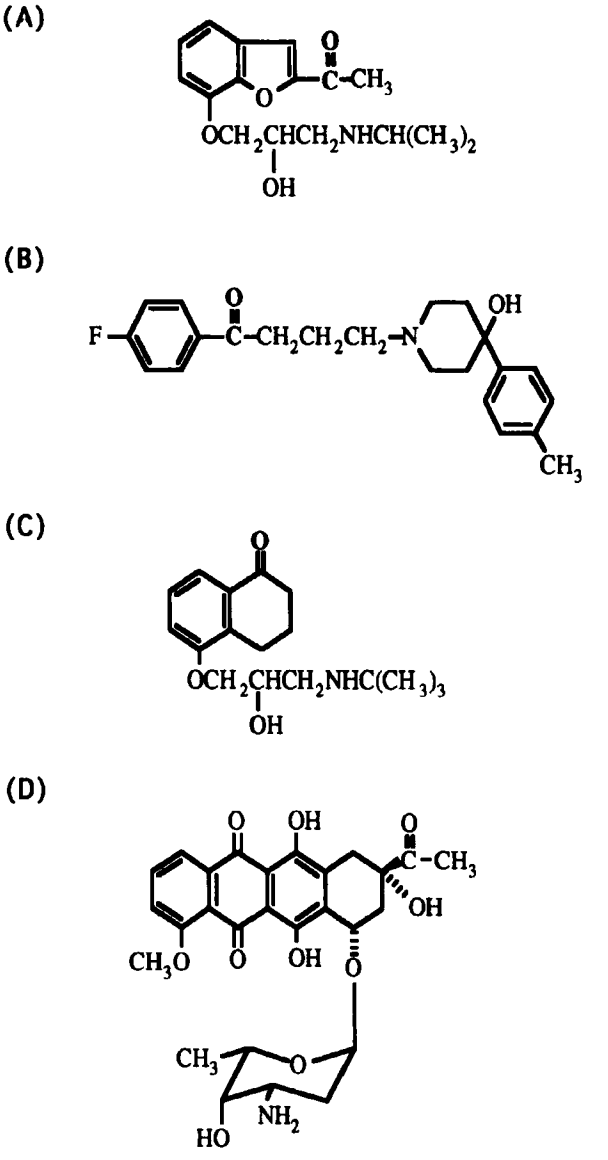


FIGURE 2 Chemical structures of befunolol (A), moperone (B), levobunolol (C) and daunorubicin (D). The chemical structure of loxoprofen is shown in Table I.

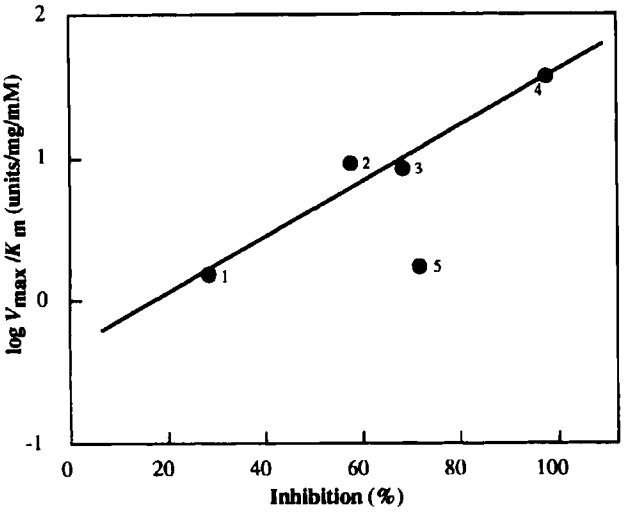


FIGURE 3 The relationship between the common logarithm of V_{max}/K_m values of carbonyl reductase from rabbit kidney and the percentage inhibition of the enzyme for befunolol, moperone, levobunolol, daunorubicin and loxoprofen. The V_{max}/K_m values are data from reference 7 and the values for inhibition (%) are the mean of three experiments. The concentration of inhibitors (drugs with a ketone group) was 1.0 mM. 1, befunolol; 2, moperone; 3, levobunolol; 4, daunorubicin; 5 loxoprofen.

Inhibition by 4-Acetylpyridine and its Analogues

It has been reported that 4-acetylpyridine and its analogues substituted in the acyl group with a straight-chain alkyl group instead of the methyl group are effectively reduced by carbonyl reductase from rabbit kidney, and that the V_{max}/K_m values of the enzyme for 4-acetylpyridine analogues are much larger than that for acetohexamide.[7] Furthermore, 4-acetylpyridine has been demonstrated to be a competitive inhibitor against acetohexamide.[7] Thus, the inhibition of the enzyme by 4-acetylpyridine and its analogues was examined. These compounds, as expected, inhibited the reduction of acetohexamide catalyzed by carbonyl reductase from rabbit kidney. The concentration of these inhibitors added was 0.2 mM because of its strong inhibitory effect. Figure 4 shows the relationship between the common logarithm of V_{max}/K_m values of the enzyme for 4-acetylpyridine and its analogues and the percentage inhibition of the enzyme by these compounds. A significant regression line was obtained from the plots for 4-acetylpyridine and its analogues ($r = 0.992$, $P < 0.001$).

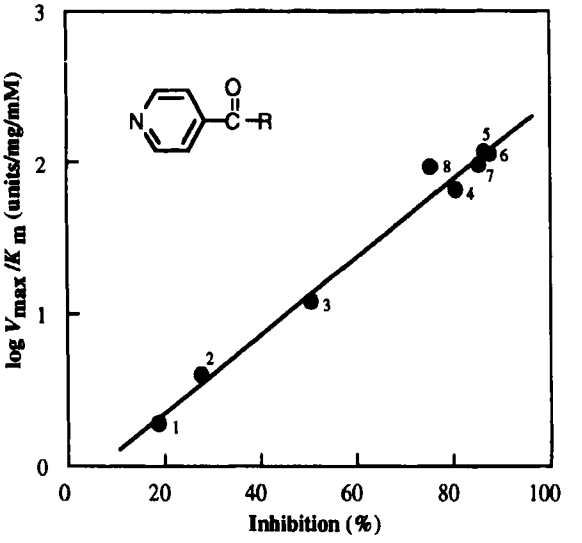


FIGURE 4 The relationship between the common logarithm of V_{max}/K_m values of carboyl reductase from rabbit kidney and the percentage inhibition of the enzyme for 4-acetylpyridine and its analogues. The V_{max}/K_m values are data from reference 7 and the values for inhibition (%) are the mean of three experiments. The concentration of inhibitors (4-acetylpyridine and its analogues) was 0.2 mM. The analogues substituted with a straight-chain alkyl group in the acyl function used in this experiment were: 1, methyl (4-acetylpyridine); 2, ethyl; 3, *n*-propyl; 4, *n*-butyl; 5, *n*-pentyl; 6, *n*-hexyl; 7, *n*-heptyl; 8, *n*-octyl.

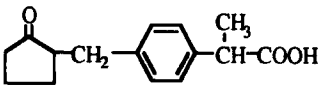
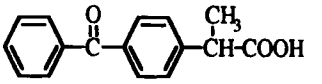
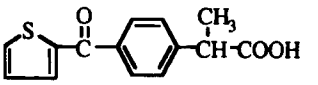
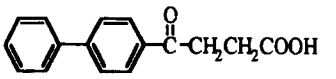
Inhibition by Nonsteroidal Anti-inflammatory Drugs with a Ketone Group

Table I summarizes the V_{max} and K_m values of carbonyl reductase from rabbit kidney for loxoprofen, ketoprofen, suprofen and fenbufen and the percentage inhibition of the enzyme by these drugs. The enzyme had little or no ability to reduce these nonsteroidal anti-inflammatory drugs with a ketone group, except loxoprofen. However, the nonsteroidal anti-inflammatory drugs strongly inhibited the reduction of acetohexamide catalyzed by the enzyme.

DISCUSSION

We have recently demonstrated that carbonyl reductase from rabbit kidney has the ability to reduce many drugs with a ketone group, and that the V_{max}/K_m values of the enzyme for moperone, levobunolol and daunorubicin are larger than that for

TABLE I Catalytic properties of carbonyl reductase from rabbit kidney for nonsteroidal anti-inflammatory drugs with a ketone group and inhibition of the enzyme by these drugs.

Drug	*Chemical Structure*	K_m[a] (mM)	V_{max}[a] (units/mg)	*Inhibition*[b] (%)
Loxoprofen		0.51	0.94	77 ± 3
Ketoprofen		—	(0.05)	80 ± 3
Suprofen		—	(0)	62 ± 2
Fenbufen		—	(0)	90 ± 4

[a]The values for K_m and V_{max} are from reference 7. The values in parentheses indicate the activity with 1.0 mM substrate (nonsteroidal anti-inflammatory drug).

[b]The concentrations of the substrate acetohexamide and inhibitor were 1.0 mM. The values of inhibition (%) are the mean ± S.D. of three experiments.

acetohexamide.[7] Thus, these drugs with a ketone group are suggested to exhibit substrate inhibition against acetohexamide reduction. Furthermore, befunolol has been reported to inhibit competitively the enzyme,[11] although the V_{max}/K_m value of the enzyme for befunolol is smaller than that for acetohexamide.[7] The present study provides new evidence that befunolol, moperone, levobunolol and daunorubicin are competitive substrates of the enzyme with respect to acetohexamide, on the basis of a significant correlation observed between the common logarithm of V_{max}/K_m values of the enzyme for these drugs and the percentage inhibition of the enzyme. This is also supported from the relationship between the common logarithm of V_{max}/K_m values of the enzyme for 4-acelylpyridine and its analogues and the percentage inhibition of the enyme.

Interestingly, the plot for loxoprofen was apparently distant from the regression line of the plots for befunolol, moperone, levobunolol and daunorubicin. It should be noted that loxoprofen, unlike befunolol, moperone, levobunolol and daunorubicin, is one of nonsteroidal anti-inflammatory drugs. Our previous work has shown that nonsteroidal anti-inflammatory drugs cause the inhibition

of carbonyl reductase from rabbit kidney by competing with NADPH in its coenzyme binding domain.[11–13] In addition, the possibility that nonsteroidal anti-inflammatory drugs interact with or near one essential arginine residue in coenzyme-binding domain of the enzyme has been reported.[14] In the present study, we demonstrate that the enzyme has little or no ability to reduce ketoprofen, suprofen and fenbufen. These nonsteroidal anti-inflammatory drugs with a ketone group may strongly bind to the coenzyme-binding domain of the enzyme and interfere with the binding of NADPH to the enzyme. We have also shown that the reduction of acetohexamide catalyzed by carbonyl reductase from rabbit kidney follows an ordered Bi Bi mechanism,[11] in which NADPH binds to the enzyme first and NADP leaves from the enzyme last.[15,16] This means that the enzyme reaction which follows an ordered Bi Bi mechanism cannot further proceed in the presence of nonsteroidal anti-inflammatory drugs with a ketone group, and consequently that these nonsteroidal anti-inflammatory drugs cannot be reduced by the enzyme. However, there is no direct evidence that these nonsteroidal anti-inflammatory drugs with a ketone group interfere with the binding of NADPH to the enzymes and further studies are necessary to elucidate why they are ineffective as substrates of the enzyme.

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