METABOLISM AND PHARMACOKINETICS OF ERGOT PEPTIDE ALKALOIDS. CONTRIBUTION BY ANALYTICAL IMMUNOLOGY.

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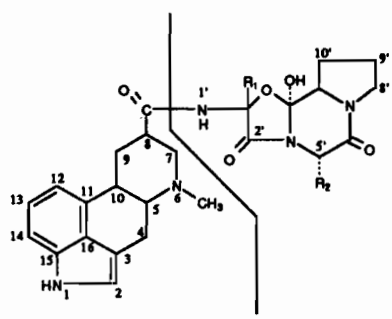
***Résumé** : les alcaloides de l'ergot de seigle représentent une classe importante au sein de l'arsenal thérapeutique moderne. Leur domaine d'utilisation est particulièrement étendu puisqu'il s'applique entre autres, à la maladie de Parkinson, aux troubles vasculaires liés à la sénescence cérébrale, aux migraines et aux hémorragies utérines. Une caratéristique des alacaloïdes de l'ergot de seigle est d'agir à faibles doses, une forte métabolisation, ce qui conduit à des taux plasmatiques très faibles. Parmi les nombreuses méthodes de détection utilisées pour les déceler dans les fluides biologiques, l'immunoanalyse apparaît être la meilleure méthode et satisfait les critères de spécificité, sensibilité et de facilité de mise en œuvre. La stratégie immuno-analytique (détection de la drogue et/ou de ses métabolites) et la synthèse de marqueurs d'activités très spécifiques offrent un outil performant pour les études de pharmacocinétique et de métabolisme.*

***Abstract** : ergot alkaloids represent an important class in the modern therapeutic arsenal. Their therapeutic uses are especially vast since they concern, among others, Parkingson's disease, vascular disturbances linked to senescence, migraine or uterin heamorrhages. A characteristic of ergot alkaloids is a low dosage and an intensive metabolization that contribute to very low plasma levels. Among the numerous methods used for their detection in biological fluids, immunoanalysis appears to be the best candidate to satisfy the criteria of specificity, sensitivity and practicability. The strategy of immunogen preparation (detection of the parent drug and/or metabolites) and the synthesis of tracers of high specific activity provides valuable tools to conduct pharmacokinetic and metabolism studies.*

The aim of this paper will be to describe, in a first part, the strategy developed to obtain sensitive immunological assays with predefined specificities in the case of derivatives of natural products. The second part will deal with the use of such tools to explore, after administration in man, the pharmacokinetics of such compounds and to contribute to the knowledge of their absorption, distribution, metabolism and excretion (ADME studies).



dihydrolysergic moiety	peptide moiety	
	R_1	R_2
Dihydroergotamine	CH_3	CH_2-Phenyl
Dihydroergotoxine		
Dihydroergocornine	CH_3	$CH_2CH(CH_3)_2$
Dihydroergocristine	$CH(CH_3)_2$	CH_2-Phenyl
Dihydroergocriptine (alpha)	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$
Dihydroergocriptine (beta)	$CH(CH_3)_2$	$CH_2CHCH_2CH_3$
Bromocriptine (Br in position C2 and 9-10)	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$

Figure 1 : chemical structures of main ergot alkaloids derivatives

Ergot peptide alkaloids constitute a chemical homogeneous group of molecules. They deriv from natural alkaloids extracted from the sclerotia of *Claviceps* species. *Claviceps* are parasitic fungi living on the ears of rye and other grasses (1). The alkaloids molecules structures may be share in two moieties (figure 1). First, the lysergic part constitutes with lysergic or Dihydrolysergic acid. The two acids differ by the presence of a double bound in C9-C10 position. This part may be substitute using bromide atom in the case of bromocriptine in position 2. The carboxylic function is engaged through a peptide bound to the peptidic moiety. This second part of the molecule is biosynthetized after reaction and cyclization of three amino-acids. In the case of Dihydroergotamine, the amino-acids are: alanine, phenylalanine and proline.

Dihydroergotamine is widely used for headache treatment. Dihydroergotoxin components (DHECT, DHEKT and DHECN), are used in the treatment of vascular disorders associated with aging. Bromocriptine is an agonist of D2 receptors ans it is a reference treatment of hyperprolactinemia and Parkinson's diseases. Metabolism of ergot peptide alkaloids takes place on peptide moiety where proline ring is submitted to hydroxylation in position 8,' 9' or 10'. Hydrolysis of the peptide bound linking cyclopeptide and lysergic ring and modifications of the lysergic part give others metabolites but in a lesser extended manner. Lysergic or Dihydrolysergic part is easely produced by using chemical degradation of ergot peptide alkaloids. Pure hydroxylated metabolites have not been yet chemically synthetized, until now, these products have been only obtained in small quantities after *in vitro* metabolisation (2).

As metabolic modifications take place mainly on peptidic ring with preservation of lysergic part, the immuno-analytical strategy was to develop two immunoanalytical procedures. One must use antibodies directed against the peptide moiety of the molecule and so able to detect only non metabolized drugs. A second series of antibodies has to be specific for the non modified part of the molecule (the lysergic ring). As a consequence, this system could be able to assay the drug but also all metabolites. By combination of the two assays, it could be possible to quantify separately the drug and its metabolites.

When injected in mammals, ergot peptide alkaloids do not elicit production of antibodies. In order to raised antibodies directed against alkaloids, it is necessary to covalently coupled them to a carrier protein. First, Dihydrolysergic acid has been linked to Bovine Serum Albumin (BSA) by formation of a peptide bound between carboxylic acid and primary amine function of BSA. Same procedure has been used to graft Bromolysergic acid to BSA in the case of Bromocriptine assay. Antibodies directed against non metabolized drug have been raised using other kind of immunogens. It was done by covalent coupling of the alkaloid (DHE, DHECT,...) to BSA (or others' carriers proteins) using formaldehyde as coupling agent. It has been shown that reaction takes place between primary amine groups of the carrier protein (e.g., lysine) and nitrogen in position 1 on the indol ring. Two groups of polyclonal antibodies have been obtained and used in combination with radioactive or enzyme labelled tracers to performe immunoassays (3,4). In all cases, immunoassays appear very sensitive. IC_{50} is around 100 pg/ml. and limit of detection of the systems may be evaluated between 10 and 20 pg/ml allowing a detection of plasma concentrations near to $3\ 10^{-11}$ M.

System A	Compound	System B
100	Dihydroergotamine	100
<0.01	Dihydrolysergic acid	55
9	Dihydroergotoxine	94
0.2	Dihydroergokryptine	87
48	Dihydroergocristine	105
32	Ergotamine	4.7
<0.01	Lysergic acid	4.5
0.2	Bromocriptine	24
<0.01	Lisuride	<0.01
<0.01	Tryptophane	<0.01

Table 1 : Specificity of two immunoassays of Dihydroergotamine (DHE). Cross reactivity index of various coumponds in the two systems are expressed in % of reractivity of DHE

The specificity of the antibodies used in these assays has been evaluated. Specificity was first evaluated using the ability of several derivatives to bind the antibodies. As exemple, we will show here the case of the assays performed to measure DHE in plasma (5). System A uses antibodies raised using BSA coupled with intact DHE as immunogen (Table 1). System B uses antibodies raised after immunization with BSA coupled to Dihydrolysergic acid. In this system, all compounds that possess intact Dihydrolysergic moiety are recognized irrespectively of the nature of the peptidic moiety (DHLS, DHE, DHEKT). When Dihydrolysergic part is modified, we observe an important loss in binding capacities toward the antibodies.

Ergotamine that differs from DHE by reduction of the C9-C10 bound is not well recognized. In contrary, in system A, peptide part appears important for recognition by antibodies. All alkaloids that possess peptide part identical or analog to DHE cross react in the system A. Loss (DHLS) or modification (DHEKT) of this part totaly abolish the recognition. Modification on lysergic ring does not imply loss of affinity. This is the case of ergotamine. Eventhrough, we have injected complete molecule coupled through its lysergic part, antibodies are predominantly directed against the peptide part of the molecule. The two systems have therefore different specificities.

One (system A) is directed against peptidic part of the molecule, one (system B) is directed against lysergic part. Metabolites of DHE have been generated using *in vitro* incubation with hepatic microsomes from rats (6). Metabolites have been purified using HPLC. It has been further demonstrated that no one metabolite is detected in system A except of course DHE. In contrary, system B assays DHE as well metabolites in the same manner.

Using: 1/ **chemical modification** of the drug, 2/ **immunochemistry** and 3/ **radiolabelling or enzyme labelling techniques**, it has been possible to obtain two kinds of powerful set of assays able, in one hand, to detect non metabolized drug and, on the other, to detect all metabolites of a same drug. Using this strategy, our laboratory has developed assays for 8 ergot peptide alkaloid derivatives already marketed or under development. This includes DHECT, DHE or Bromocriptine. Methods have been validated according Food and Drug Administration (FDA) guidelines and have been demonstrated to be suitable to determine amounts of drug in plasma.

Such assays may contribute to define the bioavailability of a drug and to investigate its biotransformations.

The kinetic profiles in plasma of DHECT after single oral (4.8 mg) or intravenous (0.6 mg) administration have been evaluated in 12 human volonteers. Assay has been performed using a system identical to system A. Non metabolized drug may be followed up to 24 hours in plasma in both cases. However, levels of DHECT are much higher after intravenous administration than after oral administration irrespective of the dose. Using these data and an appropriate mathematical model it is possible to evaluate the global bioavailability of this alkaloid. Only 3 % of the dose reach the blood circulation after oral administration. This low bioavailability may be due to low absorption and/or extensive metabolism in liver (so call first pass effect). Animal and human experiments performed using radioisotopes labelled molecules (^{3}H and ^{14}C) have been demonstrated that absorption of ergot alkaloids ranges from 30 to 80 % (7). As a consequence, in the case of DHECT, low bioavailability is mainly due to first pass effect. This is also the case for DHE or DHEKT (8).

The plasma profiles of DHE and DHE metabolites have been separately evaluated after oral administration of 5 mg (i.e. 70 µg/kg) in man. Maximal DHE plasma concentration is obtained between 15 and 30 minutes indicating that absorption of DHE takes place rapidly (5). DHE disappears very rapidly with an apparent half-life of 1/2 hour. In the same time, metabolites appear rapidly, as a consequence of first pass effect, with half time of disappearance greater than DHE, near to 4 hours. Comparison of the two curves and use of an adequate mathematical model allowed to calculate a ratio of metabolization of 95 %. Other workers have demonstrated that DHE metabolites (monohydroxylated) have a pharmacological action identical to DHE and are responsible for duration of the action observed after clearance of DHE (9).

Figure 2 shows results of pharmacokinetic studies performed after oral administration of 5 mg Bromocriptine (BKT) in man. Bromocriptine is used at this daily dose in the treatment of hyperprolactinemia.

As shown here, plasma levels of prolactine decrease rapidly after BKT administration. Maximal effect is observed at 4 hours and 24 hours after administration, substantial inhibition is still observed.

Non metabolized BKT has been evaluated in plasma. Maximal concentration is observed 1 hour after administration and at a level of 120 pg/ml (i.e. 10^{-10} M) and no BKT was detectable at 24 hours. BKT levels in plasma and effect intensity do not fit correctly. Better correlation exists when plasma levels of BKT metabolites are taken into account. Maximal plasma level is reached at 4 hours corresponding to the time when maximal effect is observed. BKT metabolites are still detectable 24 hours after administration. Correlation is only correlation and it does not implicate the existence of active metabolites. Investigations are under progress to performe a Pharmacodynamic / Pharmacokinetics (PK/PD) simulation of these data (10) as it has been already done for a dopamine agonist (11).

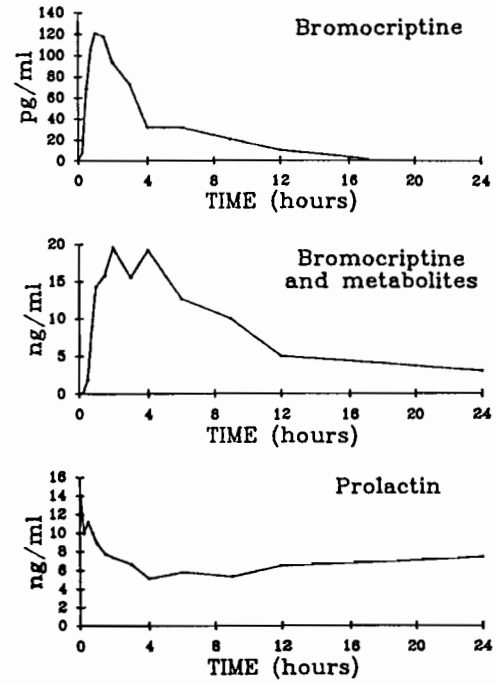


Figure 2 : Evolution of Bromocriptine, Bromocriptine metabolites and Prolactine after single oral administration of 5 mg Bromocriptine in man (mean of 12 subjects)

As a conclusion, we would like to stress that if drugs act on organism through receptor binding sites, the organism interacts also on drug using distribution, elimination and metabolism. It is a hazardous journey for a drug to reach its target into the body. And to our point of view, immunoassays are useful tools to investigate this journey and to improve new pharmaceutical preparations.

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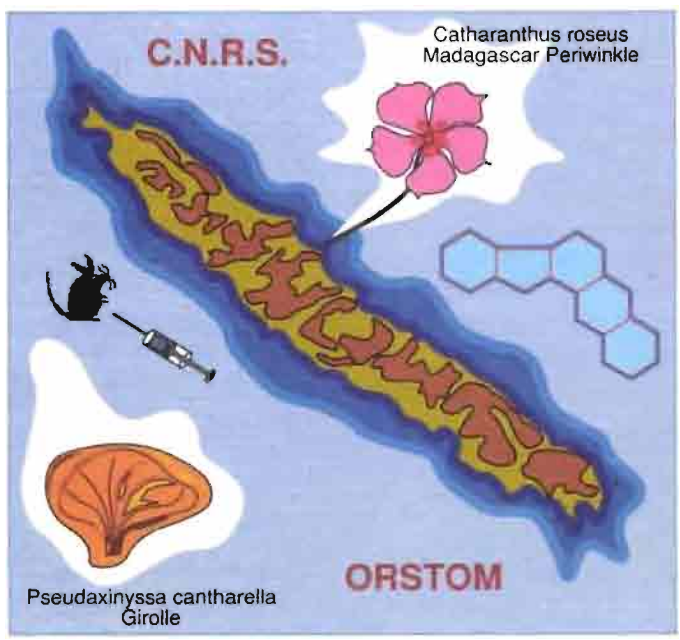

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