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Application of radioreceptor assays for systematic toxicological analysis — 2. Theoretical considerations and evaluation

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Abstract: In this paper the applicability of radioreceptor assays for systematic toxicological analysis will be evaluated on a theoretical basis as well as on the basis of the outcomes of the analysis of a large number of urine samples collected after administration of a selected number of drugs to healthy volunteers and patients. Many drugs and other substances of toxicological relevance exert their action through an interaction with one or more receptor (sub)types. Whether the number of persons are using particular drugs intentionally or unintentionally, radioreceptor assays can be a useful tool for systematic toxicological analysis in that they can be applied to the identification of entire pharmacological classes of substances as well as pharmacologically active metabolites. In part 1 of this paper detailed procedures for radioreceptor assays for benzodiazepines, anticholinergics and antihistaminics have been described in detail in order to illustrate not only the potentials but also the limitations of assay conditions. Fifteen drugs were administered to patients and volunteers and urine samples were collected and determined with the three radioreceptor assays. The results of this study underline the theoretical applicability of receptor assays in systematic toxicological analysis though sample pretreatment procedures may contribute to an improvement in sensitivity and applicability to other biofluids.

Keywords: *Radioreceptor assays; systematic toxicological analysis; screening; benzodiazepine; anticholinergic; antihistaminic; identification; therapeutic drug monitoring.*

Introduction

Systematic toxicological analysis (STA) comprises the logical chemical analytical search for potentially harmful substances, whose presence is unsuspected and whose identity is unknown. By combining specific features of various analytical methodologies, detection and identification of a.o. drugs and/or metabolites in biological material can be achieved [1, 2].

Radioreceptor assays (RRA) can contribute to STA, though this approach has not yet been evaluated in detail [3, 4].

RRA are based on the competition between a radiolabelled ligand and a drug (unlabelled ligand) for binding to a certain receptor type. When a drug is added to a mixture containing fixed concentrations of receptors and labelled ligand, the competitive drug will displace a certain amount of labelled ligand depending on its equilibrium constant, K_d, and the added concentration of competitive drug [5, 6]. RRA can be advantageous over chemical and physical as well as over immunoassays, in that they pair a sufficiently high sensitivity (directly related to the potency and affinity of these drugs) with a selective determination of biologically active compounds such as the eutomer of a racemic drug and/or active metabolites that contribute to the desired (and undesired) actions of the parent compound. Moreover, new (designer) drugs that exert their action via a particular receptor will be detected by receptor assays and most probably not by other screening methodologies.

In order to establish the theoretical impact of receptor assays in STA, three databases of pharmacologically and toxicologically relevant compounds were classified by their interaction with one or more receptor (sub)types. This classification can help to decide which particular receptor types should be applied in an RRA to be of particular interest in STA.

The applicability of three available RRA (anticholinergics, benzodiazepines and antihistaminics) for the analysis of urine samples collected after administration of 14 different

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drugs were analysed and the positive and negative outcomes were related to the registered drug use.

Materials and Methods

Assessment of receptor binding properties of compounds

All compounds of a toxicological database (database I, taken from ref. 7) containing 1791 substances have been classified according to their pharmacological action by means of literature data [8–10]. The type of receptors via which these pharmacological classes exert their action have been identified. While database I contains a large number of toxicologically less relevant compounds like plasticizers, pharmaceutical adjuvants, fatty acids and metabolites, these compoundss were rejected and the remainder formed database II. Metabolites were also rejected while there was insufficient information on the receptor binding properties (affinity as well as class of receptors) of metabolites which can differ substantially from the parent compound. For the development of screening systems in toxicology a test set of 99 basic substances has been used and forms database III (taken from ref. 11 and presented in Table 2). The number of substances, present in a particular database, interacting with one or more receptor types will be used for the selection of receptors which might be useful for systematic toxicological analysis.

Evaluation of the applicability of radioreceptor assays for anticholinergics, antihistaminics and benzodiazepines

Patients or healthy volunteers had taken one of the following drugs, mianserin, procyclidin, nitrazepam, flunitrazepam, medazepam, clomipramin, dextromoramide, butobarbital, cyclobarbital, heptobarbital, brallobarbital, clomethiazole, dothiepin or dipipanone chronically or as a single dose. Any co-mediation was registered. Urine samples were obtained from the Academic Hospital in Groningen and a 25-μl aliquot was analysed by the three radioreceptor assays. For each drug, urine samples of five persons were analysed.

The analytical procedures of the radioreceptor assays have been described in part 1 of this paper [12]. Positive test decisions were made at two levels for comparison purposes: reduction in binding of the radiolabelled ligand >50 or 25%, respectively.

Assessment of the impact of urine on binding of radiolabelled ligands

Two blank urine samples of six students were collected in the early morning. On one occasion they were allowed to take alcohol during the night, on the second occasion consumption was prohibited. Twenty-five microlitre aliquots were added to the three different radioreceptor assays and the reduction in binding of the radiolabelled ligands was determined.

Table 1
Overview of compounds from three databases interacting with pharmacological receptors

Receptor type	DI (N)	DI (%)	DII (N)	DII (%)	DIII (N)	DIII (%)
Benzodiazepine	79	4.36	42	3.27	12	12.12
Muscarinic	76	4.24	61	5.52	6	6.06
Histaminic	65	3.63	60	4.67	11	11.11
Opiate	109	6.09	77	5.99	13	13.13
Alpha-adrenergic	32	1.79	31	2.41	0	0.00
Beta-adrenergic	32	1.79	32	2.49	5	5.05
Alpha/beta-adrenergic	21	1.17	20	1.56	2	2.02
Dopaminergic	78	4.36	55	4.28	5	5.05
Nicotinic	9	0.50	9	0.70	1	1.01
Corticosteroid	5	0.28	4	0.31	0	0.00
Oestrogenic	9	0.50	9	0.70	0	0.00
rostagenic	5	0.28	4	0.31	0	0.00
Prostagenic	6	0.34	6	0.47	0	0.00
Sex hormones (other)	2	0.11	2	0.16	0	0.00
Database size	1791	100	1286	100	99	100
Receptor interaction	527	29.41	412	32.8	55	55.55

Results and Discussion

In Table 1 the number of substances which interact with a particular pharmacological receptor is presented for the three databases used. For obvious reasons the percentage of compounds which have a receptor interaction will increase unless many active metabolites exist. While benzodiazepines and opiates have many active metabolites, which are present in database I and not in database II, the number of compounds in database II, interacting with benzodiazepine and opiate receptors is substantially reduced. Evaluation of the third database with a selection of basic drugs, presented in Table 2, which are amongst the more toxicologically relevant substances also illustrates that the same pharmacological receptors are identified for screening purposes.

These data only give an indication about the

Table 2
Classification of the receptor interactions of the 99 basic substances originating from database III [11]

Substance	Code	Substance	Code
Amitriptyline		Flunitrazepam	B
Amphetamine		Flurazepam	B
Atropine	M	Haloperidol	D
Benzatropine	M	Hydrocodone	
Caffeine		Hydromorphone	O
Carbamazepine		Hydroxyzine	H
Chlordiazepoxide	B	Imipramine	
Chlormethiazol		Ketamine	
Chlorpromazine	D	Lidocaine	
Cimethidine	H	Lorazepam	B
Clomipramine		Maprotiline	
Clorazepic acid	B	Meclofenoxate	
Cocaine		Medazepam	B
Codeine	O	Mepyramine	H
Desipramine		Metamphetamine	
Desmethyldiazepam	B	Methadone	O
Dextromethorphan		Methapyrilene	H
Dextromoramide	O	Methaqualone	
Dextropropoxyphene	O	M.M.D.A.-2(#)	
Dextrorphan		Metoprolol	β-A
Diamorphine	O	Metronidazol	
Diazepam	B	Mianserine	
Dihydrocodeine	O	Morphine	O
Diphenhydramine	H	Morphine-6-acetaat	O
Dipipanone	O	Nadolol	β-A
Dipyridamole		Nicotine	N
Disopyramide		Nomifensine	
Doxepine		Nortriptyline	
Doxylamine	H	Orphenadrine	M
Ephedrine	αβ-A	Oxazepam	B
Oxycodone	O	Psilocin	
Papaverine	M	Quinidine	
Pentazocine	O	Quinine	
Pethidine	O	Strychnine	
Phenazopyridine	O	Temazepam	B
Phendimetrazine		Theophyline	
Phenethylamine		Thioridazine	D
Pheniramine	H	Timolol	β-A
Phenmetrazine		Tiothixine	D
Phentermine		Trazodone	
Phenylpropanolamine	αβ-A	Triamterene	
Phenyltoloxamine	H	Triazolam	B
Pindolol	β-A	Trifluoperazine	D
Prazepam	B	Trifluopromazine	
Procainamide		Trihexyphenidyl	M
Procaine		Trimeprazine	H
Prochlorperazine		Trimipramine	
Procyclidine	M	Tripelenamine	H
Promethazine	H	Verapamil	
Propranolol	β-A		

B = benzodiazepine; M = muscarinic; H = histaminic; O = opiate; β-A = beta-adrenergic; αβ-A = alpha/beta-adrenergic; D = dopaminergic; N = nicotinic.

usefulness of radioreceptor assays for screening. However, the prevalence is strongly affected by the number of prescriptions and should in fact be based on the actual presence of a compound of a particular pharmacological class in toxicologically relevant samples. However, this applies to any analytical technique. Moreover, large differences in prescribed drugs and prescription behaviour exist between countries. When one considers the opening of the European borders, one might expect a larger variety in drugs which might cause serious problems in STA when traditional physico-chemical methods are to be used [13].

On the basis of these data, radioreceptor assays employing benzodiazepine, muscarine, histamine, opiate, adrenergic and dopamine receptors might be meaningful in STA.

In our laboratory radioreceptor assays have been developed for anticholinergics, antihistaminics and benzodiazepines. A critical factor in the development of a bioassay is the interference caused by the biological matrix in which the drug of interest is present [5, 6].

The addition of urine (with or without alcohol consumption) caused some inhibition of the specific binding of the radiolabelled ligands as can be seen in Table 3. The intake of alcohol seems to have an inhibitory effect on the binding of ^{3}H-NMS to muscarinic receptor, while alcohol is completely converted in the human body this is an indirect effect probably caused by the presence of acetaldehyde in urine. Therefore a direct assay, without sample pretreatment, may be only possible when using small volumes of urine. It is anticipated that the addition of 25 µl of urine to the RRA for antihistaminics will not seriously affect the outcome of the assay.

Table 3
Inhibition of receptor binding of radiolabelled ligands employed in radioreceptor assays by blank urine; effect of alcohol consumption (N = 6; mean ± SD)

Urine samples	^{3}H-NMS (%)	^{3}H-FlU (%)	^{3}H-MEP (%)
No alcohol intake	2.4 ± 6.0	5.9 ± 5.7	0.0 ± 0.0
Alcohol intake	12.7 ± 2.9	7.7 ± 6.6	n.d.

n.d. = Not determined.

The 70 urine samples collected after drug intake, were analysed by means of the three radioreceptor assays, the percentage inhibition was calculated and positive/negative conclusions were based on criteria of 50 and 25% inhibition. The results are summarized in Table 4.

On the basis of pharmacological effects of the administered drugs, about 50% of all tested urine samples could theoretically give a positive test result in one or more receptor assays. Setting criteria of 25 or 50% inhibition should be based on the actual application of the methodology. In forensic toxicology detection of a particular class of drugs might be very important, which implies that the sensitivity should be as good as reasonably achievable in order to reduce the number of false negative outcomes.

In clinical toxicology and therapeutic drug monitoring determination of toxicological or therapeutic relevant concentrations should be possible in order to facilitate medical treatment. In all assays a small number of false-positive outcomes have been observed. With some samples false-positive and false-negative outcomes were obtained in more than one RRA. These false-positives might be caused by matrix interferences, which can be eliminated by more elaborate sample pretreatment procedures. If false-positives are caused by unregistered drug use, then they support the potential of RRA in STA in that we are able to detect potentially harmful substances, whose presence is unsuspected and whose identity is unknown.

Even with the 25% inhibition criterion, about 25% false-negatives were observed. This

Table 4
Percentage of positive and false-positive assay outcomes for the three radioreceptor assays. ^{3}H-NMS represents anticholinergic RRA, ^{3}H-FLU represents benzodiazepine RRA and ^{3}H-MEP represents antihistamine RRA

Test result	^{3}H-NMS (%)	^{3}H-FLU (%)	^{3}H-MEP (%)	Total (%)
Theoretical	14.3	24.3	28.6	51.4
50%-Criteria	4.3	5.7	21.4	27.1
False-positive	1.4	1.4	2.9	2.9
25%-Criteria	12.9	20.0	21.4	38.6
False-positive	1.4	4.3	2.9	5.7

is due to the use of small aliquots of urine in combination with low, subtherapeutic concentrations. The latter can be explained by the design of the study. A substantial part of the urine samples was collected after single dose administration. Increase of the sensitivity of the RRA for screening of urine samples can help to reduce the number of false-negatives.

The overall conclusion should be that these assays have a practical applicability which is in line with the theoretical estimations. In case the sensitivity of these assays should be drastically increased, sample pretreatment procedures are to be used.

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