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INHIBITORY ANTIBODIES FOR IDENTIFYING CYP2C8, CYP2C9, CYP2C19 AND OTHER MAJOR CYTOCHROME P450S

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STUDY OBJECTIVE

- Development of selective inhibitory antibodies for CYP1A1, CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 & CYP3A4
- Development of antibodies for determining the quantitative contribution of each particular P450 enzyme in xenobiotic metabolism (reaction phenotyping study)

METHOD

- Express P450s enzymes in *E. Coli*
- Purify P450s by chromatography
- Immunize SPF rabbits with purified P450
- Characterize specificity of resulting antibodies
- Improve specificity by immunoabsorption

PROBE REACTION FOR CHARACTERIZATION

CYP	Enzyme	Probe Reaction
1A1	*E. Coli* expressed CYP	7-Ethoxyresorufin O-dealkylation
1A2	*E.Coli* expressed CYP & HLM	7-Ethoxyresorufin O-dealkylation
2A6	HLM	Coumarin 7-Hydroxylation
2C8	HLM	Taxol Hydroxylation
2C9	HLM	Diclofenac 4'-Hydroxylation
2C19	HLM	S-mephenytoin 4'-Hydroxylation
2D6	HLM	Bufuralol Hydroxylation
2E1	HLM	Chlorzoxazone 6-Hydroxylation
3A4	HLM	Testosterone 6â-hydroxylation

HLM: Human Liver Microsomes

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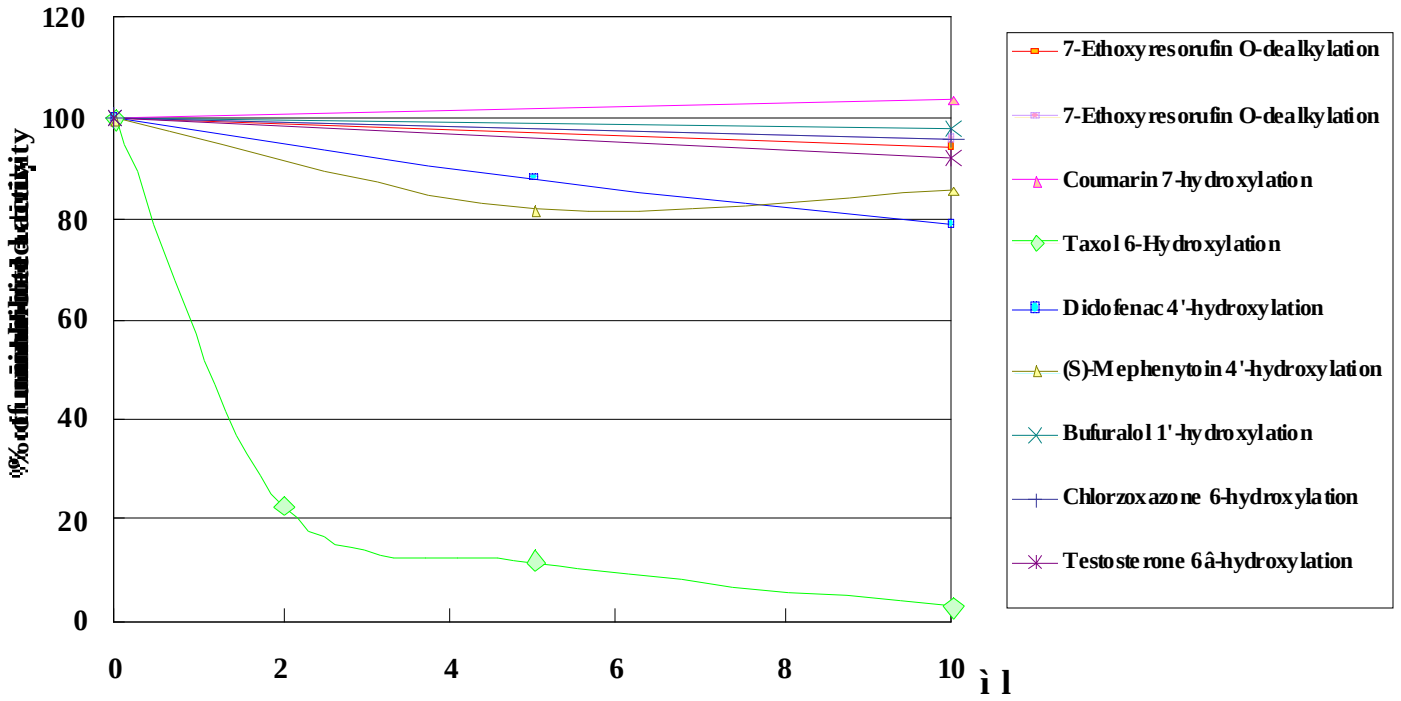
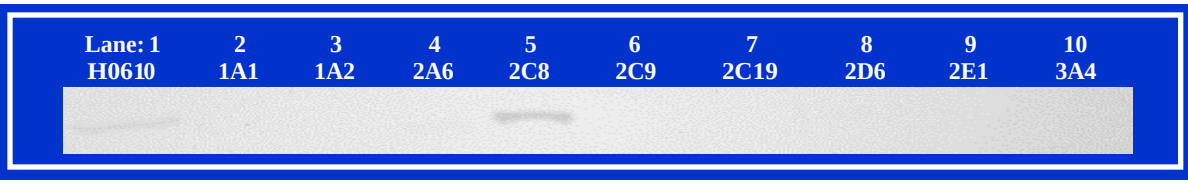
Result-1

SPECIFICITY OF ANTI-CYP2C8



ì l serum per 100 ì g human liver microsomes or 10 pmol P450



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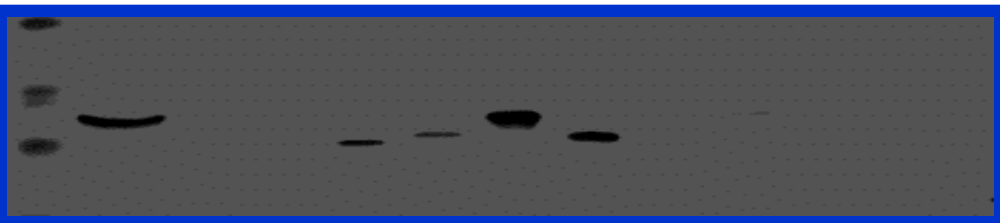
Result-2

IMMUNOABSORPTION OF ANTI–CYP2C9

- **OBJECTIVE:** Inhibitory specificity of pretreatment Anti-CYP2C9 is high. However, for immunoblotting, this antibody recognized all members of CYP2C family.
- **TREATMENT:** Immunoabsorption by incubating cDNA expressed CYP2C19 (membrane bound form) together with Anti-CYP2C9 at RT for 30 min. Non-specific immunoglobin will bound to the CYP2C19 and could be easily resolved as precipitate from the antiserum after ultracentrifugation.
- **EFFECT:** The immunoblotting specificity of Anti-CYP2C9 was improved.



PRE-TREATMENT

POST-TREATMENT

Liver microsomes 1A1 1A2 2A6 2C8 2C9 2C19 2D6 2E1 3A4

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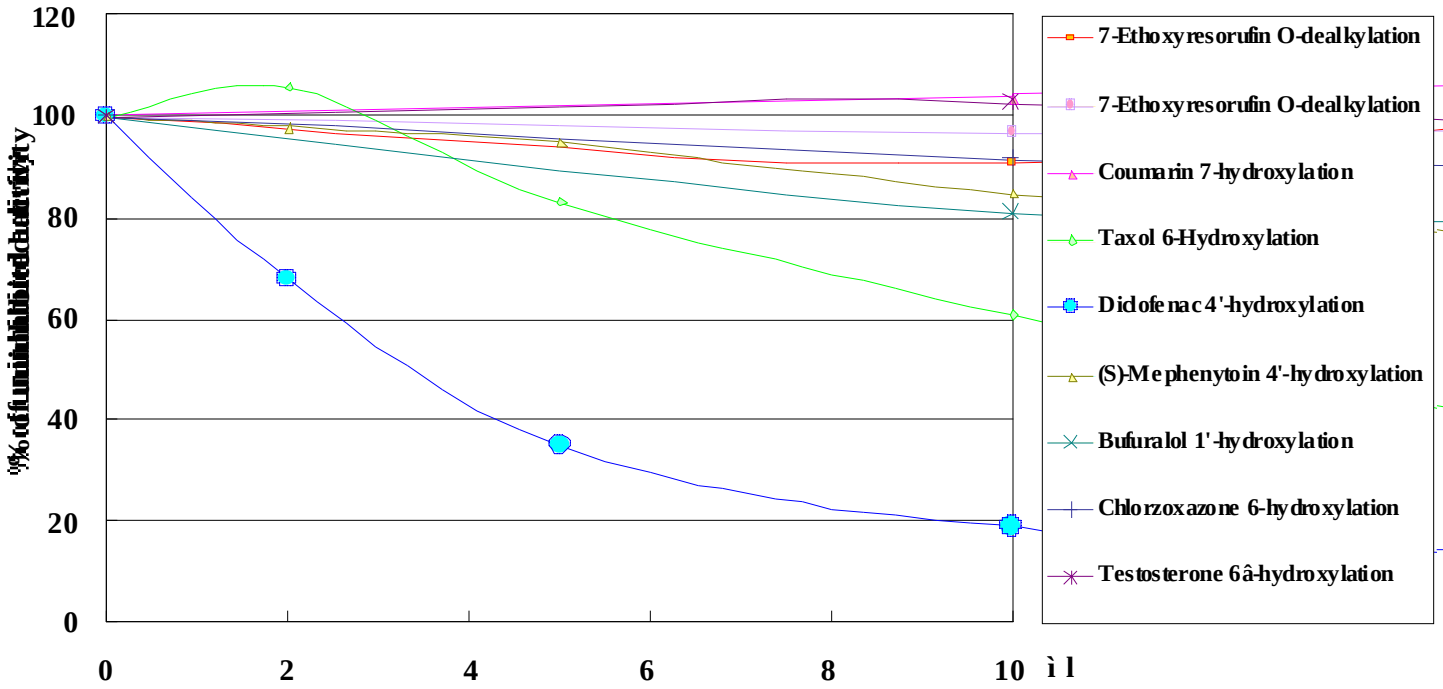
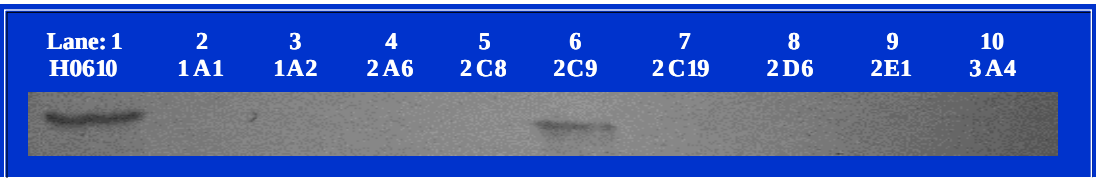
Result-3

SPECIFICITY OF ANTI-CYP2C9





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Result-4

IMMUNOABSORPTION OF ANTI–CYP2C19

- **OBJECTIVE:** Anti-CYP2C19 potently inhibiting CYP2C19 probe reaction and also moderately inhibited CYP2C9 probe reaction
- **TREATMENT:** Immunoabsorption by incubating cDNA expressed CYP2C9 (membrane bound form) together with Anti-CYP2C19 at RT for 30 min. Non-specific immunoglobin will bound to the CYP2C9 and could be easily resolved as precipitate from the antiserum after ultracentrifugation.
- **EFFECT:** The inhibitory specificity of Anti-CYP2C19 was improved.

The effect of immunoabsorption treatment for anti-CYP2C19

Immunoabsorption	µl of immunoabsorpted serum/ 100 µg pooled human liver microsomes			
	5	10	5	10
(x pmol CYP2C9/ml anti-2C19)	(S)-Mephenytoin 4'-hydroxylation (% of control)		Diclofenac 4'hydroxylation (% of control)	
0	60	24	84	58
50	57	23	87	73
100	61	37	98	93
200	70	44	94	92

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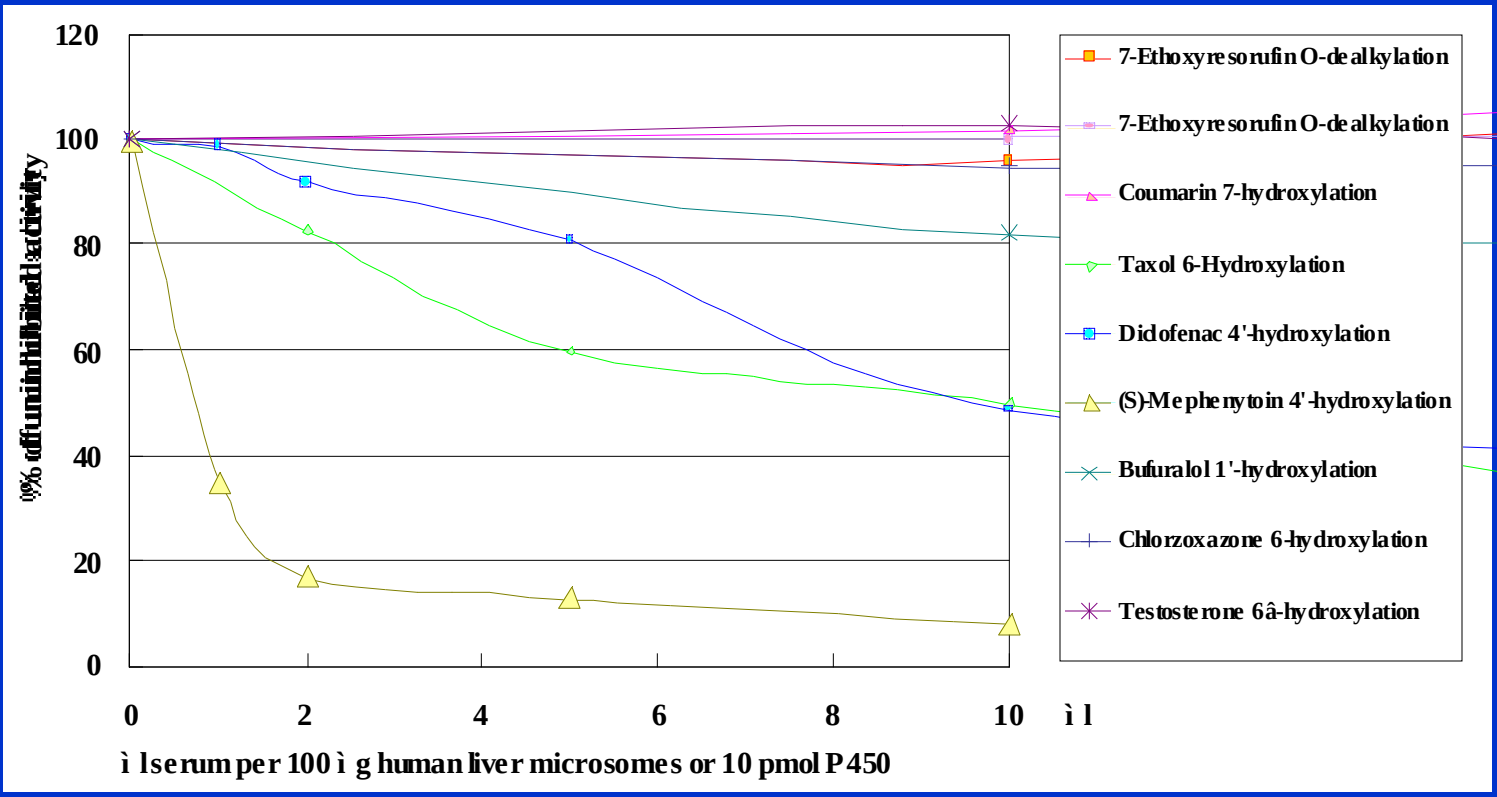
Result-5

SPECIFICITY OF ANTI-CYP2C19



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Conclusion-1

- Anti-CYP2C8, Anti-CYP2C9, Anti-CYP2C19 could precisely recognize the structurally confirmation and could inhibit enzymatic activity of the correspondence antigen
- Anti-CYP2C8 and Anti-CYP2C9 recognized a single protein band in human liver microsomes that co-migrated with the respective cDNA expressed P450.
- With the same approach, we have also developed Anti-CYP1A1, CYP1A2, CYP2A6, CYP2D6, CYP2E1 and CYP3A4
- The developed inhibitory antibodies will serve as in vitro specific tools for evaluating the quantitative contribution of individual P450 enzymes in mediating the biotransformation of xenobiotic

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Conclusion-2

Antibody	Lot	Inhibition Specificity									Western Blotting
		CYP1A1	CYP1A2	CYP2A6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4	
Anti-CYP 1A1	1	***	-	-	***	-	-	-	-	-	X
Anti-CYP 1A2	2	-	***	-	-	-	-	-	-	-	Good
Anti-CYP2A6	3	-	-	***	-	-	-	-	-	-	Good
Anti-CYP2C8	4	-	-	-	***	-	-	-	-	-	Good
Anti-CYP2C9	5	-	-	-	-	***	*	-	-	-	Good
Anti-CYP2C19	6	-	-	-	-	-	***	-	-	-	X
Anti-CYP2D6	7	-	-	-	-	-	-	***	-	-	Good
Anti-CYP2E1	8	-	-	-	-	-	-	-	***	-	X
Anti-CYP3A4	9	-	-	-	-	-	-	-	-	***	Good
Control serum	10	-	-	-	-	-	-	-	-	-	Good

***	Strong inhibition	Good	Good for Western Blotting
**	Cross reaction	X	Not suitable for
*	Weak cross reaction		Western Blotting
-	Cross reactivity not detected		