

# RADIOFREQUENCY ASSOCIATED WITH INTRATUMORAL LIPOSOMAL CHEMOTHERAPY. STUDY DESIGN FOR AN EXPERIMENTAL MODEL ON LIVER COLORECTAL METASTASES IN RATS

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## REZUMAT

**Introducere:** Cancerul colorectal (CCR) a devenit în ultimele două decenii una dintre tumorile cele mai frecvente. Distrucția locală a metastazelor hepatice nerezecabile de CCR, cu ajutorul radiofrecvenței, reprezintă o alternativă terapeutică grevată însă de o rată crescută de recidivă. **Scop:** Prezentul proiect urmărește creșterea eficienței tratamentului metastazelor hepatice nerezecabile ale CCR, prin asocierea radiofrecvenței cu chimioterapice administrate intratumoral ca soluții hipertermice lipozomale. Un scop secundar este aprecierea rolului diferiților factori de creștere în apariția recidivei locale și în raport cu tipul tratamentului. **Material și metode:** Lipozomii încărcăți cu doxorubicină, oxaliplatină sau irinotecan vor fi preparați pe baza fosfolipidelor naturale. Vor fi utilizați 70 șobolani rasa Wag/Rij împărțiți în 4 loturi, la care se vor inocula în ficat celule de CCR (cultura CC531). La primul grup se va evalua ritmul de creștere a metastazelor. În ultimele trei grupuri, metastazele vor fi distruse prin aplicarea radiofrecvenței, singură sau în asociere cu chimioterapia (hiper- sau normotermică). Eficiența terapeutică va fi evaluată prin frecvența recidivei locale și prin concentrația plasmatică și tisulară a factorilor de creștere. **Rezultate estimate:** Se va urmări ameliorarea proprietăților farmacologice ale citostaticelor. Din punct de vedere terapeutic se urmărește ameliorarea controlului tumoral local asociind chimioterapia în formă lipozomală, cu radiofrecvența.

**Cuvinte cheie:** CC531, metastaze hepatice, lipozomi, chimioterapie intratumorală, factori de creștere, model experimental

## ABSTRACT

**Background:** Colorectal cancer (CRC) has become one of the most frequent malignant diseases of the past two decades. Liver metastases which cannot be resected might be destroyed in situ by radiofrequency although the local recurrence is still very important. **Aim:** The present project aims to increase the efficiency of the unresectable CRC hepatic metastases treatment by associating radiofrequency with hyperthermic intratumoral liposomal chemotherapy. The second aim is to appreciate the role of different growth factors in local tumoral recurrence in correlation with the type of treatment. **Material and methods:** Liposomes with doxorubicin, oxaliplatin and irinotecan will be prepared from natural phospholipids. CC531 cells will be inoculated in liver in 70 Wag/Rij rats divided in 4 groups. First group will evaluate the natural tumor growth rate. In the last three groups the liver metastasis will be destroyed by radiofrequency alone or in association with chemotherapy (with and without local hyperthermia). The treatment efficiency will be evaluated by the local recurrence rate and the plasmatic and tissular concentration of growth factors. **Expected results:** From a pharmacological point of view, the aim will be to improve the pharmacokinetic and pharmacodynamic properties of cytostatics. Therapeutically, the association between radiofrequency and liposomal chemotherapy might ameliorate the local control in the metastatic colorectal cancer.

**Key Words:** CC531, liver metastasis, liposome, intratumoral chemotherapy, growth factors, experimental model

## INTRODUCTION

Nowadays the colorectal cancer (CRC) is one of the most frequent malignant diseases.<sup>1</sup> In Romania, a recent study reported an increase in the CRC incidence by 74.5%.<sup>2</sup> About 26-44% of these patients have lymphatic metastases when diagnosed and about 50% of them will recur, mostly with liver metastases.<sup>3</sup>

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Surgical resection is considered to be the most efficient treatment for colorectal liver metastases. However, surgery has several limitations: bilobare metastases and the need to leave enough hepatic parenchyma in order to ensure an efficient hepatic function.<sup>4</sup> Therefore, radiofrequency has become a widely used method for in situ destruction of the CRC hepatic metastases.<sup>5</sup> However, up to 40% of patients display local recurrences and 12% of these recur on the spot of the treatment within the first year after radiofrequency.<sup>6</sup> This high local recurrence rate might be due to the local release of tissue growth factors, such as the vascular endothelial growth factor (VEGF) or the platelet derived growth factor (PDGF) which favors the development of micro-metastases in the proximity of the destructed tumor.<sup>7</sup> Some authors

consider VEGF a predictive element for the CCR tumor evolution but its direct relationship with the tumor proliferation and neoangiogenesis is still not defined.<sup>8,9</sup>

One of the chemotherapy adverse effects is the high toxicity for the healthy cells. Therefore, several concepts such as „vectored therapy” or „target therapy” were developed in order to improve chemotherapy efficiency and to reduce its toxicity.

According to their specificity for a certain target, these vectors may be grouped on three levels: vectors with target specificity on organs, larger than a micrometer; vectors that can target tissues, characterized by dimensions smaller than a micrometer (liposomes and nanoparticles) and vectors including an element of recognition of the desired cellular or intracellular target.<sup>10</sup>

Liposomes are artificial vesicles of micron and submicron dimensions, made up of one or more phospholipids bi-layers, alternating with watery compartments. Liposome proved to be efficient carriers for chemotherapeutic drugs like doxorubicin and epirubicin. Oxaliplatin, the election drug for CCR therapy, has been recently incorporated in liposomes but its administration is only in the phase of trials.<sup>11</sup> The main feature of the liposomes is the important decrease of the transported medication toxicity.<sup>12,13</sup>

Liposomes can be administered in various ways: intravenously, orally, or directly in the affected tissue. However, the specificity of the tumor vascularization (variable diameters of vessels, chaotic ramification, intermittent and erratic flux) may determine a chemotherapy diffusion.<sup>14</sup> When liposomes were administered straight into the tumor, a decrease of the drug efficiency has been noted, due to the liposome fixation in the vicinity of the administration area.<sup>15</sup>

The association between radiofrequency and doxorubicin, administered as liposomes, shown an increase of the intratumoral chemotherapeutic concentration with subsequently increase in tumor necrosis.<sup>16,17</sup> Experimental studies have shown that mild hyperthermia increases the cytotoxic effects of the chemotherapy.<sup>18</sup> Until now, there were a few numbers of studies dealing with chemotherapeutic intratumoral administration in association with radiofrequency and the reported results are contradictory.

The present project aims to increase the treatment efficiency for the unresectable CCR hepatic metastases by associating radiofrequency with hyperthermic intratumoral chemotherapy, to increase chemotherapeutic local efficiency and to study the influence of the different growth factors on the tumor recurrence.

## **MATERIAL AND METHODS**

### **Cells**

The colon adenocarcinoma cell line CC531 will be used for tumor inoculation. Tumor cells will be cultured in RPMI 1640 supplemented with L Glutamine 2mM (Gibco, Grand Island, NY USA) 8% heat inactivated fetal calf serum, penicillin 100U/ml and streptomycin 0.1 mg/ml.

### **Animals**

Male Wag/Rij rats weighing 250-300 g will be used. All animals will be maintained in the animal facility of the University of Medicine and Pharmacy Iuliu Hatieganu Cluj-Napoca. The University Ethic Committee approved the study design.

### **Liposome/nanoparticle-packing of the cytostatics**

Doxorubicin, oxaliplatin and irinotecan will be used. Liposomes will be prepared from synthetic or natural phospholipids (lecitin), according to the Bangham method in order to achieve small unilamellar liposomes (SUV).

### **The “in vivo” study**

In the first group 10 Waj/Rij rats will be used to test the “in vivo” behaviour of the tumor. 0.1 ml suspension of tumor cells (concentration of  $2 \times 10^6$  cells/ml) will be injected under the liver capsule. The tumoral growth will be assessed by ultrasound examination until the mass reaches at least 1cm in diameter.

In the second group 20 rats will be used. Tumor cells will be injected into the liver in two lobes and when the tumor will reach 1 cm diameter, the one tumor will be destroyed by radiofrequency (RITA generator and Starbust SDE electrode, AngioDynamics® USA). The radiofrequency will be applied for 5 minutes at 60°C. The second tumor will be left in place as a negative control.

The third group will contain 20 rats with tumors obtained in the same manner as already described. Intratumoral chemotherapy preconditioned as liposomes associated with local moderate hyperthermia (41-43° C) will be used. The chemotherapy will be administrated using instillation with Starbust SDE electrode placed in the tumor centre. The unfolding of the electrode antennas will allow cytostatic diffusion till the tumor borders. Hyperthermia will be achieved with the electrode connected at the radiofrequency generator with a temperature set-up at 41°C, the power at 0 for 20 minutes.

In the fourth group of 20 animals with hepatic tumour implants, the tumour destruction will be made

by association of radiofrequency and hyperthermic intratumoral chemotherapy. The intratumoral chemotherapy will be administered in the same manner as in the third group. Subsequently, radiofrequency will be applied as described for the second group.

### **Data analysis**

The radiofrequency efficiency with or without chemotherapy and hyperthermia will be evaluated by the rate of local recurrence (euthanasia at 4 days after ablation and following this at 1 day time point or any condition of animal suffering). Tumor neovascularization will be assessed using microvascular laser Doppler (Periflux System 5000<sup>®</sup>, Perimed AB, Sweden). The plasmatic concentration of growth factors, especial those which are considered to be linked to neoangiogenesis: VEGF, PDGF, TNF will be measured using Luminex 200 system (Luminex Corp. USA). Histological probes will be obtained for histological analysis of neoangiogenesis and local recurrence. The results, especially complications and tumor recurrence will be compared according to the type of the treatment.

### **EXPECTED RESULTS**

The main aim of the project is to increase the efficiency of the un-resectable CCR liver metastases treatment through the association of radiofrequency with hyperthermic intratumoral chemotherapy.

From a pharmacological point of view, the aim will be the improvement of the pharmacokinetic and pharmacodynamic properties of cytostatics in order to increase their efficiency at the level of the tumor cells. Cytostatics, mainly oxaliplatin and irinotecan, will be conditioned as liposomes, in order to obtain medication that is specific to CRC therapy with increased tumoral toxicity, but with less collateral effects.

From an oncological point of view, the aim will be to obtain CRC tumoral cell lines maintained afterwards in cultures in order to further carry out *in vitro* studies on the efficiency of the cytostatic medication. Meantime, factors that are supposed to have a favorable effect on post radiofrequency local recurrence, especially those that stimulate angiogenesis (PDGF- growth factors derived from platelets, VEGF- endothelial growth factor and TNF- tumoural necrosis factor) will be assessed in the plasma and in the scar.

The aim will be to establish a statistic correlation between the revascularization rhythm at the level of the post-radiofrequency scar monitored through microvascular laser Doppler, the rate of local

recurrence and the plasmatic and tissue levels of the above mentioned growth factors.

From a surgical point of view, the aim will be to obtain an experimental model of hepatic metastases in rats. On the experimental model thus achieved, the tumoral behavior will be observed in order to determine the intra-hepatic tumor growth rhythm.

Eventually, the therapeutical efficiency of radiofrequency, intra-tumoral chemotherapy associated with hyperthermia will be studied, so as to determine the therapeutical efficiency of each and every method, as well as the therapeutically results of the two associated treatments.

### **DISCUSSIONS**

The colorectal cancer is one of the most frequent malignant diseases in the modern era and a majority of these tumors have already metastases when diagnosed, most of these being located in the liver.<sup>20</sup> Nowadays, radiofrequency is the most widely used method for *in situ* destruction for CRC liver metastases, but the local recurrence rate remains very high.<sup>5</sup>

It was shown that the local destruction of the tumor release tissue growth factors, especially the vascular endothelial growth factor (VEGF-vascular endothelial growth factor) which favors the development of micro-metastases in the immediate neighborhood of the destructed tumor.<sup>6</sup> However, the VEGF role as well as of other growth factors is still not well defined, the results of the clinical studies being rather contradictory.<sup>9,21</sup> The present study will try to establish if there is any correlation between the rate of local recurrence and the plasmatic and tissue levels of the above mentioned growth factors by evaluating the revascularization rhythm at the level of the post-radiofrequency scar through a microvascular laser Doppler. No data on this topic has been reported until now, even though the microcirculation monitoring has been described in experimental bowel transplantation studies.

Chemotherapy already proved to be an important factor in the management of the CRC liver metastases with significant improvement in patients' survival.<sup>22</sup> In order to improve local control and reduce recurrence rate it has been suggested to associate local destruction of the tumors with hepatic arterial chemotherapy.<sup>23</sup> However, the results were not significantly different when compared to systemic chemotherapy.<sup>24</sup> This leads to an attempt to improve the cytostatic delivery at the tumor site and in meantime to reduce its systemic toxicity.

Liposomes are structures of micron and sub-

micron dimensions, which proved to be very useful for drug delivery.<sup>25</sup> Oxaliplatin, which is actually the main cytostatic used for CRC chemotherapy, has been only recently conditioned as liposomes and there are no data on the drug efficiency, nor for its toxicity.<sup>11</sup> Regarding the irinotecan, the second cytostatic drug as importance in the CRC treatment, it only begins to be considered for being included in liposomes.<sup>26</sup> Therefore our attempt to include these cytostatics in liposomes might provide supplementary useful information on their pharmacological behavior and effects.

The particularities of the tumor vascularization cause a diffusion of the cytostatic medication and subsequently reduce its local efficiency.<sup>14</sup> The association of radiofrequency with the administration of doxorubicin liposomes has shown an increase of the cytostatic in the tumour, while the volume of the destroyed tumoral tissue increased by 25%.<sup>16,17</sup> When liposomes are administered directly into the tumor they concentrate in the neighborhood of the administration area. If moderate hyperthermia is associated, this increases the microvascular permeability of the tumoral vessels for liposomes and also the cytotoxic effects of the drugs liposomes are carrying on.<sup>27</sup> Using this kind of approach, the general toxicity of the cytostatic is practically nil.

Until now, there have been an extremely small number of studies on the cytostatic intratumoral administration in association with radiofrequency. Weinberg et al are the only group studying this therapeutic association, but they administered the cytostatic after the thermal ablation of the tumour using microtubular implants.<sup>19</sup> In the present study the cytostatic will be introduced into the tumor using the radiofrequency probe which makes the drug delivery less fastidious.

Although numerous research studies have been carried out in the field of nanotechnologically conditioned chemotherapy associated with radiofrequency, the results of the studies are partly contradictory, partly incomplete, and therefore, until now, there has been no evidence on the usage and association of these therapeutical means, let alone in the case of the CRC hepatic metastases. This, together with the increase in the occurrence of the CRC, is why we believe this research subject to be an extremely up-to-date one.

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