REVIEW

PREDICTABILITY OF CLINICAL ADVERSE REACTIONS OF DRUGS BY GENERAL PHARMACOLOGY STUDIES

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INTRODUCTION

The Guidelines for General Pharmacology Studies issued by the Japanese Ministry of Health and Welfare (MHW Guideline for short hereafter) is the only one which describes the guidelines for safety pharmacology in details in the world. While the principle pharmacology focuses on the therapeutic effects by studying effects of drugs on the intended or targeted tissues and/or organs, the general pharmacology aims to define the general pharmacological profile of drugs by systematically testing effects on the central nervous system, respiratory and cardiovascular systems, digestive system and other functions of major tissues and organs. Objectives of the general pharmacology studies are to identify the general profile of systemic pharmacological effects of a candidate drug for medical use and to provide information useful for predicting potential adverse effects in therapeutic use in humans. The general pharmacology is the same with the safety pharmacology in the sense of objectives of pharmacological studies in drug safety assessment. While the safety pharmacology sounds like studies straight forward the safety assessment, the general pharmacology literally regards the general pharmacological profile as meaningful and, therefore, recommends a set of a variety of studies.

In order to study the relationship between pharmacological findings in experimental animals and adverse reactions of respective drugs in humans, the working group members of the Subcommittee for General Pharmacology of the Japanese Pharmaceutical Manufacturers' Association (JPMA) investigated published papers on general pharmacological studies and on clinical adverse reactions observed during the clinical investigations of 141 new drugs, which were approved during a period of 1987 to 1992 in Japan. The present paper aims to introduce the results of the investigation achieved by the abovementioned working group members (JPMA Inhouse Report ; Document Non-clinical 64 ; Kitagawa *et al.*, 1994) and some additional results of an investigation done thereafter, and to discuss significance of test items written in MHW Guideline from the view point of the reliability to

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predict possible clinical adverse reactions from the pharmacological findings of the drugs.

METHODS

1. Contents filed in JPMA database of general pharmacological findings and clinical adverse effects of new drugs.

Saikin No Shinyaku (Recent New Drugs) Vol. 39–43 (1987–1992) files 141 new chemical entities approved by MHW with some brief summaries of non-clinical and clinical data of the drugs. JPMA General Pharmacology Working Group investigated 175 original papers on the general pharmacology studies of 104 new drugs. The rest of 37 new drugs were omitted, because no detailed data were available. The generic names (Japanese Accepted Name for Pharmaceuticals) and of 104 new drugs investigated are listed together with their therapeutic categories and Code No. in the Non-clinal Document 64 in Table 1.

The contents filed in JPMA database in a form of text-type database (Ninja, Version 4.0, NEC 9800 series, Something Good Inc.) are :

a) Name and category of drug ; code No., generic name, trademarks and therapeutic category (s) ;
b) Pharmacological findings of a total of 46 test items (Table 2) ; Study conditions of test animal species, route of administration and some complimentary notes for methodology if in need be, and results of study including the maximum no effect dose and/or the minimum effective dose ;
c) References for the pharmacological studies ;
d) Clinical findings of adverse reactions ; Clinical route of administration and therapeutic doses, adverse reactions with their incidence (percent of total patients investigated) ;

Out of 104 drugs investigated, the number of drugs for respective therapeutic categories were 26 drugs for cardiovascular diseases, 20 for central nervous system, 15 of antibiotics and chemotherapeutics, 7 of anti-tumor agents, 7 of drugs affecting metabolisms and so on. Sixty eight of 104 drugs were oral dosage forms, 24 intravenous injectable forms, 10 intra-muscular injectable forms and the rests were such dosage forms as intra-arterial, subcutaneous injections, inhalation and local application into the bladder, articular space and so on.

2. Test items in general pharmacology.

MHW Guideline classifies test items of the general pharmacology into two categories of A and B. Category A defines studies to be normally conducted for all test substances to assess the overall profile of the general pharmacological effects :

1) Effects on general activity and behavior which are studied using the traditional multidimensional observation, for example, according to Irvin method ;
2) Effects on the central nervous system by assessing :
   a) spontaneous locomotior activity using wheeling cages or open field method, b) potentiating effects on barbiturate anesthesia, c) anti-convulsion effects, analgesic effects, and d) effects on body temperature ;
3) Effects on the autonomic nervous system and smooth muscle by using isolated ileum and by assessing interaction with agonists such as histamine, acetylcholine and so on ;
4) Effects on the respiratory and cardiovascular systems by assessing respiration, blood pressure, blood flow, heart rate and electrocardiogram normally using anesthetized dogs ;
5) Effects on the digestive system by assessing : a) gastrointestinal charcoal-transit in mice or rats, and b) gastric emptying time, if necessary ;
6) Effects on water and electrolyte metabolisms ;
7) Other possible important pharmacological effects suspected from the chemical structure of drugs or related to their other animal and/or clinical findings.

On the other hand, Category B defines studies to be conducted, if necessary depending on the results of Category A studies. They are, for example, for studying effects on the central nervous system : a) electro-encephalogram (EEG), b) spinal reflex test, c) conditioned avoidance test, and d) coordinated locomotor

Table 1. Code No. in JPMA Document 64 Database, generic names and their therapeutic categories of 104 new drugs investigated.

Code No.	Generic name	Therapeutic category
001	Epoetin alfa	Hematopoietics, recombinant
002	Epoetin beta	Hematopoietics, recombinant
003	Doxazosin mesilate	Antihypertensives, alpha-blocker
006	Argatroban	Antithrombotics
008	Sevoflurane	Anethetics, harogenated ether
010	Lomefloxacin hydrochloride	Antibiotics, new quinolone
011	Tosufloxacin tosilate	Antibiotics, new quinolone
012	Calcitonin salmon	Hypocalcemic hormones (synthetic)
013	Levocarnitine chloride	Antihyperlipoproteinemics
016	Cefotiam hexetil hydrochloride	Antibiotics, cephalosporin
017	Cefodizime sodium	Antibiotics, aminoglycoside
018	Carboplatin	Antineoplastics, cytotoxic agent
019	Nizatizine	Antiulceratives, H_2-antagonist
020	Manidipine hydrochloride	Antihypertensives, Ca-antagonist
021	Vesnarinone	Inotropic agents
022	Lormetazepam	Sleep aids, benzidiazepine deriv.
023	Etidronate disodium	Bone metabolism regurator
025	Cilazapril	Antihypertensives, ACE inhibitor
027	Tazanolast	Antiasthmatics
031	Delapril hydrochloride	Antihypertensives, ACE inhibitor
032	Ibudilast	Antiasthmatics, cerebral vasodilator
032	Sultopride hydrochloride	Antipsychotics, benzamide deriv.
035	Batroxobin	Antithrombotics
036	Midodrine hydrochloride	Adrenergic alpha-stimulants
037	Terazosin hydrochloride	Antihypertensives, alpha-blocker
038	Propafenone hydrochloride	Antiarrhythmica
039	Pravastatin sodium	Hypolipidemics, HMG-CoA reductase inhibitor
040	Rilmazafone hydrochloride	Sleep aids, benzidiazepine deriv.
041	Zopiclone	Sleep aids
042	Zonisamide	Antiepileptics
044	Epirubicin hydrochloride	Antineoplastics, antracycline deriv.
045	Cadralazine	Antihypertensive, vasodilator
047	Dilevalol	Antihypertensives, alpha- / beta-blocker
048	Cefpodoxime proxetil	Antibiotics, cephalosporin
049	Interferon gamma-1a	Anti-neoplastics / -antivial
050	Sodium ozagrel	Antithrombotics
051	Indeloxazine hydrochloride	Antidepressants, nootropics
052	Denopamine	Inotropic agents
053	Limaprost	Antianginal agents, PGE1 deriv.
054	Tizanidine hydrochloride	Muscle relaxants

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Code No.	General name	Therapeutic category
055	Cilostazol	Antithrombotics
056	Dexamethasone palmitate	Adrenocortical steroids
057	Halopredone acetate	Adrenocortical steroids
058	Alminoprofen	Antianalgesics, antiinflamatorys
059	Isepamicin sulfate	Antibiotics, aminoglycoside
060	Cefuroxime axetil	Antibiotics, cephalosporin
061	Oxybutynin hydrochloride	Anticholinergic, for neurogenic bladder
063	Terodiline hydrochloride	For urinary incontinence
064	Nicergoline	Vasodilators
065	Amosulalol hydrochloride	Antihypertensives, alpha- / beta-blocker
066	Alacepril	Antihypertensives, ACE inhibitor
067	Midazolam	Sleep aids, benzidiazepine deriv.
070	Flomoxef sodium	Antibiotics, oxacephem
071	Pirarubicin hydrochloride	Antineoplastics, antracycline deriv.
072	Buserelin acetate	Gonad-stimulating, luteinizing hormone stimulating factor
073	Propentofylline	Cognition activator
075	Brotizolam	Sleep aids, diazepine deriv.
077	Interferon alpha	Antineoplastics, antivial
078	Irsogladine maleate	Antiulceratives
079	Ipriflavone	Antiosteopenics, Ca-regulator
080	Nipradilol	Antianginal, antihypertension, beta-blocker
081	Urapidil	Antihypertensive, alpha-blocker
082	Ethyl loflazepate	Anxiolytics, benzdiazepine deriv.
083	Proglumetacin maleate	Antiulceratives, indomethacin deriv.
085	Aprindine hydrochloride	Antiarrhythmica
086	Eptazocine hydrobromide	Narcotic analgesics
086	Ranimustine	Antineoplastics, alkylating agent
088	Aztreonam	Antibiotics, monobactam
090	Azosemide	Diuretics
092	Oxatomide	Antiallergics, antiasthmatic
093	Etoposide	Antineoplastics, podophylotoxin derivative
094	Ubenimex	Antineoplastics, aminopeptidase inhibitor
095	Aspoxicillin	Antibiotics, penicyllin, deriv.
096	Human anti-thrombin	Blood preparation
097	Ornoprostil	Antiulceratives, PG-derivative
098	Spizofurone	Antiulceratives, stimulator of endogenous PG-synthesis
099	Haloperidol decanoate	Antipsychotics, prodrug
100	Amlexanox	Antiallergic, antiasthmatic
101	Tenoxicam	Antianalgesic, antiinflammatory
103	Doxifluridine	Antineoplastics, prodrug

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Code No.	General name	Therapeutic category
104	Cefminox sodium	Antibiotics, cephamycin
105	Cefuzonam sodium	Antibiotics, cephalosporin
106	Imipenem	Antibiotics, carbapenem
107	Cefteram pivoxil	Antibiotics, cephalosporin
110	Bifemerane hydrochloride	Nootrpic
111	Repirinast	Antiallergic
113	Interferon alpha-2b	Antineoplastics, antivials
114	Interferon alpha-2a	Antineoplastics, antivials
115	Carumonam sodium	Antibiotics, monobactam
116	Omeprazol	Antiulcerative, proton-pump inhibitor
117	Tisokinase	Tissue plasminogen activator
118	Bezafibrate	Antihyperlipoproteinemics
121	Roxithromycin	Antibiotics, macrolide
122	Alteplase	Antihyperlipoproteinemics
123	Pilsicainide hydrochloride	Antiarrhythmics
124	Nemonapride	Neuroleptics, D_3-antagonist
125	Indometacin farnesil	Antianalgesics, antinflammatory
126	Clarithromycin	Antibiotics, macrolide
128	Monoethanolamine oleate	For sclerotherapy of esophageal varicose
129	Polidocanol	For sclerotherapy of esophageal varicose
130	Amezinium methylsulfate	Antihypotensives, sympathomimetic
133	Trazodone hydrochloride	Antidepressants
134	Romurtide	Antileucopenia, muramidil peptides
137	Simvastatin	Hypolipidemics, HMG-CoA reductase inhibitor

activity test. Test items listed in Category B for other systems are not detailed here (Table 2).

It is stated in MHW Guideline that the guidelines aim : a) to encourage for industry researchers to determine test items on a case by case principle, b) to recommend the essential test items (Category A) to be studied for all drugs, and c) to suggest that more than a half of test items (Category B) which have been uniformly conducted in the past for most drugs should be studied only on the necessity.

Table 2 shows the test items listed for Categories A and B in MHW Guideline, percent ratios of number of drugs where the respective test items were conducted for the 104 new drugs investigated as well as percent ratios of the number of drugs where any significant pharmacological effects were observed in the respective studies. Most of these drugs were presumably subjected to the general pharmacology studies before MHW Guideline became officially effective, because the MHW Guideline was issued in 1991, while the drugs investigated were approved by MHW during 1987–1992. Therefore, the test items conducted for the general pharmacology shown in Table 2 indicates the situation before MHW Guideline was issued. Although there are no concrete data available, the test items listed in Category B are these days conducted only on the necessity as indicated in MHW Guideline, i.e. not at the high percentage as shown in Table 2 anymore.

3. Major adverse reactions listed in 104 new drugs investigated.

The database for clinical adverse reactions in the present survey was constructed by another JPMA working group who was engaged in investigating the reliability of animal toxicity studies in predicting clinical adverse reactions (JPMA In-house Report : Document. Non-clinical 65, Hashimoto *et al.*, 1994). Summary data of clinical adverse reactions listed in *Saikin-No-*

Table 2. Items tested in the general pharmacology studies for the 104 new drugs investigated : Ratio A : the number of the drugs where the respective items studied / the total number of drugs investigated, and Ratio B : the number of the drugs with any pharmacological effects reported / the number of the drugs where the respective items studied.

Test items	Ratio A (%)	Ratio B (%)
Category A		
General activity and behavior	85.6	60.6
Spontaneous locomotor activity	86.5	47.1
Anesthetic	91.4	33.7
Convulsion	87.5	23.1
Analgesic	81.7	28.8
Body temperature	89.4	40.4
Isolated ileum*	96.2	58.7
Respiration	87.5	42.3
Blood pressure	95.1	53.8
Heart rate	91.3	50.0
Blood flow (carotid artery)	32.7	19.2
Blood flow (femoral artery)	64.5	38.5
ECG	84.7	21.2
Intestinal transport	93.2	39.4
Gastric emptying time	17.3	10.6
Urinary volume	84.6	37.5
Electrolyte excretion	83.6	41.3
Category B		
EEG (spontaneous or arousal)	82.6	28.8
Spinal reflex	58.6	14.4
Conditioned avoidance	25.9	3.8
Coordinated locomotor activity	70.2	25.0
Neuromuscular junction	80.7	16.3
Muscular relaxation	65.4	15.4
Local anesthetic**	70.2	11.5
Papillary diameter / nictitating membrane	76.9	29.8
Isolated blood vessel*	41.4	26.0
Isolated trachea*	63.4	39.4
Isolated vas deferens*	64.5	30.8
Isolated uterus*	85.8	56.7
Pressure reflex to vagal stimulation, interactions with vasoactive amines	57.7	27.9
Heart function in situ	21.2	13.5
Isolated cardiac preparation*	70.1	41.3
Isolated vascular bed*	18.2	11.5
Gastric juice secretion	78.9	48.1
Salivary secretion	27.0	13.5
Bile secretion	53.8	16.3
Pancreatic secretion	9.6	2.9

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Test items	Ratio A (%)	Ratio B (%)
Isolated stomach and intestine muscles*	28.9	20.2
Gastrointestinal movement in situ	47.2	26.0
Injury in gastro-duodenum mucus	20.2	5.8
Blood coagulation system***	69.2	20.2
Platelet aggregation***	33.6	14.4
Hemolytic potential***	40.4	8.7
Renal function (GFR, RPF)	30.8	15.4

* : *In vitro* studies.
** : Studies by topical application of test drugs.
*** : Including *in vitro* studies.

Table 3. Major clinical adverse reactions observed for the 104 drugs investigated. The number of the drugs with the respective adverse reaction was calculated at three levels of the incidence of 0.1, 0.5 and 5.0%, i. e. ratio of the number of the patients with the respective reaction to the total number of patients investigated.

Adverse reactions	Incidence of adverse reactions		
	≥0.1%	≥0.5%	≥5.0%
Rash, eruption, urticaria*	49	28	0
Itching, pruritus*	14	9	0
Headache, headache dull	32	28	5
Dizziness, light headed feeling	33	28	6
Sleepiness	16	13	6
Malaise, fatigability, weakness	25	24	9
Anorexia	29	23	12
Nausea, vomiting	62	48	12
Stomach (abdominal) ache, discomfort	27	23	2
Diarrhoea, stools loose	45	33	3
Constipation	12	8	2
Thirst	15	10	3
Palpitation, tachycardia	13	11	0
Hot flushes, feeling of warmth	12	10	1
Fever	21	11	6
Oedema swelling moon face	12	9	0

* : Interrelation with animal studies was not examined, because of no related test item in the general pharmacology.

Shinyaku (Recent New Drugs) Vol. 39 (1987)–43 (1992), *Nihon-Iyakuhin-Shu* (Collective Information of Japanese Medicines, published by Yakugyo-jiho Co., Ltd., 1991) and *Toki-No-Shinyaku* (New Drugs of Today, Medical View Co., Ltd., 1989–1991) were examined by checking the original papers published in official journals. Table 3 summarizes the major clinical adverse reactions which were observed during the clinical trials with the 104 new drugs investigated. Out of adverse reactions listed in Table 3, immuno-related reactions (rush/eruption/urticaria and itching/pruritus) were not examined for the interrelation with animal studies, because of no related test item in the general pharmacology.

Nausea/vomiting was the adverse reaction reported in the most new drugs when the number of new drugs with the respective adverse reactions were counted at the incidence of the adverse reaction higher than 0.1, 0.5 or 5.0%. On the other hand, rash/eruption/urticaria at an incidence of 0.1 or 0.5% was reported with many

new drugs but there was no drugs with these adverse reactions at the incidence of higher than 5.0%.

The other adverse reactions than listed in Table 3 were tremor and numbness of limbs, akathisia, paralysis of limbs, arrhythmia, coughing and dyspnoea (difficulty in breathing), hypertension, hypotension, dysuria and nocturia (Tables 7–10).

4. Methods for the interrelation between findings in general pharmacology and clinical adverse reactions.

Number of new drugs was put into 2×2 cross tables to examine the interrelation between pharmacological findings in animal studies and clinical adverse reactions recorded. Each test item of general pharmacology (31 *in vivo* studies and 12 *in vitro* or by topical application studies) shown in Table 2 was examined for the interrelation with the respective 13 major clinical adverse reactions shown in Table 3.

The interrelation was statistically examined by X^2 independence test. Fisher's Direct Probability test was applied for the purpose when one of figure (number of drugs) in the column of 2×2 table was less than five. Results of the statistical test at P less than 0.05 were considered as "the association positive". The coincidence rate (% ratio of the number of the positive drugs in pharmacological action and in clinical adverse reaction to the total number of the drugs investigated) was also calculated.

Test items in general pharmacological studies can be classified whether the application of test drugs is systemic or local. When the application of test drugs was systemic, i.e. by oral administration or intravenous injection, the ratio of the test dose to the clinical therapeutic dose was taken into consideration of the interrelation analysis, while the dose ratio of the test dose to the human dose was not evaluated where the test items of general pharmacological studies were conducted in a *in-vitro* system with isolated tissue and organs.

Less common adverse reactions which were reported for only several drugs, namely less than 10 out of 104 drugs, were not subjected to the above-mentioned statistical analysis because of the low incidence. Those adverse reactions were only checked up on whether or not there were any pharmacological findings accounting for such adverse reactions.

RESULTS

1. Results of examination with total 104 new drugs.

Statistical significance of association between pharmacological findings in animal studies of a total 43 items listed in Table 2 and 13 major clinical adverse reactions listed in Table 3 was examined with the 104 drugs.

Out of total 43 pharmacological test items, 31 test items were the studies conducted by systemic application of test drugs either by same or different route of clinical administration. A significant association with any clinical adverse reactions was recognized in 17 test items. Among those, the following combinations were seemed reasonable :

Spontaneous locomotor activity vs. Headache/headache dull
Spontaneous locomotor activity vs. Sleepiness
Convulsion vs. Hot flushes/feeling of warmth
Intestinal transport vs. Anorexia
Gastric emptying time vs. Anorexia
Gastric juice secretion vs. Nausea/vomiting
Gastric juice secretion vs. Anorexia
Pancreatic secretion vs. Anorexia
Injury in gastro-duodenum mucus vs. Thirst
Injury in gastro-duodenum mucus vs. Oedema/swelling

There were some statistically significant combinations of animal data and clinical adverse reactions where the pharmacological relationship was not clear. They were :

Papillary diameter vs. Abdominal pain
Pressure reflex to vagal stimulation etc. vs. Sleepiness
Pressure reflex to vagal stimulation etc. vs. Thirst
Pressure reflex to vagal stimulation etc. vs. Malaise/fatigability
Gastric juice secretion vs. Hot flushes/feeling of warmth
Gastric juice secretion vs. Thirst
Gastric juice secretion vs. Oedema/swelling

Among 12 test items of *in vitro* studies including local anesthetic test by topical application of test drugs, a significant association was

found between pharmacological findings and clinical adverse reactions in the following combinations :

Local anesthetic vs. Thirst
Isolated ileum vs. Thirst
Isolated ileum vs. Dizziness/light headed feeling
Isolated uterus vs. Dizziness/light headed feeling

The above-mentioned analysis of the interrelation between pharmacological findings and clinical adverse reactions raised some questions in the methodology. They were : a) bi-directional pharmacological effects, for example, increase and decrease in heart rate, were both classified as pharmacologically positive but they should be separately analyzed, b) to exactly take test doses in animal studies into consideration, the results of pharmacological studies conducted by the same or different route of administration with the clinical investigation should be separately from each other data analyzed, and c) there were some specific categories of drugs which were supposed to be excluded from the analysis of interrelation, as will be mentioned later.

2. Results of examination with selected 84 new drugs.

Twenty out of 104 new drugs investigated were excluded from the following analysis of the interrelation : 7 antineoplastic agents because they are generally inactive in general pharmacological studies by single dose administration while their adverse reactions were developed by the repeated dose treatment, 4 recombinant interferon preparations because of the species differences in biological-effects, and 7 new drugs to be administered through specific routes such as intra-coronary arterial injection and local application into the bladder, articulus cavity, 2 drugs because of the poor number in the clinical data.

Results of most pharmacological studies are bi-directional, although some test items such as analgesic and anti-convulsion tests generally provide only data of suppressing or inhibiting effects. The accelerating or potentiating effects and the suppressing or inhibiting effects in pharmacological studies were separately evaluated in the following analysis. Hereafter, an adverse reaction of the incidence less than 0.5% was neglected and the drug was classified as the adverse reaction negative.

As an example, Table 4 shows the interrelation of pharmacological effects on the spontaneous locomotor activity in mice and the adverse reaction of dizziness in clinical investigation. The locomotor activity was studied on 70 of 84 drugs which satisfied the conditions of the above-mentioned criteria. Out of 70 drugs, a decreased motility was reported in 41 drugs, an increased motility in two drugs. The rest of 27 drugs were not effective on the spontaneous mortility. The association between the reducing action on spontaneous motility and adverse reaction of dizziness was statistically significant ($P<0.05$) when it was examined using data at test doses less than 100 fold of the therapeutic dose. However, the association between the reducing action on spontaneous motility and adverse reaction of dizziness was not significant when the 12 cases of studies conducted with test doses over 100 fold therapeutic dose were included into the examination of significance of the relation due to the increase in the number of false positive cases. The false positive ratios (number of drugs with

Table 4. Pharmacological effects on the spontaneous motility in mice and clinical adverse reaction of dizziness. Number of drugs reported with reduced or increased spontaneous motility with (+) or without (−) clinical adverse reaction of dizziness.

Dose ratio (R)	Reduced			No effect			Increased			Total
(animal/human)	Total	(+)	(−)	Total	(+)	(−)	Total	(+)	(−)	
$R<10$	6	4	2	6	0	6	0	0	0	12
$10\leq R<30$	11	4	7	5	1	4	1	1	0	17
$30\leq R<100$	12	4	8	6	0	6	0	0	0	18
$100\leq R$	12	1	11	10	3	7	1	1	0	23
Total	41	13	28	27	4	23	2	2	0	70

negative clinical data/total number of drugs with positive animal data) were increased in a dose-dependent manner, i.e.. 2/6 (33%), 7/11 (64%), 8/12 (67%) and 11/12 (92%), when animal data were classified by test dose ratios of less than 10, 30, 100 fold and over 100 fold, respectively.

Table 5 summarizes pharmacological findings and clinical adverse reactions significantly associated each other with selected 88 new drugs administered in animals and humans by the same dosing route, respectively.

Among test items of effects on the central nervous system, in addition to dizziness, the reduced spontaneous locomotor activity had a

Table 5. Pharmacological findings and clinical adverse reactions associated each other ($P < 0.05$) in the examination of the selected 88 new drugs.
A) The same route of administration in animals and humans.

Pharmacological findings Test dose (ratio to clinical dose)	Clinical adverse reactions <10 fold	<30 fold	<100 fold
Reduced spontaneous motility mice.	Dizziness Sleepiness	Dizziness Thirst	Dizziness
Potentiation of anesthetic effect mice, barbiturate anesthesia		Thirst	
Anti-convulsion mice	 Thirst	Anorexia Thirst	Anorexia
Analgesic effect mice, acetic writhing	Sleepiness Thirst Flushes	 Thirst	Dizziness
Reduction in body temperature rats, rabbits or mice	Headache Malaise	Dizziness Anorexia Thirst	Dizziness Anorexia Thirst
Reduced arousal wave in EEC rabbits or cats		Malaise	Malaise
Reduced coordinated locomotion mice	Dizziness Sleepiness	Dizziness Sleepiness	Dizziness
Enhanced intestinal transport mice			Diarrhea Abdominal pain
Reduced intestinal transport mice	Constipation	Constipation Anorexia Nausea Stomach pain	Constipation Anorexia
Reduced gastric secretion rats		Anorexia	Anorexia
Injured gastro-duodenum mucosa rats		Stomach pain	Stomach pain
Reduced urine / Na excretion rats	Oedema	Oedema Dizziness	Oedema Dizziness

significant association with sleepiness at doses of less than 10 fold of the therapeutic dose and with thirst at doses of less than 30 fold. Adverse reactions such as dizziness, sleepiness and thirst were not specific with the drugs reducing spontaneous motility but common with central depressing effects such as potentiation of barbiturate anesthesia, anti-convulsion, anti analgesic, body temperature lowering, inhibiting effects on coordinated locomotion, and arousal EEC wave reducing. Adverse reactions such as anorexia and malaise could be classified in these categories. The effects of drugs on spontaneous locomotor activity seems to be representative of the test items of studies affecting the central nervous system.

Charcoal transportation in mice was one of the most popular pharmacology tests. The enhanced intestinal transport had significant association with diarrhea and abdominal pain, while the reduced intestinal transport were related to the adverse reactions of constipation, anorexia and others. These results may suggest that the simple and classical pharmacological study of the effects on charcoal transportation in mice can be representative of studies of the effects on the gastrointestinal tracts.

It was interesting to find a significant association between water and sodium retaining effect and adverse reaction of oedema, while it was not quite easy to relate the renal pharmacological action to the adverse reaction of dizziness.

Table 6 summarizes the interrelation between the findings in general pharmacological studies conducted by intravenous injection and the adverse reactions observed in clinical investigation conducted by oral administration. The pharmacological studies classified in Table 6 were characteristic in need of electronic instruments to measure electronic activity, organ movement or blood stream. Anesthetized dogs or cats were mainly used for these studies. For example, effects on blood pressure were determined on 26 drugs of this category in anesthetized dogs. Six drugs including 3 vasodilators reduced blood pressure at an intravenous dose less than 0.1 fold of the oral therapeutic dose. Out of these 6 drugs, dizziness or light headed feeling was reported in all 6, headache in 5, malaise, fatigability or weakness in 3 and flushes in 2. These incidence of adverse reactions were significantly higher than those for 13 drugs which reduced blood pressure at a higher dose, 3 drugs which enhanced blood pressure and 4 drugs which did not affect blood pressure. Interrelation of vasodilating effects (blood flow increase through the

Table 6. Pharmacological findings and clinical adverse reactions associated each other ($P < 0.05$) in the examination of the selected 88 new drugs.
B) Clinical trials conducted by oral administration but animal studies by intravenous injection.

Pharmacological findings	Clinical adverse reactions		
Test dose (ratio to clinical dose)	<0.1 fold	<0.3 fold	<1.0 fold
Blood pressure reduction anesthetized dogs	Flushes Dizziness Headache Malaise	Flushes Dizziness Headache Malaise	Flushes
Increase in heart rate anesthetized dogs	Palpitation	Palpitation	Palpitation
Increased blood flow anesthetized dogs	Flushes Headache	Flushes Headache	Flushes
Depressed spinal reflex anesthetized cats			Sleepiness
Depressed gastro-intestinal movement, anesthetized dog	Anorexia	Anorexia	Anorexia

Table 7. Pharmacological findings for less common clinical adverse reactions.
1) Central and peripheral nervous disorders.

Adverse reaction (incidence %) 〈Category of drug〉 Pharmacological findings (animal, dose ratio or different route of administration)
Tremor limb (36), Akathisia (20), Muscle rigidity (28) 〈Psychotropics (benzamide deriv.)〉
Intermitted tremor and ataxia in behavior analysis (dog, 1.1)
Potentiation of monosynaptic spinal reflex (cat, iv)
Tremor limb (15), Akathisia (18), Sleep disturbed (13) 〈Psychotropics (butylophenone deriv.)〉
Catalepsy (mouse, im), Potentiation of electronic convulsion (mouse, im)
Tremor limb (15), Akathisia (15), Muscle rigidity (11) 〈Psychotropics (benzamide deriv.)〉
Catalepsy (monkey, 8.3), Potentiation of pentetrazole convulsion (mouse, 25)
Potentiation of spinal reflex (rat, iv)
Muscle relaxation (3) 〈Sleep inducer (diazepam deriv.)〉
No related finding
Sleep disorder (0.1), tremor (0.1) 〈Anti asthma (inhibitor of mediator release)〉
No related finding
Numbness of limbs (0.7) 〈Anti-tumor (platinum complex deriv.)〉
No related finding
Tremor limb (0.8) 〈Drug for micturition disorder〉
Tremor and ataxia (dog, 60), Potentiation of picrotoxine convulsion (mouse, 20)
Tremor limb (8.5) 〈Anti arrhythmic agent〉
Epileptic discharge in amygdala and hippocampus (cat EEG, iv)

Table 8. Pharmacological findings for less common clinical adverse reactions.
2) Coughing and respiratory disturbances.

Adverse reaction (incidence %) 〈Category of drug〉 Pharmacological findings (animal, dose ratio or different route of administration)
Coughing (6.1) 〈Antihypertensive (ACE-inhibitor)〉
No related finding
Coughing (1.1) 〈Antihypertensive (ACE-inhibitor)〉
No related finding
Influenza-like symptom (69), Respiratory disturb. (0.2) 〈Recombinant Interferon〉
No related finding
Influenza-like symptom (47), Respiratory disturb. (0.6) 〈Recombinant Interferon〉
No related finding
Respiratory disturb. (0.4) 〈Anti asthma (inhibitor of mediator release)〉
No related finding
Respiratory disturb. (1.7) 〈Anti-thrombin〉
No related finding
Respiratory disturb. (0.8) 〈Cardiotonic〉
No related finding

carotid artery or femoral artery) with adverse reactions of flushes and headache seemed reasonable. Anorexia was reported in 6 of 10 drugs with depressed gastrointestinal movement *in situ*, but only 1 of 6 drugs in which gastro-intestinal movement was activated, and none of 7 drugs which did not affect the gastro-intestinal movement.

3. Animal findings for less common clinical adverse reactions.

In addition to the adverse reactions listed in Table 3, there were some less common adverse reactions which were reported for only several drugs.

a) Central and peripheral nervous disorders :

As summarized in Table 7, some specific adverse reactions such as tremor limb, akathisia and muscle rigidity were reported at a high incidence in the clinical trials with some types of psychotropic agents. These reactions were recognized also in some other drugs. Pharmacologically related findings were tremor, ataxia and ataxia in behavior analysis, enhanced spinal reflex and potentiating effects on experimental convulsions.

b) Coughing and respiratory disturbances :

As summarized in Table 8, there were reported no closely related pharmacological find-

Table 9. Pharmacological findings for less common clinical adverse reactions.
3) Cardiovascular disturbances.

Adverse reaction (Incidence %) 〈Category of drug〉 Pharmacological findings (animal, dose ratio or different route of administration)
Arrhythmia (2.6) 〈Inhalation anesthetic〉
No related finding
Arrhythmia (0.7) 〈Antihypertensive (alpha-blocker)〉
No related finding
Arrhythmia (3.2) 〈Antitumor (anthracycline-deriv.)〉
Tachycardia (dog, iv)
Arrhythmia (4.5) 〈Antitumor (anthracycline-deriv.)〉
No related finding
Extrasystoles (1.1), Ventricular, tachycardia (0.5) 〈Cardiotonic (dopamine-deriv.)〉
Tachycardia (dog, iv)
Arrhythmia (0.6) 〈Sleep-inducer (benzodiazepine-deriv.)〉
No related finding
Arrhythmia (3.7) 〈Antitumor (Podophyllotoxin deriv.)〉
No related finding
AV block (0.7) 〈Antiarrhythmic (Class 1)〉
ECG findings (dog, cat, iv)
Arrhythmia (10.2) 〈Recombinant-product (Interferon)〉
No related finding
Reperfusion arrhythmia (34.1) 〈Recombinant plasminogen activator〉
No related finding
Hypertension (6.9) 〈Recombinant-production (erythropoietin)〉
No related finding
Hypertension (4.6) 〈Recombinant-production (erythropoietin)〉
No related finding
Hypotension (3.8) 〈Psychotropics (benzamide deriv.)〉
Hypotension, inhibition of vaso-reflex (dog, iv)
Unstable blood pressure (2.6) 〈Inhalation anesthetic (ether deriv.)〉
Hypotension (rabbit, 0.4)

Table 10. Pharmacological findings for less common clinical adverse reactions.
4) Micturition disorders.

Adverse reaction (Incidence %) 〈Category of drug〉 Pharmacological findings (animal, dose ratio or different route of administration)
Pollakiuria (0.5) 〈Antihypertensive (Ca antagonist)〉 No related finding
Pollakiuria (40.9), Micturition painful (40.3) 〈Antitumor (antracycling-deriv.)〉 No related finding
Dysuria (2.0), Urinary retention (0.4) 〈Drug for dysuria (bladder relaxant)〉 No related finding
Dysuria (2.2), Urinary retention (0.4) 〈Drug for dysuria (bladder relaxant)〉 No related finding
Micturition disorder (0.6) 〈Antiarrhythmic (Class 1)〉 No related finding
Pollakiuria (0.1), Cystitis (0.2) 〈Psychotropics (butylophenone deriv.)〉 No related finding
Pollakiuria (0.1), Cystitis (0.2) 〈Anti asthma (inhibitor of mediator release)〉 No related finding
Dysuria (incidence unknown) 〈Antidepressant〉 No related finding

ings with clinical adverse reactions of respiratory disturbances. No animal findings were reported for those respiratory disturbances. For example, although it is generally known that the clinical adverse reactions of respiratory disturbance by use of angiotensin converting enzyme inhibitor are generally related to the pharmacological effects (interaction with kinins) of those drugs, some specific pharmacological procedures should be employed for the detection of these kinds of specific adverse reactions.

c) Cardiovascular disturbances :

Although a variable types of arrhythmias were reported as adverse reactions, there were no related pharmacological findings observed for most cases of such adverse reactions (Table 9). Because of the variability in mechanism of the abnormal cardiac conduction and automaticity, a variable sets of electrophysiological systems will be needed to relate the adverse reactions with animal data. Two cases of hypertension developed by repeated treatment with erythropoietin preparation are accounted for by an exaggerated effect of red cell formation, although there were no findings by single dose pharmacological studies.

d) Micturition disorders :

Micturition disorders such as pollakiuria and dysuria seem specific for human and difficult to relate with the animal data, as shown in Table 10.

DISCUSSION

1. Needs of international guidelines for the safety pharmacology.

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has made a great substantial progress in drawing up harmonized guidelines in the field of quality, safety (toxicology studies) and efficacy (clinical investigations). The establishment of international guidelines for the reproductive and developmental toxicity studies, pharmacokinetic validation of toxicology studies (toxicokinetics), dose-selection in carcinogenicity studies and core-battery for genotoxicity were those in the field of safety research. The timing of toxicity studies to support clinical trials will be one of the highlights at the third ICH (1994 in Yokohama). The safety pharmacology study is internationally regarded essential as one of pre-clinical studies. There-

fore, what kind of pharmacological studies, and at what timing in relation to the clinical investigation, should be conducted became an important subject to be internationally discussed.

2. Further discussion on the association between pharmacological findings and clinical adverse reactions.

Some associations with clinical adverse reactions were found in several test items of general pharmacology studies defined in MHW Guideline as summarized in Tables 4, 5 and 6. The results of the present investigation indicate the following points to be noteworthy :

a) In the present investigation, the association between animal data and human adverse reactions were statistically analyzed using X^2 test or Fisher's direct probability test, because not only the coincidence ratios between animal and human data but also the incidence of false positive and negative data should be carefully evaluated. Not all but most of pairs of animal data and human adverse reactions statistically associated with each other seemed to be pharmacologically accountable.

b) In most studies, animal data obtained with extremely high dose reduced the association with clinical reactions, because the higher the test dose in animal studies (ratio to the therapeutic dose) was, the higher the incidence of false positive cases (animal data positive but human data negative) was. Therefore, high test doses in animal studies should be recommended only when it can be fairly expected that the false negative incidence will be decreased by increasing test doses in animal studies.

c) Although there were not a few test items which had a significant association with some major clinical adverse reactions, they were not specific for any adverse reactions. For instance, dizziness, sleepiness, thirst, anorexia and malaise were the major clinical adverse reactions which were related to a variety of central depressing effects in animal studies. Spontaneous locomotor activity in mice was the representative of such pharmacology studies affecting the central nervous system.

Similarly, intestinal charcoal-transportation in mice was supposed to be the representative of study procedures for effects on the digestive system.

3. Specific adverse reactions.

Some less common clinical adverse reactions shown in Tables 7–10 were defined as "specific adverse reactions", which were specific for some categories of drugs or related to some specified pharmacological actions. They were, for instance, convulsions, muscle rigidity, coughing, arrhythmias and micturition disorders. For most cases, there were no related pharmacological findings possibly accountable for those specific adverse reactions, suggesting needs of a wide variety of and specified models for the assessments. Some kind of those specific adverse reactions can be suggested from their pharmacological action and/or adverse reaction reported for the related compounds. The adverse reactions which were unexpectedly found in the clinical trials should be as matter of course clarified by additional pharmacology studies.

4. What studies, and at what timing in relation to the clinical investigation, should be conducted for the safety pharmacology ?

MHW Guideline issued in 1991 clearly defined a lot of test items, which were uniformly conducted for all drugs in the past, as studies to be conducted on a case by case principle, resulting in a marked reduction in number of the essential test items. However, because of poor reliability and specificity of most of test items in predicting clinical adverse reactions as indicated in the present investigation, the essential studies to be conducted for all drugs can be defined to some representative studies.

As a conclusion of the present investigation, we would like to propose the following studies as the minimum battery for the general or safety pharmacology to be conducted before the first application of a candidate drug to humans :

(a) Since blood pressure, heart rate and ECG are usually monitored in Phase I clinical studies. Pharmacological studies on these cardiovascular parameters should be conducted beforehand. The same route of administration and use of not anesthetized animals with implanted cannulas and instruments for the measurement are desirable, if feasible.

(b) The traditional pharmacology studies of spontaneous locomotor activity and intestinal transportation can be representative of the

study procedures for effects on the central nervous system and digestive system, respectively. Both studies are simple and not resource demanding but useful to provide a quantitative and qualitative assessment of the drug safety.

5. Importance of profiling general pharmacology.

MHW Guideline recommends a list of studies with isolated tissues and organs. Although there are some difficulties in quantitative evaluation (dose ratio in animals studies to therapeutic doses), such data obtained by *in vitro* studies, especially, studies on the interaction with intermediators, are useful to understand the general pharmacological profiles, and provide assessment of drug safety. For these kind of studies, modern technologies such as a set of drug-receptor binding assay are to be encouraged. Those studies providing comprehensive data of pharmacological profiles of candidate drugs should be conducted in an early stage of new drug development.

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* : Document Non-clinical 64 (in p. 75–79) lists 175 original papers on the general pharmacology studies of 104 new drugs investigated. The references are not listed in the present paper, because almost all those papers are in Japanese, and the present paper does not aim to describe details of individual pharmacological studies.