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SHORT COMMUNICATIONS

Interindividual variability of carbonyl reductase levels in human livers

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Abstract—Interindividual variability of carbonyl reductase levels in human livers (N = 11) was examined by measuring reductase activity toward various substrates and by western blot analysis using anti-rat ovarian carbonyl reductase CR2 antibody. The carbonyl reductase activity toward *p*-nitrobenzaldehyde (PNBA) (58.1 ± 5.4 nmol/mg protein/min, mean ± SE) was highest among the substrates examined, followed by 4-benzoylpyridine (4BP) (14.4 ± 2.0 nmol/mg protein/min) and *p*-nitroacetophenone (PNAP) (2.00 ± 0.37 nmol/mg protein/min). The reductase activity (6.33 ± 0.56 pmol/mg protein/min) toward 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (15KD-$PGF_{2\alpha}$), which is a diagnostic substrate for rat ovarian carbonyl reductases, was relatively high compared to that in other species. Western blot analysis revealed that each human liver contained several immunoreactive proteins to anti-CR2 antibody. The activities toward 15KD-$PGF_{2\alpha}$ ($r = 0.85$, $P < 0.01$) and 4BP ($r = 0.84$, $P < 0.01$), but not PNBA ($r = 0.53$, not significant) or PNAP ($r = 0.52$, not significant), were closely correlated with the relative amounts of the high molecular weight immunoreactive proteins determined with a densitometer. Thus, the major carbonyl reductases in human liver are similar to those of rat ovarian enzymes.

Carbonyl reductase (EC 1.1.1.184) is a cytosolic, monomeric oxidoreductase that catalyses the NADPH-dependent reduction of a large number of biologically and pharmacologically important endogenous and xenobiotic carbonyl compounds, such as prostaglandins (PGs*), steroids, quinones and anthracycline antibiotics [1–3]. Based on its broad substrate specificity, the enzyme is distinguished from alcohol dehydrogenase (EC 1.1.1.1), aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC 1.1.1.21). Multiple forms of the enzyme differing in size and charge have been isolated from various sources and even from the same tissue [1–3].

Recently, we have purified from rat ovary two isoforms designated as carbonyl reductases CR1 and CR2, which are very similar to each other in terms of substrate specificity and immunological cross-reactivity, and we demonstrated that the isoforms were specific for PGs rather than steroids, i.e. they were able to catalyse the reduction of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (15KD-$PGF_{2\alpha}$) to 13,14-dihydro-$PGF_{2\alpha}$, as well as the interconversion of PGE_2 to $PGF_{2\alpha}$ [4]. Western blot analysis using anti-CR2 antibody revealed that immunoreactive proteins, which migrated to the same position as CR1 and CR2, were present in various tissues [4, 5]. The immunoreactive proteins were found only in tissues which exhibited detectable 15KD-$PGF_{2\alpha}$ reductase activity [4, 5], suggesting that 15KD-$PGF_{2\alpha}$ may be a diagnostic substrate for isoforms of carbonyl reductase from rat ovary.

Human liver possesses considerable carbonyl reductase activity toward 15KD-$PGF_{2\alpha}$, as well as toward *p*-nitroacetophenone (PNAP) and *p*-nitrobenzaldehyde (PNBA), which are non-specific substrates for carbonyl reductases [5]. In the present study, we investigated interindividual variability of carbonyl reductase levels in human livers by measurement of 15KD-$PGF_{2\alpha}$ reductase activity and by western blot analysis using anti-CR2 antibody, and examined the correlation between the activities and the immunoreactive proteins.

* Abbreviations: PG, prostaglandin; 15KD-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$; PNAP, *p*-nitroacetophenone; PNBA, *p*-nitrobenzaldehyde; 4BP, 4-benzoylpyridine; HMW, high molecular weight components; LMW, low molecular weight components.

Materials and Methods

Materials. Portions of human livers were obtained from 11 cadavers during medico-legal autopsy and stored at −70° until use. Each liver was homogenized with 3 vol. (v/w) of ice-cold 1.15% KCl. Cytosolic fractions were prepared, as described previously for laboratory animals [4, 5]. 15KD-$PGF_{2\alpha}$ was obtained from Upjohn Pharmaceuticals Ltd (Kalamazoo, MI, U.S.A.) and [5,6,8,9,11,12,14-^{3}H]15KD-$PGF_{2\alpha}$ (sp. act. 80 Ci/mmol) from Amersham International (Amersham, U.K.). 13,14-Dihydro-$PGF_{2\alpha}$ was kindly supplied by the Ono Pharmaceutical Co. (Osaka, Japan). Other chemicals of reagent grade were products of either Wako Pure Chemical Industries (Osaka, Japan) or the Japan Bio-Rad Lab. Co. (Tokyo, Japan).

Enzyme assay and protein determination. Two different techniques were employed to measure reductase activities. One involved radiochemical measurement of [^{3}H]13,14-dihydro-$PGF_{2\alpha}$ formed by the enzymatic reduction of [^{3}H]-15KD-$PGF_{2\alpha}$ as described by Inazu *et al.* [6]. The other involved spectrophotometric measurement of the oxidation rate of NADPH at 340 nm and 37° [4, 5]. The standard assay mixture consisted of 100 mM phosphate buffer (pH 6.5), 0.1 mM NADPH, enzyme and either PNAP, PNBA or 4-benzoylpyridine (4BP) at 1.0 mM in a total volume of 1.0 mL. The reaction was initiated by addition of cofactor to the assay mixture. Blanks without substrate or enzyme were routinely included. Water-insoluble substrates were dissolved in ethanol and the final concentration of ethanol in the assay mixture did not exceed 2%, a concentration which had no effect on the catalytic activity of the enzymes. One unit was defined as the amount of enzyme that catalysed the oxidation of one micromole of cofactor at 37°. Protein concentration was determined by the method of Lowry *et al.* [7].

Western blot analysis. Western blot of human liver cytosolic proteins (10 μg) was performed by a modification of the method of Towbin *et al.* [8] using polyclonal antiserum raised against the purified rat ovarian carbonyl reductase CR2 as described previously [4, 5]. The relative intensity of each band was calculated as a percentage of that of sample 1, based on densitometric measurements (λ_s 400 nm, λ_r 650 nm).

Statistical analysis. Results were correlated using linear regression analysis. Student's *t*-test was used and the cor-

Table 1. Carbonyl reductase activities and relative contents in human livers

Specimen (age, sex)	Reductase activity				Content of immunoreactive proteins		
	15KD-$PGF_{2\alpha}$ (pmol/mg protein/15 min)	PNAP (nmol/mg protein/min)	PNBA (nmol/mg protein/min)	4BP (nmol/mg protein/min)	Total	HMW (Relative intensity)	LMW (Relative intensity)
1 (47, M)	8.36	3.25	68.5	22.9	100	74	26
2 (36, M)	2.97	1.06	37.0	5.9	37	27	10
3 (40, M)	6.64	0.83	51.6	19.4	68	48	20
4 (40, M)	5.22	1.76	51.9	14.2	49	28	21
5 (69, M)	6.01	1.72	32.5	11.8	73	54	19
6 (22, M)	7.80	4.73	64.7	19.2	91	46	44
7 (50, M)	8.01	2.31	76.3	20.6	107	76	31
8 (51, F)	5.88	2.06	77.2	13.7	96	37	59
9 (47, M)	5.02	0.73	45.5	4.9	53	23	30
10 (51, M)	9.14	2.73	87.9	20.8	89	NS	NS
11 (55, M)	4.52	0.68	46.5	5.5	45	19	27
Mean ± SE	6.33 ± 0.56	2.00 ± 0.37	58.1 ± 5.4	14.4 ± 2.0			

NS, not separated.

The detection limit for reductase activity toward 15KD-$PGF_{2\alpha}$ is 0.75 pmol/mg protein/15 min and that toward PNAP, PNBA and 4BP is 0.02 nmol/mg protein/min.

Table 2. Correlation coefficients

	15KD-$PGF_{2\alpha}$	PNAP	PNBA	4BP	CR (HMW)	CR (LMW)
CR (total)	0.86‡	0.70*	0.78†	0.81†	0.83†	0.60
15KD-$PGF_{2\alpha}$		0.70*	0.77†	0.90‡	0.85†	0.38
PNAP			0.57	0.69*	0.53	0.47
PNBA				0.71*	0.52	0.73*
4BP					0.84†	0.23
CR (HMW)						0.06

Statistically significant; * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.
CR, carbonyl reductase.

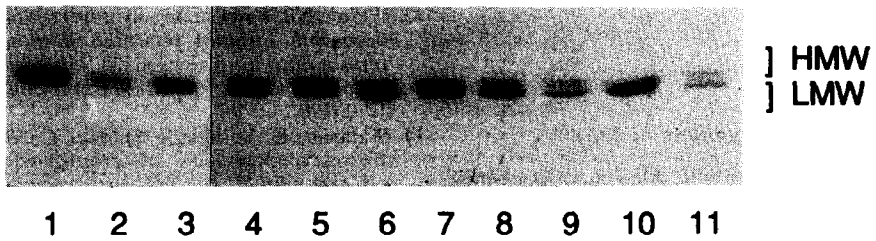

Fig. 1. Interindividual variability of human liver immunoreactive carbonyl reductases by western blot analysis. Human liver cytosolic proteins (10 μg) were electrophoresed on 10% polyacrylamide gel containing SDS, transferred to nitrocellulose membrane and then immunostained with anti-CR2 antiserum as described in Materials and Methods.

relations were considered to be statistically significant at $P < 0.05$.

Results and Discussion

Figure 1 shows the result of western blot analysis using anti-CR2 antibody. The human liver cytosolic proteins contained several immunoreactive proteins having similar electrophoretic mobility to those of the ovarian carbonyl reductases we examined previously [5]. Relative intensity of each band was variable in individual human livers. In samples 1–7, high molecular weight bands were more intense than low molecular weight ones, whereas in samples 8–11, low molecular weight bands were more intense. The results of the cytosolic carbonyl reductase activity determinations and the relative intensity determined by densitometric scanning of western blots in Fig. 1 are summarized in Table 1. As we could not separate several bands in each lane by densitometric scanning, for convenience, we designated higher molecular weight components, low molecular weight components and the sum of them as HMW, LMW and total, respectively. The carbonyl reductase activity toward PNBA (58.1 ± 5.4 nmol/mg protein/min, mean ± SE) was the highest among those toward the substrates examined, followed by 4BP (14.4 ± 2.0 nmol/mg protein/min) and PNAP (2.00 ± 0.37 nmol/mg protein/min). Carbonyl reductase activity toward 15KD-$PGF_{2\alpha}$ was higher (6.33 ± 0.56 pmol/mg protein/min) than in livers of other species such as mouse, hamster, guinea pig, rabbit, cow, dog and monkey [5]. We found that total immunoreactive proteins to anti-CR2 antibody were correlated significantly with activities toward both 15KD-$PGF_{2\alpha}$ and 4BP, but not PNBA or PNAP (Table 2). Further, the correlation coefficients suggested that the former activities may be mostly due to HMW, but not LMW. That is, the immunoreactive HMW recognized by anti-CR2 antibody may contribute predominantly to the carbonyl reductase activities toward 15KD-$PGF_{2\alpha}$ and 4BP. In contrast, no significant correlation of immunoreactive HMW with the enzyme activities toward either PNBA or PNAP was found. Thus, it seems that carbonyl reductases having similar characteristics to those of the rat ovarian enzyme exist in human liver.

In general, the carbonyl reductases have been classified into two groups according to their substrate specificity. One is a group that shows higher affinity for steroids and is reported to be identical with 3α- or 3(17)β-hydroxysteroid dehydrogenases, which have been isolated mostly from hepatic tissues [9–12]. The other is a group having higher affinity for PGs and functions as 15-hydroxyprostaglandin dehydrogenase, PGE_2 9-ketoreductase or PGD_2 11-ketoreductase [4, 13–18]. The carbonyl reductases from rat ovary belong to the latter group [4, 6]. These enzymes are considered to play important roles in the metabolism of both endogenous and xenobiotic carbonyl compounds. When we examined species differences of rat hepatic 3α-hydroxysteroid dehydrogenase in various animals, anti-3α-hydroxysteroid dehydrogenase cross-reacted with proteins in hepatic cytosols of mouse, hamster, guinea pig and rabbit, but not dog, pig, cow, monkey and human, which all possess reductase activities toward PNBA and PNAP [5]. In contrast, the immunoreactive proteins to anti-CR2 antibody were found in livers from all of the above species, all of which exhibit distinct hepatic 15KD-$PGF_{2\alpha}$ reductase activity [5]. Thus, similar enzyme forms to those purified from rat ovary are present in human liver, and appear to be preserved and functionally important in all species examined. The purified carbonyl reductases from both the testis [18] and liver [19] in humans show charge heterogeneity. In this study, there were no great interindividual differences in the carbonyl reductase activities of human livers (Table 1). The heterogeneity of carbonyl reductases in human liver may arise from small structural

modifications, resulting in differences in molecular weight and isoelectric point.

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