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In Vitro and *In Vivo* Effects of $[Ni(M5FTSC)_2Cl_2]$ Complex in Cancer: Preliminary Tests

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Abstract. *The human colon adenocarcinoma - derived cell line CaCo-2, male Swiss mice and male Sprague - Dawley rats were used as model systems to study the effects of nickel (II), 5 - methyl - 2 - furaldehyde thiosemicarbazone ligand and their complex. The stimulation effect of the complex was obtained with low concentrations from $3.7.10^{-7}$ to $3.7.10^{-6}$ M, while inhibiting effects occurred from $3.7.10^{-5}$ to $3.7.10^{-4}$ M. This study showed that Ni (II) is toxic for cultured cells. Our data suggest that the complex, according to the toxicity assays in mice and carcinogenesis assays in rats, could be used as an antimitotic agent.*

We recently described the potent cytotoxicity of the complex $[Cu(H_2L)(H_2O)_2]$ $Cl_2.4H_2O$ towards various leukemia, lymphoma and carcinoma cells (1). We have demonstrated that this complex causes a 10% reduction in DNA topoisomerase II inhibition at 150 μM. The cytotoxicity of some copper complexes has been characterised in our laboratories. However, copper (II) by itself is toxic according to our experimental conditions (2). In our investigations of new antimitotic metal complexes, we have described the stability *versus* time of a nickel salt ($NiCl_2$) and its complex with 5 - methyl 2- furaldehyde thiosemicarbazone ligand (3). We have also determined and compared their cytotoxicity in cultured CaCo-2 cells, their toxicity in Swiss mice and their anatomical effects in male Sprague-Dawley rats treated with 5-methyl N-nitrosourea, a colon carcinogen.

Materials and Methods

Chemicals. The synthesis and the chemical characterisation of the ligand (Figure 1) 5-methyl 2-furaldehyde thiosemicarbazone (M5FTSC) and of the complex $[C_{14}H_{16}N_6NiO_2S_2]$ have been previously described (3). Thiosemicarbazone, hexahydrated nickel (II) chloride, 5 - methyl 2 - furfural and 5 - methyl N - nitrosourea of analytical grade were purchased from Merck, Prolabo (Paris, France) and Aldrich (St. Quenting, France), respectively and were used without further treatment. Dulbecco's modified Eagle essential medium (DMEM) was from Jacques Buy (Paris, France). All other biological chemicals were from Eurobio (Less Ulis, France.

Cell cultures. CaCo-2 cells, from passage 75 to 100, were routinely cultured in a culture flask at 37°C, in a humid atmosphere containing 90% O_2 and 10% CO_2, and in DMEM containing 20% foetal calf serum, 1% penicillin - streptomycin and 1% non essential aminoacids (AANE). The culture was fed every 4 hours. The medium was changed and new treatments were added to the cultures in aqueous solutions. The cells were trypsinised and counted using a hemocytometer.

Cytotoxic activities test. To perform cytotoxic activity, the cells were plated in 96 - well tissue culture plates (10^{10} per cm^2). After overnight growth, the medium was changed to DMEM with the studied molecules at various concentrations. The cells were washed with phosphate-buffered saline (PBS), fixed with 20% (V/V) trichloracetic acid and the plates were washed with PBS five times and then dried for 30 minutes. Sulforhodamin B (SRB) in acetic acid (0.04% W/W) was added and left for 30 minutes. The plates were rinsed three times with 1% acetic acid. SRB was extracted with tris - base buffer at pH = 10.4. The incorporation of SRB to the cells depended on protein synthesis.

Toxicity test. Swiss mice from Debre (St. Doulchard, France), weighing from 20 to 25 g, were used because of their regular weight gain and the absence of oestral cycle with changes in hormonal status, which could lead to some errors in *in vivo* experiments. The animals had free access to both water and a standard diet containing 0.8% Ca, 0.7% P and 3 IU.g -1 vitamin D. They were placed at room temperature (20 ± 2°C) and exposed to a 12-hour light / 12-hour darkness cycle. After being administered by intraperitoneal injection *(i.p.)* with various doses of the studied compounds, the animals were kept under the same conditions and were observed for 7 days.

Carcinogen treatment. Male Sprague - Dawley rats from Debre (St Doulchard, France), weighing from 125 to 150 g., were used for the same reasons as the mice. They were kept under the same conditions as described above. The animals were *i.p.* injected as follows: 5 received NaCl 0.9 $g.L^{-1}$ while 10 were treated with carcinogen (20 $mg.kg^{-1}$) (4). They were kept under the same conditions for 20 days, after which the complex was orally injected to 5 carcinogen rats for 10 days. The rodents were killed by asphyxiation and the sigmoid flexure and rectum were removed, opened and placed on a Petri dish and photographed with numerical Nikon Coolpix 950 apparatus.

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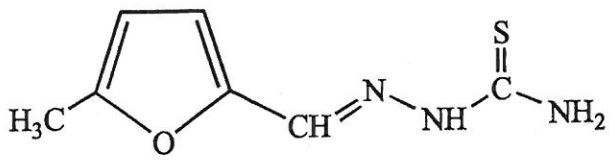

Figure 1. *Chemical structure of the ligand.*

Results

Cytotoxic test. The main results are presented in Table I and Figure 2. It may be noted that the mitosis of cultured CaCo-2 cells was inhibited by concentrations higher than $3.7.10^{-7}$ M ($NiCl_2$), $3.7.10^{-7}$ M (ligand) and $3.7.10^{-5}$ M (complex).

Toxicity in mice. 1 - $NiCl_2$: The effects of this compound on male Swiss mice proved that this salt is toxic. The lethal dose zero (LD_0) was 0.65 $mg.kg^{-1}$ body weight (bw); LD_{25} was 0.95 $m.kg^{-1}$; LD_{50}: 2 $mg.kg^{-1}$ bw; LD_{75}: 2.85 $mg.kg^{-1}$ bw and LD_{100} was 4 $mg.kg^{-1}$.

Table I. *Cytotoxicity of Ni (II), ligand and their complex on CaCo -2 cells in culture (mean ± SD).*

	Viability percentages		
Concentration (mol. L^{-1})	Metal	Ligand	Complex
$3.7{\cdot}10^{-12}$	75 + 12	57 + 5	73 + 20
$3.7{\cdot}10^{-11}$	50 + 15	56 + 25	70 + 23
$3.7{\cdot}10^{-10}$	42 + 9	71 + 15	43 + 20
$3.7{\cdot}10^{-9}$	138 + 8	47 + 8	40 + 17
$3.7{\cdot}10^{-8}$	85 + 7	13 + 1	27 + 8
$3.7{\cdot}10^{-7}$	162 + 17	49 + 6	19 + 2
$3.7{\cdot}10^{-6}$	64 + 2	40 + 6	55 + 14
$3.7{\cdot}10^{-5}$	56 + 5	29 + 2	80 + 12
$3.7{\cdot}10^{-4}$	41 + 8	30 + 9	8 + 2

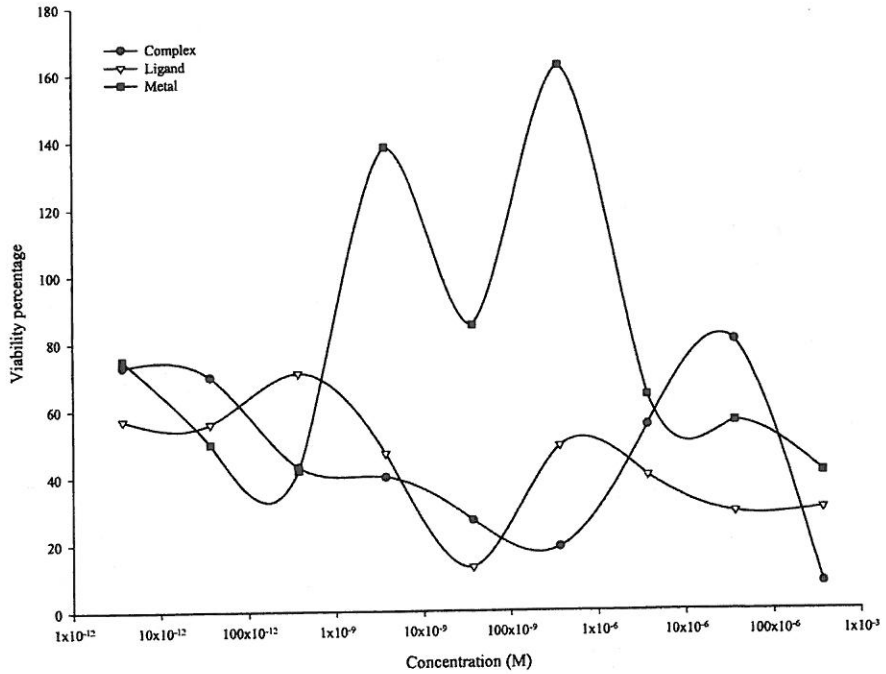

Figure 2. *Viability percentages versus concentration (logarithmic scale).*

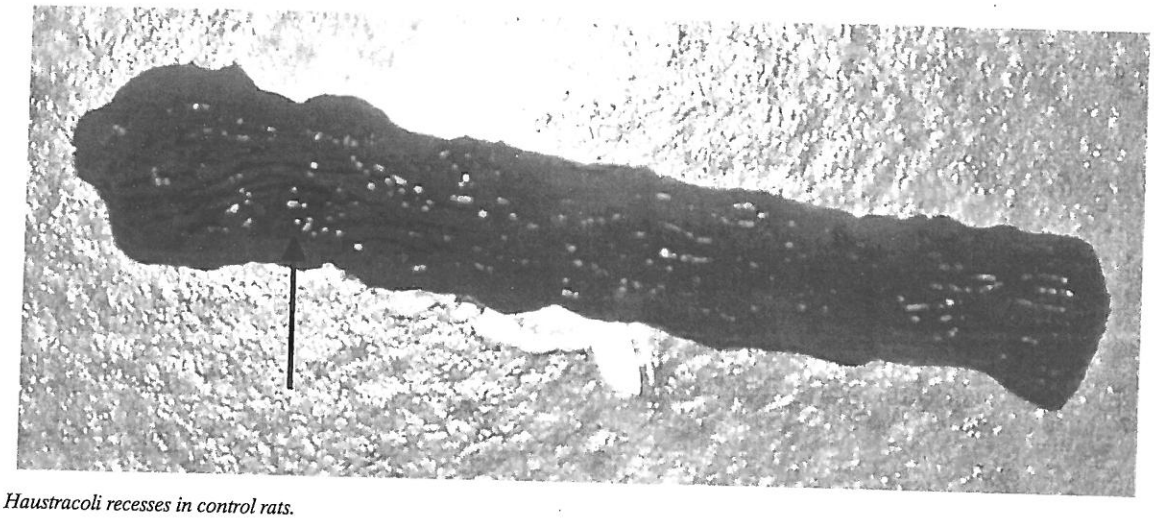

Figure 3. *Haustracoli recesses in control rats.*

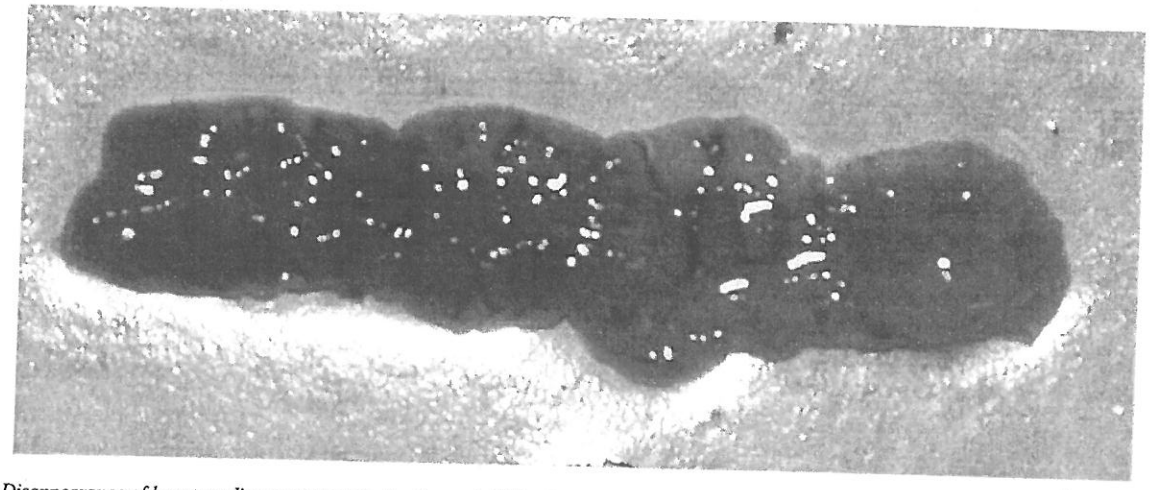

Figure 4. *Disappearance of haustracoli recesses in rats after 5 - methyl N - nitrosourea treatment.*

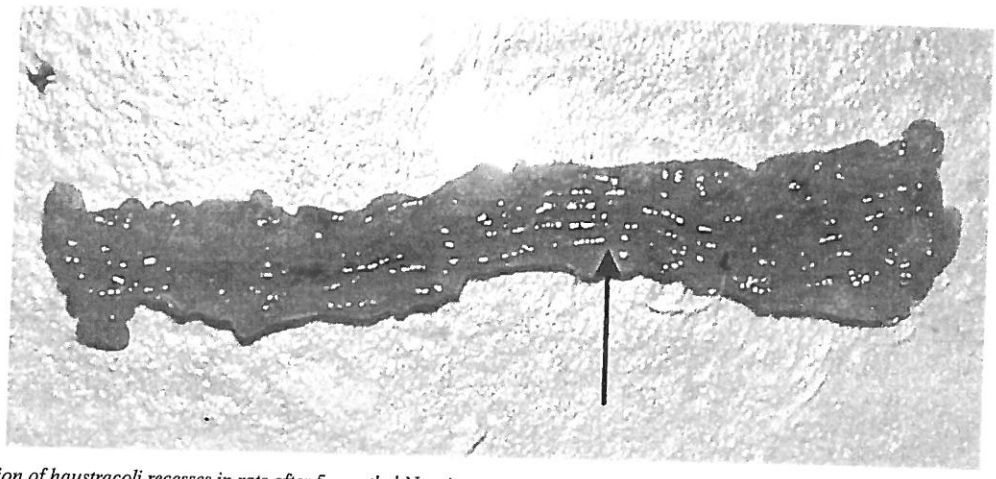

Figure 5. *Restoration of haustracoli recesses in rats after 5 - methyl N - nitrosourea and complex treatments.*

2 - Ligand: LD_0: 0.9 $mg.kg^{-1}$ bw; LD_{25}: 1.8 $mg.kg^{-1}$; LD_{50}: 2.7 $mg.kg^{-1}$ bw; LD_{75}: 5.4 $mg.kg^{-1}$ bw and LD_{100} 5.5 $mg.kg^{-1}$.
3 - Complex: LD_0 : 5.5 $m.kg^{-1}$ bw; LD_{25}: 6.8 $mg.kg^{-1}$; LD_{50}: 8.2 $mg.kg^{-1}$ bw; LD_{75}: 11.0 $mg.kg^{-1}$ bw and LD_{100} 16.0 $mg.kg^{-1}$.

Carcinogen treatment in rats. 1 - Treatment with NaCl 0.9 $g.L^{-1}$ did not affect the haustracoli recesses (Figure 3).
2 - In rats receiving 5-methyl N-nitrosourea, the disappearance of nearly all the haustracoli recesses was observed (Figure 4).
3 - Treatment by both 5-methyl N-nitrosourea and the complex restored nearly all the recesses (Figure 5).

Discussion

We have already tested cobalt (II) and copper (II) cations as anticancer treatment with some interesting results (5, 6). The Ni (II) cation, when taken orally, is about 10% absorbed by the gastric mucosa and can have some antimitotic properties (7). Unfortunately, Ni (II) salts are toxic by themselves as well as oxides and nickel carbonyls. When chronically absorbed, they could induce carcinogenesis and, therefore, they cannot be used as anticarcinogens. CaCo - 2 cells are a cell line deriving from human colon adenocarcinoma of malignant origin (8) and the complex $[Ni(M5FTSC)_2Cl_2]$ was used to control colorectal cancer cell growth.

Figure 2 shows that the ligand and Ni (II) inhibited cell proliferation at $3.7.10^{-7}$ M, while the complex stimulated the cancer cultured cells mitosis from $3.7.10^{-5}$ to $3.7.10^{-4}$ M. These facts suggest that the complex is less toxic than the free ligand and $NiCl_2$ in cultured cells. These *in vitro* observations are corroborated by our toxic studies in mice: Ni (II) had a LD_{100} up to 4 mg.kg -1 bw, while the LD_{100} ligand was 5.5 $mg.kg^{-1}$ bw and their complex did not kill any animal at this dose. These results, obtained from mice, allowed us to continue the study with male Sprague - Dawley rats.

Anatomical observations showed that haustracoli recesses disappeared 30 days after carcinogen administration and were restored when the rodents were treated with the complex. Our experiments indicated that a period of thirty days was not sufficient to initiate significant colorectal cancer; however the results obtained up till now are encouraging for the pursuit of our work.

Finally, we can assume that the complex $(Ni(M5FTSC)_2Cl_2]$ can be used to inhibit $CaCo^{-2}$ cell mitosis, because of its lower toxicity and on account of the fact that it helps in restoring haustracoli recesses in male Sprague - Dawley rats.

In further experiments, we should take into account these data in experiments with rats in which induction of colorectal cancer is delayed (160 days at least) and then study the carcinogen and complex effects histologically.

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