The Role of Lipiodol in the Treatment of Hepatocellular Carcinoma (HCC) through Transarterial Chemoembolization (TACE)

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TACE has become the standard treatment in selected HCC patients, not eligible for curative surgery, due to advanced tumor stage or poor hepatic status. TACE combines the effect of targeted chemotherapy with the effect of ischemic necrosis induced by arterial embolization, based on the embolic and drug-carrying characteristics of Lipiodol. In this article we present the basic principles of TACE using Lipiodol, our experience and recent advances in obtaining new Lipiodol formulations for enhancing the treatment’s effect.

Keywords: lipiodol, TACE, biodistribution, emulsion

Hepatocellular carcinoma (HCC) (fig. 1) is one of the most common solid organ tumors, being the most common of the primary liver tumors (80-90%), with a higher prevalence in Asia and Africa, where more than 500,000 new cases are reported annually, in Western Europe and USA being responsible for 0.5-2% of cancer mortality [1].

The preferred treatment option for patients with HCC is liver transplantation, as it addresses both the tumor and the underlying liver disease [2], but the shortage of donors and long waiting period allows for the tumor to progress. Surgical resection remains the principal method of treatment; however, only 10–30% of patients are suitable for surgical resection at presentation [1, 3, 4]. Various other methods of treatment have been developed to address HCC, but they are all considered palliative. Transarterial chemoembolization (TACE), percutaneous ethanol injection, systemic chemotherapy, radiofrequency ablation and radiotherapy have all been used with varying success. Internal radiotherapy with radiolabelled Lipiodol has been also used with some success in treatment of unresectable HCC and has been documented to reduce the rate of progression of the tumor [5].

Lipiodol, is an oily mixture of polyunsaturated fatty acid esters, enriched in iodine which has a specific tropism for hepatocarcinoma nodules, when injected in the hepatic artery. This property may be due to the fact that HCC is mainly nourished by the hepatic artery, receiving no or little portal blood, in comparison to normal liver. [6] The two major effects of Lipiodol are the temporary embolic effect, and penetrating the HCC tumor cells. These effects have been studied and exploited for several years in the treatment of HCC. Although a variety of techniques and agents have been used to treat HCC with transarterial therapy [7], transarterial chemoembolization (TACE) and transarterial embolization (TAE) using Lipiodol are widely accepted for the treatment of HCC. The need for improvement is continuous as HCC prognosis is still poor. The main challenge in TACE remains the grade of tumoral uptake and its variations in using different Lipiodol formulations.

Experimental part

Patients’ selection and preparing for TACE

The first transcatheter embolization of the hepatic artery for unresectable liver cancer was conducted by a group of French doctors in 1974. After Yamada and colleagues [8, 9] have reported their experience in a number of cases in Japan, the method has spread throughout Asia and later in Europe and the USA. Since then, this type of treatment has become widely used in most countries.

The liver has the advantage of having a dual blood supply from both the portal vein and the hepatic artery. The liver parenchyma receives 2/3 of its necessary blood supply from the portal vein and 1/3 from the hepatic artery. However, it is well-known that vascularization of hepatocellular carcinoma (HCC) is mostly dependent on the hepatic artery [10]. This characteristic of HCC justifies both the diagnostic use of contrast enhanced CT (fig.2) and MRI, and also enables the method of transarterial therapy as an effective treatment of HCC [11].

To select an appropriate treatment for HCC one must take into consideration not only the tumor burden but also

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Doxorubicin is predominantly eliminated by metabolism (>50%) and excretion into the bile by carrier-mediated transport [26]. The anticancer drugs are vigorously mixed with the Lipiodol in order to prepare an emulsion. The emulsified Lipiodol and drug mixture is injected into the tumor’s supplying vessels and the drug is slowly released from Lipiodol and remains in high concentrations within the nodules for a long period [27]. Johnson et al. [28] stated that there was no difference with regard to pharmacokinetic parameters or toxicity of intra-arterial administration of doxorubicin with or without Lipiodol compared to intravenous doxorubicin. This study is considered technically flawed as the volume of normal saline (25 mL) used to dissolve doxorubicin was excessive compared to the volume of Lipiodol (10 mL) [13].

**Fig. 3. Doxorubicin [29]**

### Table 1

<table>
<thead>
<tr>
<th>Okuda Stage</th>
<th>Tumoral volume</th>
<th>Ascitis</th>
<th>WBC</th>
<th>Total Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt; 50% of hepatic vol.</td>
<td>-</td>
<td>&gt; 3 g/dl</td>
<td>&lt; 3 mg/dL</td>
</tr>
<tr>
<td>II</td>
<td>50% of hepatic vol.</td>
<td>+</td>
<td>&lt; 3 g/dl</td>
<td>cca. 3 mg/dL</td>
</tr>
<tr>
<td>III</td>
<td>&gt; 50% of hepatic vol.</td>
<td>++</td>
<td>&lt; 3 g/dl</td>
<td>3-6 mg/dL</td>
</tr>
</tbody>
</table>

#### Embolic effect of Lipiodol

Lipiodol (iodized oil; Guerbet Laboratories, Roissy, France), is an iodinated ethyl ester of poppy seed oil, used initially for lymphangiography studies as an oily contrast medium. During TACE, Lipiodol is injected into the hepatic artery. It has an embolic effect on small vessels and it can remain in tumor nodules for a long period of time, starting from several weeks to over a year. This effect is determined by the siphoning effect of hypervascularization of the tumor vessels and also due to the absence of Kupffer cells, which are not present inside tumoral tissue [15, 16]. Talking about the normal liver parenchyma, when Lipiodol is injected into the hepatic artery, it accumulates in the portal system’s venules due to arterio-portal communication and is gradually released into the systemic circulation via the hepatic sinusoids. Another mechanism of cleansing the Lipiodol in a normal liver is through phagocytosis by Kupffer cells, and usually being cleared within a week. [17, 18]

#### Drug delivery systems

An important role of Lipiodol is to act as a carrier and localize chemotherapeutic drugs into a tumor, thus forming a drug delivery system composed of various chemotherapeutics drugs emulsified in the excipient, Lipiodol [19]. The excipient consists of iodized and ethylated esters of long chain fatty acids (C16 and C18) such as linoleate, oleate, palmitate and stearate [20]. It appears that the ethylated and iodinated long chain fatty acids are endocytosed into the cells to be partly eliminated through bile [21]. Long chain fatty acids are thought to be transported across biological membranes via a flip-flop mechanism through passive and facilitated carrier-mediated transport processes [22]. In addition, the excipient Lipiodol is retained in tumor tissue where it is visible on X-ray for months [23]. Doxorubicin and Cisplatin are the most widely used drugs for TACE [7]. Doxorubicin, C2H29N011 (fig. 3), enters cells by both passive diffusion and carrier-mediated transport, via the OCT6 (SLC22A16) transporter [24, 25]. Doxorubicin is predominantly eliminated by the hepatic status and the patient’s general status. TACE is mainly used in a selected group of patients who cannot benefit from curative treatment. TACE is not an option in patients with a severely compromised liver function such as Child-Pugh C or late stage B. Whenever planning a TACE, the residual liver function should always be evaluated and correlated with the extent and characteristics of a tumor.

We selected our patients to correspond with the first two Okuda stages [12], as we consider the procedure to be most effective in this cases.

We performed CT or MRI examinations before any segmental or sub-segmental TACE session, in order to perform a correct anatomical evaluation. Prior to TACE, we consider that an angiographic examination is mandatory to locate all of the feeding arteries of a tumor including any possible extra-hepatic arteries, which may be predicted also from CT or MRI findings [13]. Such findings may reveal the presence of large tumors located at the surface or peripheral areas of the tumor with no Lipiodol retention and visualization of hypertrophied extra-hepatic arteries [14].

#### Stability of Lipiodol based emulsions

Major attention was given on how to prepare a stable emulsion with water-based preparations for anticancer drugs and Lipiodol, as the stability of the mixture can greatly influence the pharmacokinetic of the antitumoral agent [13]. Several studies showed that the stability of the emulsion is mostly influenced by the mixture ratio between Lipiodol and the contrast medium used to dissolve the anticancer agents, this leading to improved pharmacokinetic outcomes of TACE. Sakaguchi et al. determined the highest stability of such mixture to be obtained at a ratio of 2:4:1 [30], while Nakamura et al [27] recommend adjusting the mixing volume of Lipiodol and doxorubicin to a 2:3:1 ratio. De Baere et al. demonstrated in an experimental study that an excess volume of Lipiodol over contrast medium results in water-in-oil emulsions with increased tumor uptake of Lipiodol, while minimizing non-tumor or lung uptake of Lipiodol compared to an oil-in-water type emulsion [31]. A clinical study by Nakamura et al. [27] has shown that doxorubicin was released slowly from a water-in-oil emulsion resulting clearly in a lower blood doxorubicin concentration compared to intraarterial injection of the drug without Lipiodol. Another study showed that using gelfoam embolization in combination with Lipiodol-Doxorubicin provides a better kinetic effect, slowing the release of doxorubicin along with an increase of the drug into the tumor by preventing washout [32].

#### TACE technique in our experience

Selective TACE is the main endovascular treatment used in our hospital on patients with HCC and hepatic metastasis. The interventional treatment represents an effective alternative for the unresectable tumors (multiple metastasis, voluminous tumors, altered hepatic function, advanced cirrhosis). Also, many authors consider that TACE could be very useful prior to hepatectomy, due to the influence they have on micro-metastasis, which are not visualised prior to hepatectomy, but our experience in this field is limited.
The technique used by us for selective tumor chemoembolisation (TACE) was based on the Seldinger method with the characterisation of the common femoral artery. A 5F catheter was guided under fluoroscopic control to the common hepatic artery. At this level, we introduced, supraselective, a microcatheter (2.5-3 F) to access the tumor vessels. Through the microcatheter we subsequently introduced the emulsion of Lipiodol and antitumoral drug.

The „cocktail” of cytostatic substances used by us was represented by:
- Farmarubicin (Adriamycin, Adriblastin)/Doxorubicin 50-90 mg, depending on the size of the tumor
- mixed with 4.8 ml Lipiodol

The standard dose of doxorubicin used in the realization of hepatic chemoembolization therapy was 60 mg (40-100 mg, a mean of 61.2 mg). Doxorubicin was dissolved in 3-5 mL of fluid with specific gravity equal to that of Lipiodol (or non-ionic contrast agent having a concentration of 370 mg/mL) and subsequently mixed with Lipiodol 5-10 mL at a ratio of 2:3/1 (Lipiodol/Doxorubicin) by stirring for 10 min (classic system) or, more efficiently and rapidly, through the use of two syringes together with a three-way valve. The end point was represented by a red emulsion that appears uniform to the naked eye.

**Lipiodol visualization in portal vein**

HCC encapsulated nodules are irrigated mostly from the hepatic artery, while nodules with extracapsular invasion can have a dual blood supply, also from the portal vein, thus leading to an incomplete tumor necrosis. [33] Lipiodol has the capacity to reach also the portal veins nourishing the tumors due to arteriportal communication through the peribiliary vascular plexus, the vasa vasorum, thus strengthening the anti-tumor effect of TACE. [13] A clinical study conducted by Miyayama et al. showed that there is a significant correlation between the degree of portal vein visualization with Lipiodol and tumor recurrence, a better portal vein visualization demonstrated during TACE correlates to a lower tumor recurrence. [34]

At this stage we followed a protocol to dissolve Doxorubicin hydrochloride in 2-6 mL of fluid with a specific gravity equal to the that of lipiodol and a volume 1/5 distilled water. This solution was mixed with 2-3 volumes of Lipiodol. Mixing the two fluids, having equal specific gravity, is determined the stability of the solution. The emulsion was injected into the hepatic artery or proper hepatic artery, right or left. As the effect varies with location of injection, a correction factors may be used (for example, 3 to the left hepatic artery and a factor of 1.5 for the right hepatic artery). These correction factors are used because the right and left lobe volumes have a ratio of 1:2. Arterial embolization with a relatively large amount of lipiodol (around 17 mL) and Doxorubicin hydrochloride, with or without particulate Gelaspon / TachoComb has not been practiced in many patients with hepatocellular carcinoma. After intra-arterial injection of lipiodol combined with Doxorubicin hydrochloride, Lipiodol was viewed in the portal vein, the visualisation being related to the amount injected, even without arteriovenous portal shunt. The arteriportal communications happen at the sinusoids level and through the peribiliary vascular plexus. In healthy patients the arteriportal flow can not be revealed during a hepatic angiography, and the small quantity of Lipiodol injected in the artery will not appear in the portal vein. If a larger quantity of Lipiodol remains at the sinusoids’ level, this modifies the microcirculation mechanisms and each additional volume of Lipiodol blows into the portal vein through arteriportal communications.

**Results and discussions**

In our experience, from 2002 until 2012, the use of Lipiodol proved to be very effective both as an embolic agent and as a drug carrier. In our clinic, there are over 300 interventions each year adressing HCC patients. We can report a median survival period of 18 months, but we have patients with a survival of over 36 months still in treatment.

By mixing it with lipiodol, the cytostatic forms a stable emulsion (having “slow release” effect). The method is based on the synergy between the chemotherapeutic drug and the embolic agent (Lipiodol). The cytostatic is retained in cancer cells, with the effect of “slow-release” and the neoplastic tissue becomes ischemic due to the blocked blood flow.

The peritumoral necrosis is directly proportional with the amount of injected Lipiodol, which in turn depends on the size of the tumors, as shown in the table 2.

Regarding the arterio-portal communications and Lipiodol’s migration in the portal vein, we observed the effect of the emulsion by injecting 3 mL of the emulsion described above (dissolved Doxorubicin hydrochloride combined with 2-3 volumes of Lipiodol) in the right hepatic artery, after which the TachoComb particles were introduced to complete embolization. Portal vein was visualized on abdominal images immediately after emulsion injection. When applying TachoComb, images were captured after embolization. Images obtained immediately after injection of the emulsion revealed peripheral branches of the portal vein very frequently. Because the portal vein branches were seen not only around the tumor but also in free zones, correlated visualization of the portal vein branches was made to the amount of lipiodol injected. We found that Gelaspon or TachoComb were not required to be used when the lipiodol was used in larger quantity.

We evaluated the length of the visualised vena porta using the following scale:
- Over 50 mm  +++
- 50 to 20 mm  ++
- 20 to 5 mm  +
- < 5 mm  -/–
- Unvisualised  –

We compared these values with the quantity of Lipiodol used. It was concluded that the branches of the portal vein are better visualised when a greater quantity of Lipiodol is injected. This tendency was found both in HCC and hepatic metastasis.

Although embolic effects of Lipiodol have been widely investigated in vitro and in vivo, [35-37] and the drug distribution system using Lipiodol has been used for several decades, a standardized operating procedure for the preparation of formulation and its clinical use is not yet available [38]. This is due to the fact that an exact mechanism of Lipiodol retention in HCC is not completely elucidated, but is clear that tumor retention is due to

<table>
<thead>
<tr>
<th>Lenght of the tumor (cm)</th>
<th>Lipiodol (ml)</th>
<th>Possility of peritumoral necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>3-5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>6-8</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>8-11</td>
<td>+++</td>
</tr>
</tbody>
</table>

Table 2

**RELATIONSHIP BETWEEN TUMORAL NECROSIS AND THE QUANTITY OF INJECTED LIPIODOL**

http://www.revistadechimie.ro  REV. CHIM. (Bucharest)  •  66  •  No. 3  •  2015
 retention difference between normal capillaries and neovessels. Tumorous neovessels have a muscular and nervous deficit, causing a flow variation in comparison with normal capillaries. In normal vessels, Lipiodol is rapidly eliminated, whereas it stays longer in neovessels [6]. More and more authors focus on enhancing the effects of TACE, in order to better address HCC patients and improve their survival.

Several authors recently studied the biodistribution of Lipiodol and various Lipiodol-based formulation in order to determine the best embolic effect and retention within the tumor. It seems that comparing Lipiodol-ethanol, Lipiodol alone and Lipiodol followed by gelfoam, the first formulation has a better tumor retention [39]. In opposition with previous studies describing the mixture with a proportion of 50-70% ethanol [40, 41], Yu et al. adopted a formulation with a 33% proportion of ethanol [39], studying the decomposition and tumor retention of a Lipiodol-ethanol mixture using iodine-131 as a radioactive tracer and measuring its radioactivity in blood and urine.

In vitro studies have suggested that lipid excipients (in our case Lipiodol) in a drug delivery system increases the transport of drugs across biological membranes [42, 43]. A number of hypotheses regarding the mechanisms for this increase have been proposed: increased membrane fluidity, inhibition of carrier-mediated efflux transporters, increased aqueous solubility and/or decreased enzymatic hydrolysis [42-44]. Dubbelboeur et al. [19] tried to examine the effects of the Lipiodol on the local and systemic disposition of doxorubicin, in a study on pig-models. They got to the conclusion that Lipiodol, which consists of esters of long chain fatty acids, did not significantly alter the hepatobiliary disposition or plasmatic pharmacokinetics of doxorubicin, ruling out the possibility of direct membrane transport interaction with Lipiodol. They considered that the properties of the total Lipiodol-based emulsion, rather than those of the excipient itself, are of importance for tumor directed delivery. [19].

Another interesting hypothesis was the increase of tumor uptake when viscosity of oils is increased [45, 46]. Becker et al. [6] reported an increased uptake at 72 h, when using a Lipiodol- 0.8% stearic acid formulation and possibly a decrease in pulmonary and hepatic secondary effects. Similar conclusions were drawn by Hamuro et al. [46] who show a 14-day tumor uptake three to five times greater with a Lipiodol/triolein mixture compared with Lipiodol. Also, Oda et al. describe, in a study conducted on rabbits, a longer tumor retention at 7 days with a Lipiodol fluid compared with Lipiodol ultra-fluid, whose viscosity is three times lower. [47].

Conclusions

In conclusion, we might say Lipiodol has several functions. It acts as an embolic agent on blood vessels, as a transporter for chemotherapy drugs and as an augmenter of antitumor effects in TACE, by efflux into the portal veins. Though the use of Lipiodol in TACE has been challenged [7] and new products have been developed, there is substantial evidence confirming that the use of Lipiodol is efficient, so it is still widely adopted in TACE protocols.

The combination of embolic-therapy and regional chemotherapy has synergistic, anti-tumor effects with a high objective response rate. [13] Also, when talking about the benefits of this combined therapy, we must note the reduced toxicity, due to local action and reduced systemic drug level.

It is highly important that studies, such the ones noted in the present paper, continue, in order to better understand the mechanisms through which Lipiodol determines the “slow-release” effect of antitumoral-agents, and to continue the develop of new formulation to enhance this effect.

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