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STEREOSELECTIVITY IN THE HYDROLYSIS OF SYNTHETIC ESTERS BY CULTURED CANCER CELLS AND NORMAL TISSUE EXTRACTS OF RAT

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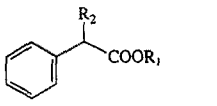
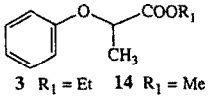
Abstract. Racemic 2-substituted phenylacetic acid esters and the related esters were hydrolyzed in different stereoselectivity by rat cancer cells and the corresponding rat normal tissue extracts. The stereoselectivity was dependent on the structure of the α-substituents.

Esterification is widely used to convert drugs to the prodrug form with the aim of improving the pharmacological properties, *e.g.*, stability and bioavailability.[1] The prodrugs are usually inactive or less active than the parent drugs, but its activity is recovered by hydrolyzing the esters nonenzymically[1c,f] or enzymically[1b,c,m] in the cells. The enzymic process is very interesting because enzyme specificity may be diverse with specific tissues and cells,[1c,m,n] thus leading to a possibility of dynamic drug targeting. This is an attractive strategy for anticancer chemotherapy. Some studies have been reported on the difference between the esterases of cancer and normal cells in their specific activity and substrate specificity,[2] but little attention has been paid to stereoselectivity of the enzymes.[3] This paper is the first to report that the stereoselectivity in enzymic hydrolysis of synthetic chiral esters is different between the cancer cells and the related normal cells.

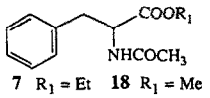
At first, we treated the racemic phenylacetate derivatives (**1** ~ **11**) with mouse sarcoma S180 cells and investigated the stereochemisty of the formed carboxylic acids.[4,5] Table 1 is the summary of their absolute configuration, enatiomeric purity and conversion rate. Of the hydrophobic ester (**1**), the *R* enantiomer was predominantly hydrolyzed by the cancer cells. This remarkably high stereoselectivity decreased with the α-substituents becoming polar as found with esters **2**, **3**,[6] and **4**. The stereoselectivity was reversed to *S* for the esters having an α-amido group (**5** ~ **7**) . These facts indicate that the stereoselectivity in the hydrolysis by S180 cells is dependent on the structure of α–substituents of the acyl group. The alcohol moiety did not influence on the stereoselectivity in the case of 2-phenylpropanoates **1**, **10**, and **11**. Ethyl 2-chloro-2-phenyl-



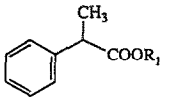
R_1 = Et		R_1 = Me
1	$R_2 = CH_3$	12
2	$R_2 = OCH_3$	13
4	$R_2 = OH$	15
5	$R_2 = NHCHO$	16
6	$R_2 = NHCOCH_3$	17
8	$R_2 = Cl$	
9	$R_2 = CH_2CH_3$	



3 R_1 = Et 14 R_1 = Me



7 R_1 = Et 18 R_1 = Me



10 $R_1 = (CH_2)_7CH_3$

11 $R_1 = (CH_2)_2$–⬡

Table 1. Stereoselectivity in enzymic hydrolysis of synthetic esters by mouse sarcoma S180 cells

Substrate	Preferentially formed acid enantiomer		Conversion rate (%)
	Config-uration	Enantiomeric purity (%e.e.)	
1	*R*	95.1	18.1
2	*R*	64.0	4.3
3	*S*	32.8	38.9
4	*R*	38.7	21.2
5	*S*	5.4	3.6
6	*S*	14.1	4.3
7	*S*	86.2	12.9
10	*R*	94.6	11.0
11	*R*	97.6	6.1

Table 2. Stereoselectivity in enzymic hydrolysis of synthetic esters by rat and human cancer cells and normal rat tissue extracts

Substrate	Absolute configuration†, optical purity (%e.e.)†, and conversion rate (%)									
	Rat cancer cells‡					Normal rat tissue extracts				
	XC	IA-XsSBR	NRK49F	Anr4	ARIP	Muscle	Intestine	Kidney	Liver	Pancreas
1	*R* 98.3 0.6	*R* 90.9 1.4	*R* 96.7 8.2	*R* 99.1 0.5	*R* 97.5 6.9	*R* 56.8 1.0	*R* 17.8 5.0	*R* 32.0 11.2	*R* 26.5 11.5	*R* 30.8 1.2
3	*S* 27.0 23.0	*S* 48.9 12.2	*S* 29.9 4.5	*S* 73.8 14.2	*S* 64.4 5.7	*S* 7.6 18.0	*S* 11.9 5.0	*S* 2.6 53.0	*S* 6.3 52.5	*S* 39.7 33.3
5	*S* 35.8 1.2	*S* 5.3 1.3	*S* 39.5 2.2	*S* 15.7 1.2	*S* 34.1 1.6	*S* 4.3 5.9	*S* 29.7 1.4	*S* 65.9 7.6	*S* 42.2 2.7	*S* 83.9 14.3
7	*S* 99.2 42.0	*S* 98.7 30.5	*S* 98.1 35.1	*S* 97.9 30.6	*S* 98.0 37.2	*S* 95.5 31.9	*S* 98.6 37.3	*S* 78.1 23.2	*S* 87.6 48.2	*S* 98.5 43.8

	Human cancer cells								
	U937 (histiocytic lymphoma)			Colo320 (colon adenocarcinoma)			MIA PaCa-2 (pancreatic carcinoma)		
1	*R*	98.0	1.4	*R*	96.1	1.2	*R*	99.6	11.9
3	*S*	58.9	n.t.	*S*	55.5	n.t.	*S*	53.2	35.9
5	*S*	39.1	3.5	*S*	13.3	3.8		n.t.	
7	*S*	93.0	17.5	*S*	92.9	13.8	*S*	99.1	42.0

†Concerned with the preferentially formed acid enantiomer.

‡Derived from sarcoma (XC), small bowel adenocarcinoma (IA-XsSBR), normal kidney fibroblast (NRK49F), EJ-ras oncogene transformed liver cell (Anr4), or pancreatic tumor (ARIP). NRK49F is not a cancer cell line, but included in this panel.

acetate (**8**) and 2-phenylbutanoate (**9**) were resistant to the enzymic hydrolysis, probably because of the limit in size or hydrophobicity of the α–side chain acceptable by the enzymes.

Then, four representative esters (**1**, **3**, **5**, and **7**) were chosen and tested further for the stereoselectivity in their hydrolysis by human and rat cancer cells.[7] The results (Table 2) were very similar to those of S180 cells. The high *R*-selectivity for **1** and *S*-selectivity for **7** appears almost a rule, in spite of the difference concerning animal species and source organs.

Finally, the stereoselectivity of rat cancer cells was compared with that of the corresponding normal tissues of rat. They were considerably different with each other as summarized in Table 2. The hydrolysis of **1** with the tissue extracts also proceeded with the *R*-enantiomer preference, but the degree of this stereoselectivity had decreased as compared with that by the cancer cells. The stereoselectivity in the hydrolysis of ester **3** was also lower with the normal tissue extracts than with the cancer cells. In contrast to these two examples, the stereoselectivity for (±)-*N*-formylphenylglycine ethyl ester (**5**) was usually higher with the normal tissues than with the cancer cells. The largest difference was found between normal pancreas and the pancreatic cancer cells (ARIP). The high *S*-selectivity for **7** was shown by the normal tissues as well as by the cancer cells. Thus, we have found three cases of stereoselectivity in the hydrolysis of synthetic esters by cancer and normal cells. No complete inversion of the stereoselectivity (*R*-preference ↔ *S*-preference) has been found between the cancer cells and the corresponding normal tissue, but it should be noted that the result with ester **5** and the pancreatic cells is important in terms of the stereoselective activation of prodrugs in cancer cells. The rat

pancreas was considerably selective to (*S*)-**5**, while the rat pancreatic cancer cells (ARIP) was less selective to the (*S*)-enantiomer. This fact implies that the counter enantiomer, (*R*)-**5**, would be hardly hydrolyzed in the normal pancreas cells, but it would be hydrolyzed relatively easily in pancreatic cancer cells. If we administer an anticancer drug as an inactive form modified with an (*R*)-*N*-formylphenylglycyl group to a rat bearing pancreas cancer, the prodrug might be preferentially activated at the site of the cancer.

However, we should elucidate many fundamental factors such as catalytic activity of the enzyme per cell and substrate penetrating rate into the tissue to achieve the above strategy of drug targeting actually in vivo. It is also important to know what and how many enzymes are responsible for the hydrolysis of the esters by the cancer cells.[8] Every tissue is constructed of several species of cells. The rat tissues were respectively homogenized as a whole in this experiment, and consequently the corresponding relation between the tissue and cancer cells might be not strict here, even though the cells were derived from that tissue. A significant comparison would be possible only between an orginal solid tumor and its surrounding tissue. Detailed study to resolve these problems and to develop chiral acyl groups maximizing the stereoselectivity difference between the cancer and normal cells for actual drugs is in progress. The present work emphasizes the importance of using the enzymic stereoselectivity in anticancer prodrug design.

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3. Some chiral acyl groups were introduced to anticancer drugs for prodrug synthesis,[1j,k] but the relation between the chirality and the effect on targeting to cancer cells have not been fully examined. The effect of stereochemistry on the prodrug activation has been discussed with antibacterial quinolone prodrugs[1i] and modified minor tranquilizers.[1n]
4. Each reaction mixture was made of 0.15 ml of 0.1 M phosphate buffer (pH 7.5), 10 μl EtOH containing 1 mg substrate ester, and S180 cells (1 X 10^6), which were cultured for 60 hr and harvested according to the previous procedure.[5] After stirring at 30 °C for 12 hr (for **1** and **8** ~ **11**) or 6 hr (for **2** ~ **7**), the mixtures were acidified and extracted with EtOAc. The formed acids were isolated by preparative TLC,

methylated with CH_2N_2, and subjected to HPLC analysis with Daicel chiral columns (Chiralcel OB, OD, OJ, and OK) for stereochemistry determination: columns and capacity factors for the methyl esters, OB for **17** (k'_R =7.52, k'_S =5.54), OD for **13** (k'_R =2.66, k'_S =1.67), **15** (k'_R =3.61, k'_S =6.19), **16** (k'_R =6.25, k'_S =5.66), and **18** (k'_R =3.28, k'_S =4.31), OJ for **12** (k'_R =3.04, k'_S =2.69), and OK for **14** (k'_R =3.25, k'_S =4.85); solvent, 10% 2-PrOH/hexane; flow rate, 0.5 ~ 1 ml/min. The authentic specimens were prepared from the commercially available optically active acids, except for (*R*)-(+)-2-phenoxypropionic acid, which was obtained by the lipase-mediated optical resolution: Yamazaki, Y.; Hosono, K. *Agric. Biol. Chem.* **1990**, *54*, 3357. Hydrolysis rates were determined by GC with the crude EtOAc extracts after methylation with CH_2N_2. Control reactions without the cells indicated that the nonenzymic hydrolysis rate was less than 0.2 %.

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6. (*S*)-**3** has essentially the same spacial arrangement of the α–substituents as (*R*)-**1**. This difference of *S* and *R* is a matter of the representation rule.
7. U937 cells were cultured in the same way as described before.[5] The cell lines Anr4 and NRK49F were obtained from RIKEN Cell Bank and COLO320 from Japanese Cancer Research Resources Bank. IA-XsSBR, XC, ARIP, and MIA-PaCa2 were purchased from DAINIPPON Pharmaceutical Ltd. These cells were cultured according to the supplier's catalogue. Rat organs were taken from a male Wister rat, 4 weeks old and weighing 124 g. Tissue extracts were prapared by homogenizing 0.1 ~ 0.2 g of the organ sections and 0.1 ml of the phosphate buffer with a Potter homogenizer and then centrifuging the homogenates at 15000 rpm for 3 min. The hydrolysis was carried out in the same way as described before,[4] but the tissue extracts were used by 50 μl and the reaction time was 12 hr for **1** with the cultured cells, 6 hr for **3**, **5**, and **7** with the cultured cells and for **1** with the tissue extracts, or 2 hr for **3**, **5**, and **7** with the tissue extracts.
8. In a preliminary experiment, the extracts of Anr4 cells and normal rat liver were analyzed by electrophoresis. The electrophoretograms were actively stained with α-naphthyl acetate to indicate that several esterases were contained in these extracts and that the zymograms were different between the two samples.

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