GIRODAZOLE :
"FROM THE LAGOON OF NOUMEA TO CANCER PATIENTS"

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Résumé : le Girodazole est une nouvelle molécule antitumorale isolée à partir de l'éponge
Pseudaxinyssa cantharella *récoltée en Nouvelle Calédonie. Cette molécule a été sélectionnée pour des études cliniques du fait de sa structure nouvelle, de son mécanisme d'action (inhibition de la biosynthèse des protéines) et de son activité antitumorale expérimentale. Des études cliniques Phase 1 ont été effectuées. La toxicité limitante est une sévère hypotension. Les études cliniques ont été arrêtées.*

Abstract : Girodazole is a new antitumor agent from the sponge Pseudaxinyssa cantharella *collected in New Caledonia. It has been selected for clinical trials due to its new structure, mechanism of action (inhibition of protein synthesis) and experimental antitumor activity. Phase 1 clinical studies were done and the dose limiting toxicity was a severe hypotension. Clinical development has been stopped.*

1. Introduction

The first evidence of *in vivo* experimental antitumor activity on P388 leukemia has been obtained in 1980 with an aqueous extract of the sponge *Pseudaxynissia cantharella* collected by ORSTOM in Noumea. The chemical structure of the active compound was identified three years later (1). In 1985, RHONE-POULENC SANTE made the decision to go ahead and to have clinical studies with Girodazole ("girolline", RP 49532A). 150 kg of sponge were collected by ORSTOM in Noumea and one year later, our colleagues of CNRS provided us with 100 g of "pure natural Girodazole" for preclinical and early clinical studies. At the same time, the first total synthesis of the racemate was done in CNRS, opening the way for an alternative source of active compound. Clinical studies were started in 1989 and were stopped one year later.

2. Experimental antitumor properties (2)

2.1. Antitumor properties: Girodazole has *in vitro* antiproliferative properties on several murine and human cell lines. The IC_{50} values range from 0.01 µg/ml to 0.04 µg/ml. Girodazole is as active on murine leukemia cells P388 than on P388 cells resistant to anthracyclines and vinca alkaloids. This is an important point since drug resistance is one of the major causes of failure of chemotherapy in patients. New drugs active against resistant cells are highly needed.

The important antitumor activity is obviously the *in vivo* one. Activity is evaluated against a panel of murine grafted tumors including leukemias and solid tumors. Girodazole given i.p. or i.v. is active on some of these models : P388 and L1210 leukemias where significant increases of life span were observed in treated animals ; MA16/C mammary adenocarcinoma adn M5076 histiocytosarcoma where inhibition of tumor growth were obtained

after i.v. administration. Girodazole has remarkable activity against M5076 histiocytosarcoma which leads to a massive and rapid metastatic spread to the liver. Girodazole is able to induce complete regressions of advanced M5076 tumors grafted s.c. and it can also inhibit the formation of liver metastases (Table 1).

2.2. Mechanism of action : the second objective of the pharmacological studies is to get information about the mechanism of action. When we looked for the first time at the chemical structure of Girodazole, we hypothesised that Girodazole would be a DNA alkylating agent. 1- The imidazole ring contains a guanidinium moeity which is known to have a high affinity for DNA. 2- The lateral chain has functions able to generate epoxide or aziridine entities.

We started our investigations by cellular experiments of inhibition of DNA, RNA and protein synthesis (Table II). P388 cells were labelled with radioactive thymidine, uridine and leucine in the presence of different concentrations of Girodazole. The macromolecular incorporations of radioactive thymidine, uridine and leucine reflect respectively DNA, RNA and protein synthesis. The values are of inhibition are indicated into brackets. A reproductible and preferential inhibition of protein synthesis was noted in P388 cells. At concentrations of 38 µM and 7,6 µM, maximal inhibition was noted on protein synthesis ; at 1.5 µM, only protein synthesis inhibition was observed.

The inhibition of protein synthesis observed in cells was confirmed in several acellular models. The results obtained in one of these models are presented on Fig.1. RNA from Tobacco Mosaic Virus (TMV) is used as a model of mRNA. The ribosomes and the different proteic factors required for eucaryotic protein synthesis were prepared from a rabbit reticulocyte lysate. TMV-RNA and rabbit reticulocyte lysate were incubated for 30 minutes without and with different concentrations of Girodazole. The profile of synthetised viral proteins was analysed by acrylamide gel electrophoresis. Control corresponds to Lane 1. Lane 5 corresponds to the mixture in the presence of 10 µM Girodazole. A full inhibition is noted. Lane 6 corresponds to 1 µM Girodazole and the inhibition is still important. We established that Girodazole has no direct action on DNA and RNA and acts directly on the proteic components and inhibits the elongation/termination steps.

Therefore, Girodazole was selected for 3 reasons 1- Girodazole has experimental antitumor activity *in vivo*. This activity is limited to some models but it is always difficult to extrapolate from the lab to the clinic with new coumpounds. 2- Girodazole has a chemical structure different from those of all the other antitumor compounds. 3- Girodazole has an unusual mechanism of action : it blocks the translation at the elongation/termination steps.

3. Toxicological studies

Toxicological studies have two objectives :
1- To provide clinicians with information about potential toxic effects of the compound.
2- To determine a safe starting dose for administration in humans.

Toxicological studies are done in mice and in dogs. Acute and subacute protocols of administration are used.

The determination of the starting dose in clinic is one of the issues of the toxicological studies. According to NCI, the LD_{10} in mice is determined. This value is expressed in mg/body surface area and 1/10 of the murine LD_{10} is administred in dog. If no toxicity occurs, this dose is used as starting dose in clinic. On the other hand, if toxicity occurs, lower doses are administred in dogs until determination of a non toxic dose. Then 1/3 of the non toxic dose in dog is used as starting dose in clinic.

The toxicities observed in mice and in dogs with Girodazole are listed in Table III. As opposite with intercalating and alkylating agents, only mild hematotoxicity was noted with Girodazole. The main toxicity observed in both species was hepatotoxicity. Hepatotoxicity

occurs as microvesicular fatty liver. This toxicity is cumulative, however it is reversible in dog. Hepatotoxicity can be understood for a protein synthesis inhibitor, since protein synthesis occurs predominantly in liver. Inhibition of synthesis of lipoproteins which are required for the transport of lipids out of the liver could effectively result in abnormal lipid accumulation in the liver and explain the progressive development of microvesicular fatty liver.

LD_{10} in mice is equal to 21 mg/m^2. 1/10 of the LD_{10} administered in dogs caused minimal toxicity. Therefore 2.1 mg/m^2 was choosen as the starting dose for clinical studies.

4. Clinical studies (3)

Clinical studies in oncology comprise Phase I and Phase II studies. The issue of Phase I is not to observe clinical activity per se but to determine the Maximal Tolerated Dose (MTD) which can be administered. The tolerance study is done in patients refractory to conventional therapies. Phase II deals directly with clinical activity. Patients with various tumor types are treated at the MTD and clinical efficacy is evaluated in terms of tumor regressions and of survival benefit.

Three Phase I studies were done in Europe. The first one at the Institut Gustave Roussy in France in which Girodazole was given as an i.v. 1 hour infusion every 3 weeks. The second one in Centre Léon BERARD in which Girodazole was given as an i.v. 24 hours infusion every 3 weeks. The third one in Geneva in which Girodazole was administered weekly for 6 consecutive weeks.

Starting dose was 3 mg/m^2 The MTD was considered to be 15-17 mg/m^2/dose in the different schedules. The dose limiting toxicity observed during dose escalation was hypotension. This hypotension was severed delayed, of long duration and uncontrolled by antidotes. Other toxicities include fatigue, myalgias, nausea and vomiting and alterations of hemostasis. Hepatotoxicity was expected according to animal studies but appeared to be minimal.

Pharmacokinetic studies were done. At the MTD, the peak plasma levels of Girodazole are equal to 0.7 µg/ml. The value is much lower than that of 4 µg/ml which is required for having antitumor activity in mice. At the MTD, the biodisponibility of Girodazole is low, probably due to the polarity of Girodazole and its fast excretion in urine. Finally, no hint of clinical activity was noted among the 50 treated patients. For all these reasons, the recommandation of the three Investigators was not to proceed to Phase II studies.

5. Conclusion

Since the beginning of medical oncology fourty years ago, breakthroughs have been always obtained with compounds having new structure and/or new mechanism of action. Girodazole was an attractive potential candidate due to its new structure and mecanism of action.

We consider that natural compounds still offer today the best chances to discover next innovative antitumor compounds. Indeed, the most promising compounds undergoing clinical evaluation are two compounds from natural origin.

1- Taxol and taxotere, antimitotic drugs inhibiting the depolymerisation of microtubules.

2- Water soluble derivatives of camptothecin, and old drug which has been recently revisited and which inhibits topoisomerase I.

References
1.   *C. R. Acad. Sci. Paris* **307** (II) 145-148 (1988)
2.   *Investigational New Drugs* **9**, 233-244 (1991)
3.   *Proceedings of the 27th Annual Meeting of the American Society of Oncology, Abstract* **310** (1991)

Tumor, graft, criteria of evaluation	Route of treatment and schedule	Optimal daily (mg/kg)	dose Optimal effect	Activity rating (NCI criteria)
P388 leukemia,i.p. % increase of life span	i.p. daily days 1-9	0.36	81	++
P388 leukemia resistant to doxorubicin,i.p. % increase of life span	i.p. daily days 1-4	0.72	51	+
L1210 leukemia,i.p. % increase of life span	i.p. daily days 1-9	0.36	43	+
MA16/C mammary a denocarcinoma s.c. tumor growth inhibition (%TGI)	i.v.daily days 1-4	0.72	94	++
M5076 histiocytosarcoma i.f.p., metastases growth inhibition (%MGI)	i.v. daily days 14,18,20,22	1.45	100	++

Table I : *In vivo* experimental antitumor activity of Girodazole.

Group	Incorporated radioactivity (cpm ; mean + sd)		
	DNA	RNA	Protein
Control	11700 ± 2300	68700 ± 6300	14000 ± 1400
Girodazole 38mM	9000 ± 1400 (32)	44500 ± 2300 (35)	4500 ± 200 (68)
Girodazole 7.6mM	12800 ± 700 (0)	52000 ± 4000 (24)	6300 ± 150 (55)
Girodazole 1.5mM	12900 ± 1300 (0)	69000 ± 5000 (0)	9200 ± 1300 (34)

TABLE II : Effect of Girodazole on DNA, RNA and protein synthesis in P388 cells

Type of toxicity	Mice	Dog
Myelosuppression	+	-
Hepatotoxicity	+	+
Digestive toxicity		+
ECG changes		+
Testicular toxicity	+	+
Skeletal muscle toxicity	+	-
Nephrotoxicity	-	+
Pancreas toxicity	-	+

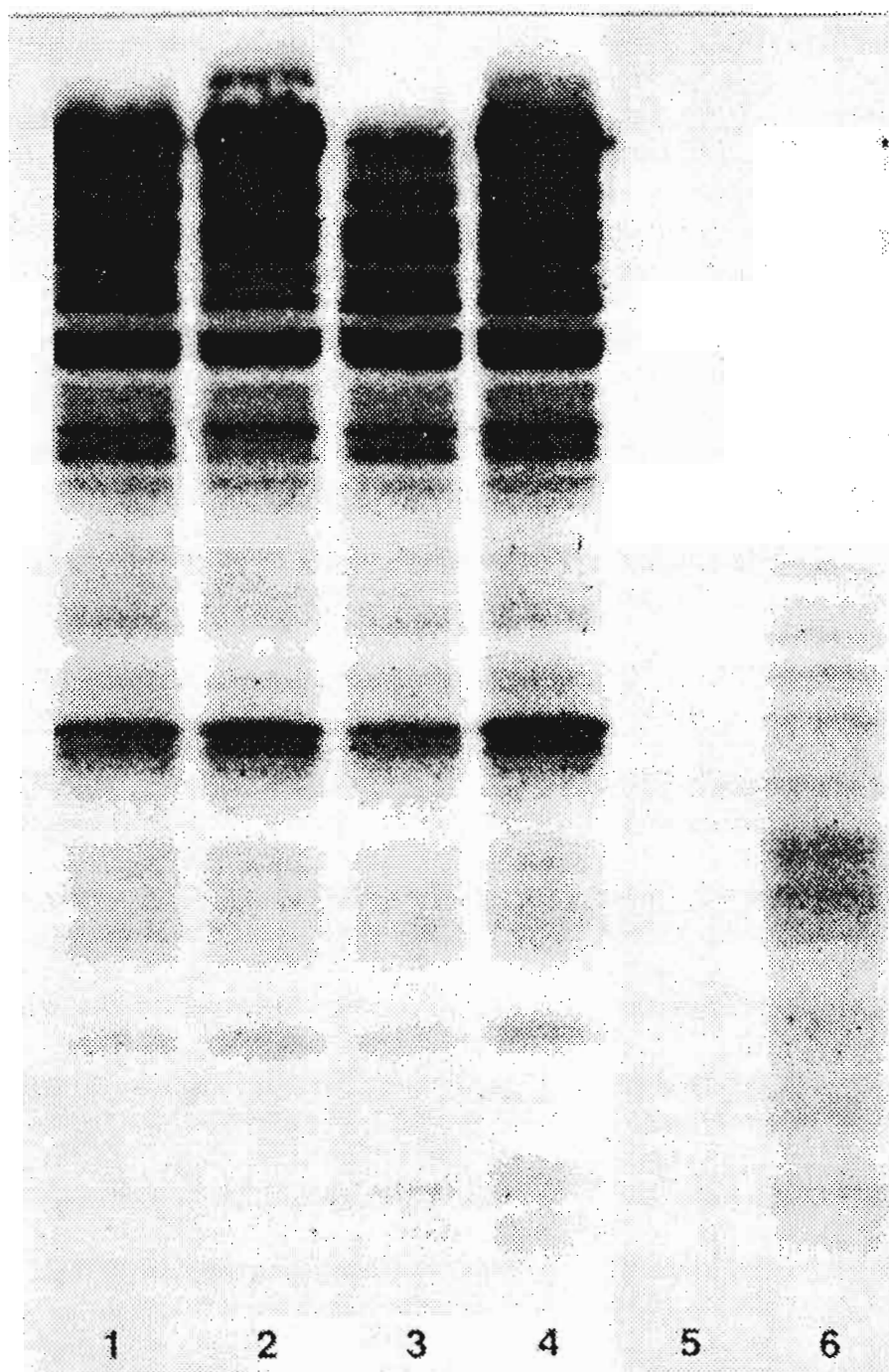TABLE III : Toxicities of Girodazole

Figure 1 : Effect of Girodazole on translation of RNA from TMV.

Translation of RNA from tobacco mosaic virus is done for 15 min (lanes 1,3,5) or 30 min (lanes 2,4,6).
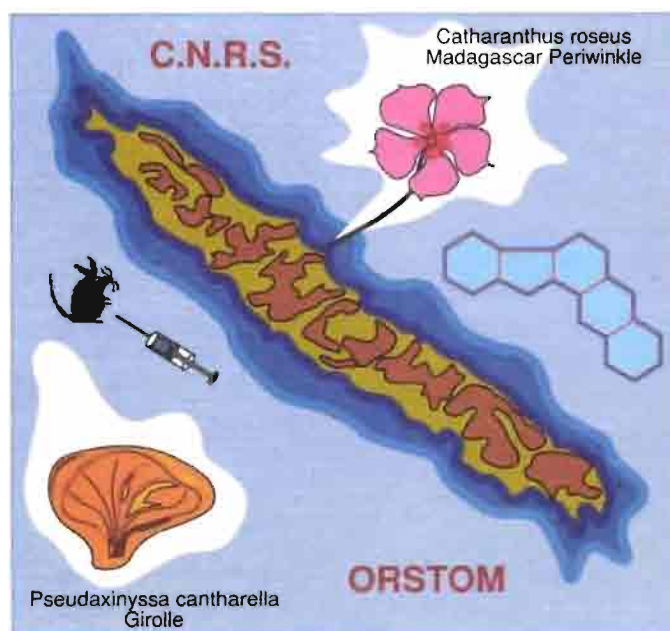
Lanes 1,2 : RNA translation without RP 49532A.
Lanes 3,4 : RNA preincubation for 30 min with 10mM RP 49532A followed by purification of RNA and by translation without RP 49532A.
Lanes 5,6 : RNA translation with 10mM RP 49532A.

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ACTES

C.N.R.S.

Catharanthus roseus
Madagascar Periwinkle

Pseudaxinyssa cantharella
Girolle

ORSTOM

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