Use of Lipiodol as a drug-delivery system for transcatheter arterial chemoembolization of hepatocellular carcinoma: A review

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1. Introduction

Interventional radiology can be defined as all medical procedures performed by radiologists using medical imaging guidance to treat diseases under minimally invasive conditions. Image-guided procedures result in more rapid recovery, fewer complications and reduced medical costs [1]. Interventional radiology has become an essential aspect in oncology and the subject of very active research involving distinct fields such as drug delivery, pharmacology, medical devices and medical imaging [1].

Transarterial chemoembolization (TACE) plays an essential role in the management of unresectable hepatocellular carcinoma (HCC) [2–5]. Since its inception in the early 80s, this technique has been based on the use of the iodinated oil Lipiodol. In TACE, Lipiodol is used not only for its radiopacifying properties but also for its drug delivery and tumour-seeking properties and its ability to induce plastic (adapted to the size of the vessels) and transient embolization of the tumour microcirculation [6–8]. However, many uncertainties remain unresolved. The aim of this review is to critically discuss the physical properties, tumour uptake behaviour and drug delivery effects of Lipiodol for the intraarterial treatment of HCC as well as future prospects.

Lipiodol is also widely used in many other interventional procedures, for example mixed with surgical glue (cyanoacrylates) or with ethanol for various fluoroscopy-guided embolization procedures [9]. These indications will not be discussed here.

2. Lipiodol

2.1. Characteristics

Lipiodol is an oily contrast medium consisting of a mixture of long-chain (C16 and C18) di-iodinated ethyl esters of fatty acids from poppy seed (Papaver somniferum var. nigrum) oil, which contains 98% unsaturated fatty acids [10]. The predominant fatty acid is linoleic acid (70%). Lipiodol is a pale yellow to amber, clear liquid, containing 37% (w/w) iodine (i.e. an iodine concentration of 480 mg/mL). The viscosity of Lipiodol Ultra-Fluid at 37 °C is approximately 25 mPa·s (and 50 mPa·s at 20 °C) and its density is 1.28 [11,12]. Lipiodol is marketed as a solution for injection in 10 mL glass ampoules. Throughout this article, Lipiodol Ultra Fluid® is referred to as Lipiodol. Historically, Lipiodol was called “Ethiodol®” in the USA until 2012.

2.2. History

Lipiodol was first synthesized by the French pharmacist Marcel Guerbet (1861–1938), early in 1901 in the Paris School of Pharmacy Chemistry Laboratory and was first presented by his friend and colleague Laurent Lafay at the May 1901 meeting of the French Society of Dermatology and Syphilography [13]. Lipiodol was first marketed for therapeutic purposes. The clinical indications were syphilis, pulmonary (asthma) and cardiovascular (angina, pericarditis) diseases, impetigo, rheumatism, etc. [14]. Its X-ray opacifying properties were discovered in 1921 by Jean-Athanase Sicard, professor of radiology in Paris, and his resident, Jacques Forestier. The first myelography was performed on 13th October 1921 in a patient suffering from paralysis, probably caused by a spinal cord tumour [15]. Epidural injection of Lipiodol had no adverse effects and X-ray imaging performed on the days following the procedure showed that Lipiodol did not remain at the injection site, but travelled along the spinal canal. Many radiological applications for Lipiodol were subsequently developed (bronchography in 1922, dacryography in 1923, hysterosalpingography in 1924, sialography and fistulography in 1928, lymphography in 1960), and even urethrography and cystography [16], to the

Abstract

Hepatocellular carcinoma (HCC) remains a major public health problem. Transarterial chemoembolization (TACE) is recognized as the standard of care for patients with unresectable, asymptomatic, noninvasive and multinodular HCC. This procedure is based on percutaneous administration of a cytotoxic drug emulsified with Lipiodol followed by embolization of the tumour-feeding arteries. The standard procedure involves Lipiodol, an oily contrast medium which consists of a mixture of long-chain di-iodinated ethyl esters of poppy seed fatty acids. The aim of this review is to discuss the physical properties, tumour uptake behaviour and drug delivery effects of Lipiodol, the parameters influencing tumour uptake and future prospects.

Lipiodol has a unique place in TACE as it combines three specific characteristics: drug delivery, transient and plastic embolization and radiopacity properties. Substantial heterogeneity in the physicochemical characteristics of Lipiodol/cytotoxic agent emulsions might reduce the efficacy of this procedure and justifies the current interest in Lipiodol for drug delivery.

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Keywords: Lipiodol; Transcatheter arterial chemoembolization; Hepatocellular carcinoma; Drug delivery; Theranostics; Cytotoxic drugs; Interventional radiology
point that radiological indications subsequently outnumbered therapeutic indications. The notable exception is prophylaxis and treatment of endemic goitre [10,17], based on the use of Lipiodol as a long-lasting source of iodide [10,18]. Tubal flushing with Lipiodol has been proposed for the treatment of unexplained infertility [19].

The use of Lipiodol for chemoembolization of HCC by Toshimitsu Konno and coworkers in the early 1980s was a major step forward and the dawn of a new life for this venerable compound [20–22]. Lipiodol-based TACE is classically used as the gold standard for comparative studies with other TACE procedures (using drug-eluting beads), radioembolization or targeted therapy (sorafenib) in intermediate or advanced HCC patients.

Treatment of HCC by the polystyrene co-maleic acid-conjugated neocarzinostatin (SMANCS)/Lipiodol method has been approved in Japan since 1995 [23]. Lipiodol is also approved for TACE in Italy and Mexico. Although widely used worldwide, the use of Lipiodol for TACE is not registered in other countries according to local marketing authorization in force.

3. Hepatocellular carcinoma

In 2008, the worldwide prevalence of liver cancer was 750,000 cases with an estimated 695,000 deaths. Due to its high mortality rate, liver cancer is the third most common cause of death from cancer worldwide [24,25]. Five-year prevalence is 613,000 cases [24]. HCC has a high prevalence in Southeast Asia and sub-Saharan Africa. Cirrhosis is present in 80–90% of cases and is therefore considered to be the single most important risk factor. Other risk factors are chronic viral hepatitis B, hepatitis C (both diseases account for the vast majority of HCC worldwide), exposure to toxins such as aflatoxin B1, or diethylnitrosamine, as well as diabetes and obesity (non-alcoholic steatohepatitis) [26].

In 2002, 82% of cases of liver cancer occurred in developing countries, with 55% in China alone [27]. HCC rates also change over time, at least in certain countries: incidence rates tripled in the US between 1975 and 2005 (1.6 and 4.9/100,000 respectively). This growth has been attributed to an epidemic of hepatitis C virus during the 1960s and rising rates of obesity and diabetes [27].

Hepatocellular carcinoma (HCC) represents about 80% of all primary liver cancers [28]. The presence of underlying cirrhosis makes the treatment of HCC very challenging, due to the intricate association of two different diseases with a poor prognosis. The 5-year cumulative risk for the development of HCC in patients with cirrhosis ranges between 5% and 30%, depending on the cause, region or ethnic group and the stage of cirrhosis [29].

The Barcelona Clinic Liver Cancer (BCLC) classification [3] (Fig. 1) is widely acknowledged and used for the management of HCC in Europe and the USA. In Japan, a different algorithm was proposed in 2007 [30] and revised in 2010 [31].

4. Transarterial chemoembolization

4.1. Definition

Chemoembolization is classically defined as “percutaneous introduction of a substance to occlude a vessel in combination with a chemotherapeutic agent, used in the treatment of cancer to deliver sustained therapeutic levels of the agent to a tumour” [32]. TACE is recognized as the primary treatment for BCLC stage B, asymptomatic, non-invasive and multinodular HCC [3,5]. It is also used to treat cholangiocarcinoma and metastases of colorectal cancer, neuroendocrine tumours, breast cancer or melanoma [33].

The rationale for TACE (as for all hepatic intra-arterial therapies) is the preferential blood supply of liver malignancies derived from the hepatic artery (~95%), thereby allowing local chemotherapy and embolization without damaging the surrounding healthy parenchyma, which receives three quarters of its blood volume from the portal vein, and thus only one quarter from the hepatic artery [34–36].

TACE must be distinguished from other procedures: transarterial oily chemoembolization (TOCE) (or “chemoli-piodolization”), in which the cytotoxic agent is mixed with Lipiodol without an embolic agent; bland transarterial embolization (TAE), in which no cytotoxic agent is delivered and transarterial chemotherapy (TAC), in which Lipiodol is not used and no embolization is performed [3] (Table 1).

It is often recommended to repeat the TACE procedure 3–4 times per year [3]. However, the optimal schedule in terms of clinical benefit/risk (liver failure, vascular lesions) remains unclear [37] and is generally based on the individual response to treatment. This variable frequency of TACE also accounts for the considerable heterogeneity of treatment schedules between centres.

4.2. Cytotoxic drugs

Basically, the TACE procedure consists of transcatheter hepatic arterial administration of one or several cytotoxic drugs.

In a recent review of the literature, the agents most commonly used were, in decreasing order of frequency: doxorubicin, cisplatin, epirubicin, mitoxantrone, mitomycin C (these drugs are sometimes diluted in a water-soluble contrast medium (CM) to adjust densities) and (in Japan) SMANCS, and subsequently emulsified with an equivalent volume of Lipiodol [37] or, sometimes, a smaller volume with the objective of administering a water-in-oil (W/O) emulsion. Other drugs, such as pirarubicin [38], nemorubicin [39] miriplatin [40] or idarubicin [41], are or have been used. It is worth mentioning that cytotoxic agents are not approved
Fig. 1. Updated Barcelona Clinic Liver cancer (BCLC) staging system and treatment strategy. CLT, cadaveric liver transplantation; LDLT, living donor liver transplantation; OS, overall survival; PEI, percutaneous ethanol injection; PS, performance status; RF, radiofrequency ablation; TACE, transarterial chemoembolization.
From [3] with permission.

by health authorities for the loco-regional treatment of HCC (except SMANCS and miriplatin in Japan).

The Lipiodol/drug emulsion is usually prepared extemporaneously. In their princeps articles, Konno et al. dispersed and solubilized the high-molecular weight (15 kDa) and lipophilic agent SMANCS [42,43] in Lipiodol [20–22]. The highly lipophilic agent SMANCS is soluble in a number of organic solvents (such as pyridine or acetone) and in water and Lipiodol [21,42]. Miriplatin is suspended in Lipiodol at a concentration of 20 mg/mL [40]. Successful attempts of solubilization of the lipophilic molecule paclitaxel in Lipiodol have also been reported [44,45].

Unfortunately, there are no evidence-based data regarding the optimal selection of cytotoxic molecule(s) and dosages [37]. The lipophilic anthracycline, idarubicin, was recently found to be more cytotoxic in vitro on three HCC cell lines (including the chemoresistant SNU-449 cell line) than all other cytotoxic molecules classically used in TACE [46]. Idarubicin was reported to be safe and effective in a pilot clinical study [41].

Most cytotoxic molecules used in TACE procedures are metabolized by the liver [47,48].

Considerable heterogeneity of TACE procedures is observed between centres and interventional radiologists with respect to the cytotoxic drug, and the dose of this drug also varies considerably (some clinicians use a fixed dose, while others base the dosage on the body surface area, tumour size, bodyweight or bilirubin level) [37]. For example, the dose of doxorubicin used for TACE ranges between 50 and 150 mg among centres, without evidence-based pharmacological rationale [37]. Such heterogeneity is uncommon in oncology. The absence of the studies that are usually conducted to determine the dose-limiting toxicity and then the optimal dose for TACE could explain this lack of rationale for the dose used. The issue of the choice of drug for TACE can be even more complex, as some US authors use a combination

<table>
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<tr>
<th>Procedure</th>
<th>Acronym</th>
<th>Cytotoxic agent</th>
<th>Lipiodol</th>
<th>Embolization agent</th>
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<tr>
<td>Transarterial chemoembolization (conventional)</td>
<td>TACE</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Transarterial oily chemoembolization (chemo-Lipiodolization)</td>
<td>TOCE</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Transarterial embolization (bland, transcatheter embolization)</td>
<td>TAE</td>
<td>No</td>
<td>Yes or no</td>
<td>Yes</td>
</tr>
<tr>
<td>Transarterial chemotherapy</td>
<td>TAC</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>DEB-Transarterial chemoembolization</td>
<td>DEB-TACE</td>
<td>Yes</td>
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of doxorubicin, cisplatin and mitomycin C [49]. A recent randomized clinical trial (RCT) performed in 365 HCC (BCLC stages B and C) patients demonstrated that the choice of chemotherapy may affect survival outcomes: a significantly better survival was observed in patients receiving triple-drug (lobaplatin, epirubicin and mitomycin C) chemolipiodolization with embolization than in patients treated by single-drug (epirubicin) chemolipiodolization with embolization [50].

However, the clinical value of adding drug-induced cytotoxicity to embolization-induced ischaemia during locoregional procedures remains debated [37]. Some authors speculate on a potentiation of drug-induced cytotoxicity by tumour ischaemia [4]. On the contrary, it must be underlined that there was no statistical evidence of a better survival associated with TACE compared with bland embolization (TAE) in a prospective RCT that was prematurely stopped because of higher survival rate associated with TACE compared with best supportive care [51]. Similarly, no difference was reported between (cisplatin-based) TACE and TAE (Lipiodol and gelfoam) in terms of survival rates, in a small RCT [52]. Additional studies are warranted to better understand the respective roles of tumour ischaemia (including pro-angiogenic factors) and drug-induced cytotoxicity.

4.3. Embolic agents

Chemoembolization is commonly followed by transient embolization of the tumour-feeding vessels with agents such as gelatin sponge particles, polyvinyl alcohol (PVA) or calibrated microspheres (trisacrylgelatin, PVA, etc.) to induce or enhance tumour ischaemic necrosis and prevent washout of the drug, thereby maintaining a high local concentration [4,49,53]. These embolic agents can be spherical or non-spherical, resorbable or non-resorbable, calibrated or non-calibrated (Table 2).

None of these agents has been demonstrated to be clinically superior to any of the others [37], although no RCT have been specifically conducted to date.

4.4. Procedure

TACE is carried out after performing a complete vascular radiological investigation. In general, catheterization is performed via a femoral artery followed by selective embolization of the hepatic artery branches feeding the tumour (Fig. 2). The radiologist extemporaneously prepares a solution (for lipophilic drugs) or an emulsion of the drug and Lipiodol. The emulsification procedure is therefore operator-dependent. One of the most commonly used techniques consists of approximately 15–20 pushes and pulls through a three-way stopcock between two syringes. Some clinicians first push the cytotoxic drug solution into Lipiodol in order to form a W/O emulsion. Depending on the tumour size, vascularity, and fluoroscopic findings, a total volume of about 10–15 mL of Lipiodol is injected. In general, the mixture is injected until stasis in the second- or third-order branches of the right or left hepatic artery is achieved [49] (Fig. 3). Based on retrospective multivariate analysis of 219 patients with HCC who underwent TACE, the optimum dose of Lipiodol (mL) has been proposed to be 1–1.5 times the absolute value of the tumour diameter (expressed in cm) [54]. However, this approach is rarely used in clinical practice.

Injection of the solution or emulsion/suspension is followed by intra-arterial injection of a mixture of an embolic agent and a water-soluble contrast medium in order to retain the drug in the tumour and to exert embolic effects.

The cytotoxic drug/Lipiodol extemporaneous mixture described above is unstable (except for SMANCS and miriplatin). Various techniques have been used in pre-clinical studies, basically blending [6] or ultrasonication [55], to improve the stability of the emulsion/suspension.
Conceptually, enhanced stability of the drug/Lipiodol emulsion could result in increased accumulation of the drug in the oily phase, thereby improving Lipiodol vectorization properties [56]. Interestingly, one study reported that ultrasonication achieved a stable suspension of doxorubicin and Lipiodol with very small droplets (mean diameter 7 nm) (calculated half-life 25 ± 3 days). A retrospective analysis of clinical data suggested the superiority of this formulation over conventional TACE in terms of survival [57]. However, no prospective RCT have been conducted so far to support the benefit of these techniques.

Of note, Lipiodol may cause cracks in certain types of plastics and rubber stoppers, and is therefore always packaged in sealed ampoules. In a comparative study in which four types of stopcocks were soaked in Lipiodol for 6 h and were then checked for damage and subsequently used for the ‘pumping’ procedure under the usual conditions of use, crazing and cracking occurred only on polycarbonate three-way stopcocks, while polypropylene, polyamide, and polysulphone three-way stopcocks had a high durability [58].

### 4.6. Clinical efficacy

The survival benefit associated with TACE has been a controversial issue for a long time, as contradictory results have been reported [51,62,63]. In a RCT involving 112 patients, 1- and 2-year survival probabilities were 75% and 50% for bland transarterial embolization with gelatin sponge particles, 82% and 63% for TACE (using doxorubicin as cytotoxic agent) and 63% and 27% for control (symptomatic treatment), respectively \( (p = 0.009 \text{ for TACE vs. control}) \). This study was terminated prematurely because of the significant benefit associated with TACE over symptomatic treatment [51]. A second RCT based on 80 patients with unresectable HCC compared TACE (emulsion of cisplatin in Lipiodol, embolization with gelatin particles) versus symptomatic treatment (control group). Survival was better in the TACE arm (estimated 1-year, 2-year, and 3-year survival rates were 57%, 31% and 26% in the TACE group and 32%, 11% and 3% in the control group, \( p = 0.002 \)) [63]. Two meta-analyses demonstrated the clinical benefit of TACE for unresectable HCC. The first analysis [64] was based on RCTs published between 1980 and 2000 and concluded on a significant improvement of 2-year survival for TACE compared with conservative management (18 RCTs were pooled, odds ratio was 0.54 [0.33–0.89, \( p = 0.015 \)]. A subsequent meta-analysis assessing overall survival for primary treatment of HCC (core group of 6 RCTs, 503 patients) showed a significant 2-year survival benefit associated with TACE compared with conservative management (odds ratio 0.53 [0.32–0.89], \( p = 0.017 \)) [65]. According to the European guidelines, levels of evidence for chemoembolization are 1 (strength of evidence) and A (clinical endpoints) [3].

### 4.7. Drug-eluting beads

Alternatively, non-resorbable, embolic microspheres loaded with cytotoxic drugs [66] can be selectively administered into the lesions. These spherical, deformable particles, usually referred to as drug-eluting beads (DEB) have a calibrated diameter (in the 40–900 μm range). They were
developed with the goal of overcoming the difficulty in obtaining reproducible Lipiodol/doxorubicin emulsions with standard droplet size. DEBs are composed of a hydrophilic, ionic polymer that can bind anthracyclines via an ion exchange mechanism [66]. DEBs are loaded extemporaneously with the cytotoxic agent (the loading procedure takes 20–90 min, depending on the size of the beads) [67]. They allow simultaneous embolization and administration of the cytotoxic drug. DEBs can also be loaded with irinotecan for the treatment of colon cancer liver metastases [68]. In patient liver explants, doxorubicin was found to impregnate an area of at least 1.2 mm in diameter around the occluded vessel. Doxorubicin was detected in the tissue surrounding the beads at all time-points of liver surgical explantation after DEB-TACE (i.e. TACE in which the cytotoxic drug is loaded into DEBs instead of being mixed with Lipiodol) (from 8 h to 36 days) [69].

One study compared the doxorubicin pharmacokinetic profiles following injection of DEBs loaded with doxorubicin versus conventional TACE protocol (i.e. Lipiodol + doxorubicin + bland beads), doxorubicin + bland beads and doxorubicin administered intra-arterially, in the rabbit VX2 model [70]. $C_{\text{max}}$ values of doxorubicin and its principal metabolite, doxorubicinol, were significantly lower when the drug was administered with DEBs than when it was administered intra-arterially. The order of plasma doxorubicinol concentrations was intra-arterial $>$ conventional TACE $>$ doxorubicin + embolic bland beads $>$ DEBS. However, this difference was only transient, as no differences between groups were observed 2 h after the procedure. The tumour doxorubicin concentration was almost undetectable following intra-arterial administration, low in the TACE (12–36 nmol/g) and doxorubicin + bland beads (5–25 nmol/g) groups but high in the DEBs group (413.5 nmol/g at 3 days). Interestingly, at day 3, tumour necrosis was identical in the DEBs and TACE groups, suggesting that necrosis cannot be exclusively attributed to doxorubicin [70].

In a clinical study comparing the pharmacokinetic profile of doxorubicin in patients undergoing TACE ($n = 5$ patients with Lipiodol + gelatin sponge particles and $n = 2$ patients with Lipiodol + doxorubicin but no gelatin sponge particle-based arterial occlusion) or DEB-TACE (500–700 $\mu$m) ($n = 13$ patients), doxorubicin $C_{\text{max}}$ and AUC values were significantly lower in DEB-TACE patients than in conventional TACE patients [71]. However, no comparative clinical endpoints were studied to investigate whether this difference in pharmacokinetic profiles had a toxicological or clinical impact.

No significant evidence of a difference in clinical efficacy between DEB-TACE and TACE has yet been published, based on prospective RCT [72]. The phase II PRECISION V trial (212 patients) compared doxorubicin-loaded DEBs and conventional TACE (the primary endpoint was tumour response according to the amended EASL criteria [73]). At 6 months, the complete response rate was 27% vs. 22% with conventional TACE; the objective response rate was 57% vs. 44% and the disease control rate was 63% vs. 53%, $p = 0.11$ [74]. However, it is worth noting that, in a recent retrospective study of consecutive patients referred for TACE for metastatic neuroendocrine tumours ($n = 120$) or HCC ($n = 88$), the occurrence of biloma (i.e. encapsulated collection of bile) or liver infarct was found to be significantly associated with DEB-TACE irrespective of tumour type. At least one liver/biliary injury was observed after 30.4–35.7% of DEB-TACE sessions versus only 4.2–7.2% of conventional (Lipiodol-based) TACE sessions ($p < 0.001$) [75]. Subsequently, an unexpectedly high incidence of biloma in DEB-treated patients led to forced interruption of a clinical trial [76]. Clinical use of DEBs is relatively recent and knowledge on their safety is, logically, maturing. Lipiodol-based TACE remains the standard of care for patients meeting the criteria for the intermediate stage of the BCLC staging classification [3].

### 4.8. Selective internal radiotherapy

Incorporation of a radionuclide with Lipiodol was rapidly considered to be an interesting approach for the local treatment of HCC [77]. The $\beta$-emitting radionuclide $^{131}$I was selected. However, the long physical half-life of this radionuclide (8.04 days) and the high $\gamma$-ray emission constitute a serious drawback for radiotherapy with $^{131}$I-Lipiodol because patients must be kept in isolation for at least 8 days [49,78]. However, the absence of particle embolization following injection of $^{131}$I-Lipiodol allows this procedure to be performed in the presence of portal vein thrombosis [49].

Radioembolization with resin or glass microspheres coated with the $\beta$-emitting radioisotope $^{90}$Y (half-life 64 h) is becoming an attractive approach to treat intermediate-stage HCC [5,49,79]. The radioisotope $^{188}$Re has also been used to label Lipiodol (as $^{188}$Re-4-hexadecyl 1,2,9,9-tetramethyl-4,7-diaza-1,10-decanethiol (HDD)-labelled Lipiodol) [80]. In conclusion, the potential benefits associated with selective internal radiotherapy justify the numerous ongoing clinical trials.

### 5. Lipiodol distribution following intra-hepatic arterial injection

In normal rats, following slow injection into the hepatic artery, Lipiodol rapidly appeared in the portal venules, through the peribiliary plexus [81]. The first oily droplets typically appeared in the portal venules only a few seconds after the start of injection. When sufficiently small, Lipiodol droplets rapidly passed through the terminal portal venules into the sinusoids. Following filling of peripheral portal branches, patchy sinusoidal congestion occurred, and was associated with necrotic areas surrounded by inflammatory cells. It was also observed that Lipiodol could pass through the sinusoids from portal veins into the hepatic veins.
Lipiodol has a “plastic” (as it adjusts to the size of the microvessel) and transient embolic effect [6,81].

In the event of overdosage, Lipiodol may possibly appear in the systemic circulation, and is therefore associated with a risk of systemic embolization. In rats, arterial circulation resumed relatively soon after intrahepatic Lipiodol injection. The oil cleared from the portal vessel in 2–3 days and from the sinusoids in 7 days. By the 15th day following selective injection, no oil could be detected in the portal venules and only a few droplets remained in the sinusoids [81]. The amount of oil observed in the portal vein was dose-dependent. This effect was confirmed in other animal species [82,83]. The hepatic arterial blood washed Lipiodol into the portal vein through the peribiliary plexus and not directly into the sinusoids. It is generally admitted that opacified vessels in Lipiodol-treated HCC are portal veins or venules, since the oil does not remain for a sufficiently long time in the tumour arterial branches [48].

Phagocytosis by activated Kupffer cells is probably involved in Lipiodol clearance [83]. The presence of oil droplets in Kupffer cells has been demonstrated by electron microscopy. In rats, Lipiodol was completely cleared in 30 days (0.1 mL/kg) or 60 days (0.2 mL/kg). At these time-points, the microcirculation recovered and the number of Kupffer cells returned to normal [48].

In healthy pigs, extensive necrosis of the hepatic parenchyma was observed after re-embolization of the restored arterial flow with gelatin particles. Hepatic artery embolization performed with gelatin particles alone did not produce any necrotic changes in the liver parenchyma [83]. As in pigs, combined administration of Lipiodol and gelatin beads into the rat hepatic artery severely affected the normal hepatic microcirculation (decrease in blood flow velocity through terminal portal venules [TPV] and terminal hepatic venules). However, hepatic artery embolization with Lipiodol alone, which was associated with oil accumulation in the TPV, did not adversely affect the hepatic microcirculation [84].

5.1. Tumour uptake

5.1.1. Preclinical data

Following the early observation of Lipiodol globules in HCC cells of patients [85], it was reported that Lipiodol droplets accumulate in both fibroblasts and a human HCC cell line (Hep cells) and bind to cell membranes, suggesting a non-specific phenomenon. However, the final amount of Lipiodol accumulated in Hep cells was greater than that in fibroblasts and the proportion of Lipiodol-laden cells was higher with tumour cells than with fibroblasts [86]. Uptake has been reported for both the HepG2 cell line and endothelial HUVEC (human umbilical endothelial cells) line [87], thus confirming the absence of specificity for cell loading. The uptake of Lipiodol by HUVEC is of interest, as the incorporation of Lipiodol in endothelial cells of HCC vessels in patients has been reported [87]. It has been suggested that a cell membrane pump may be involved in the absorption of Lipiodol by tumour cells [7]. The presence of membrane-bound Lipiodol-filled vesicles suggests that pinocytosis is the most likely mechanism of uptake [87].

Membrane cell fluidity of HCC cells is lower than that of normal hepatocytes because of an increase in the phospholipids/cholesterol level [88]. The addition of both dexamethasone and tamoxifen concentration-dependently increased the membrane fluidity of rat tumour N1S1 cells in vitro. In rats, pretreatment with these molecules was found to increase the tumour uptake of 99mTc-SSS-Lipiodol (i.e. super six sulphur Lipiodol composed of a lipophilic complex of 99mTc-PhCS2 (PCS3)2 solubilized into Lipiodol) compared to controls, while no effect was observed on the distribution of the radiotracer in the lungs [89].

5.1.2. Hepatocellular carcinoma microvascularization and rationale for Lipiodol embolization

HCC is typically a highly angiogenic cancer [90]. The expression of biomarkers for microvessel density is associated with development and progression of the disease [91]. HCC microvasculature is typically less dense than normal liver vasculature. Tumour microvessels have an abnormal blood flow and are very leaky. These characteristics lead to hypoxia and/or necrosis [90]. Schematically speaking, blood from the hepatic artery goes through the veno-portal compartment before it perfuses the tumour.

Basically, arterio-portal communication occurs at four levels: (a) peribiliary plexus (a dense microvascular network surrounding the bile ducts); (b) terminal arteriosinus twigs (i.e. small branches arising from the hepatic arterioles to connect the sinusoids at their origin); (c) vasa vasorum on the wall of the portal vein and (d) functional arterio-portal anastomoses (the least frequently reported of the four types) [92]. These shunts are typically located at the margin of the tumour (Fig. 4).

In healthy liver, the inner diameter of portal venules is 50–100 μm, that of terminal portal venules is 15–50 μm, that of the sinusoid network is 5–8 μm, that of the post-capillary terminal venules is ~25 μm [92] and that of the hepatic arterioles is 35–45 μm [93].

Tumour vasculature communicates with surrounding portal venules and sinusoids. Importantly, the hepatic arterial blood accesses the tumour via the portal venules and sinusoids surrounding the lesion [92]. As demonstrated after injection of a fluorescent tracer in animal models, arterial blood enters the tumour through the portal venules without resistance, while blood from the portal vein meets great resistance at the tumour margins, thereby accumulating in the sinusoids surrounding the tumour [48]. Interruption of either the hepatic arterial or portal venous blood flow (e.g. by embolization) does not eliminate tumour blood perfusion [92]. A likely explanation for this phenomenon is that a high pressure in the peribiliary plexus prevents portal blood flow from entering the tumour. Following arterial embolization, as arterial blood flow is impaired, the peribiliary plexus blood
pressure drops, thus allowing portal perfusion of the tumour [92]. Because of its oily nature, Lipiodol allows transient dual (arterial and portal) embolization of HCC [48], unlike DEBs which only block hepatic arterioles. These results could favour Lipiodol-based TACE, as they demonstrate that embolization of only the hepatic artery without concomitant embolization of the portal vein is insufficient. This is clinically important in extracapsular infiltrative tumour and in surviving frontier regions, as the majority of local sinusoids are perfused via the portal vein [92].

When sinusoidal spaces are filled beyond a certain threshold, any additional volume of oil may flow back into the portal vein via arterio-portal communications [92,94–96]. Although encapsulated liver tumours are almost exclusively perfused by arterial blood, infiltrative lesions and daughter nodules are perfused via peripheral sinusoids, i.e. with portal blood [92,94,96] (Fig. 5).

Apart from rats, many studies have also been performed in rabbits implanted with the VX2 tumour [97]. Table 3 summarizes the results of distribution of Lipiodol in this rabbit model. In the VX2 rabbit model, 3 days after selective injection of SMANCS/Lipiodol solution, the ratio of Lipiodol concentration in the lesion to that in plasma was >3000 (∼10 in liver adjacent to the tumour, ∼30 in liver parenchyma distant from the tumour and ∼100 in lung, brain and kidney) [98].

A clinical study conducted in patients treated by transarterial injection of Lipiodol alone or Lipiodol + doxorubicin followed by gelatin sponge particles and subsequent hepatectomy, compared dynamic computed tomography (CT) findings (within 3 weeks before TACE and within 4 weeks before surgery) with pathological findings of resected tumours. Tumour specimens and the necrosis rate measured on CT showed a significant correlation, an effect consistent with the retention of Lipiodol in the necrotic area of the tumour [102].

It was subsequently shown that complete Lipiodol uptake without enhancement by water-soluble contrast medium on serial helical CT (Fig. 6) was predictive of complete necrosis in the explanted liver treated by TACE followed by liver transplantation [103]. The volume of Lipiodol deposited in the tumour observed on CT 24 h after TACE was significantly correlated with subsequent necrosis and with the reduction in total tumour volume. Furthermore, a significant association was observed between Lipiodol volume measured on CT and survival time since diagnosis and since treatment [104]. In a retrospective analysis of 84 HCC patients, Mondazzi et al. reported that the early degree of Lipiodol labelling of the tumour was an independent prognostic parameter for survival [105]. This was confirmed in other studies [106–108].

Histological analysis of HCC surgically resected or discovered incidentally after transplantation in TACE-treated patients, revealed the presence of oily droplets both in endothelial cells and in tumour cells surrounding these vessels [87]. In one explant liver perfused ex vivo with Lipiodol shortly after surgery and immediately fixed thereafter, Bhattacharya et al. [87] observed brown intracellular vesicles of Lipiodol in balloononed, foamy tumour cells as well as in endothelial cells. The presence of oily droplets in HCC tumour cells within 15 min after resection (at this time point, cells were still considered viable) suggests that Lipiodol cellular uptake is a very rapid (and possibly active) process [87].

The distribution of Lipiodol or 131I-Lipiodol was evaluated (CT scan or gamma-camera) in 6 patients with HCC who underwent hepatic lobectomy or segmentectomy. Lipiodol was retained in the tumour in all cases, for as long as 3 months post-infusion. One- to two-thirds of the tumour mass was necrotic. Most necrotic areas were replaced by inflammatory tissue, as well as fibrosis in some cases. Only a few oily globules were observed in cirrhotic nodules and in the connective tissue septa. Intracellular lipid globules were located in the cytoplasm of tumour cells. Tumour cells were enveloped by non-globular lipid along the cell membrane, giving the HCC a honeycomb-like appearance. It is noteworthy that, in this study, Lipiodol and 131I-Lipiodol were administered in the absence of any cytotoxic drug [85].
5.2. Parameters influencing tumour uptake

Basically, the rationale for local treatment of the tumour is to achieve a high and selective concentration of the cytotoxic agent associated with embolization-induced ischaemia. It is also crucial to release the cytotoxic agent locally under standard conditions. These goals are obviously closely related. Anatomical (size, density and architecture of tumour microvessels, tumour size, ischaemia, necrosis, etc.), physiological (local flow, shear stress, blood pressure, permeability, etc.), and physicochemical parameters associated with the drug itself [22] or the emulsion, are all involved in tumour uptake [4,6,109].

In terms of anatomical parameters, Lipiodol (pure or as an emulsion) tends to follow large diameter arterial branches at each bifurcation [6]. This phenomenon probably plays a crucial role in the selectivity of Lipiodol for HCC.

Although each parameter cannot be interpreted independently of the others, an extensive body of work has been published in the past 25 years and is summarized below.

5.2.1. Lipiodol-cytotoxic drug emulsion

Most cytotoxic molecules are more soluble in water than in Lipiodol, which is why emulsions (mixture of two phases that are insoluble in each other) are usually used. However, depending on their hydrophilic–lipophilic balance (HLB), cytotoxic molecules may present different distribution ratios in aqueous or oily phases. When the cytotoxic drug can be directly suspended in Lipiodol, the suspension may be used without the need to prepare an emulsion. A considerable heterogeneity of the physicochemical characteristics of the emulsion is observed when emulsions are used for TACE, as the type of emulsion (water-in-oil [W/O] or oil-in-water [O/W]) and the size of the emulsion depend on many
parameters which are mostly beyond the control of the clinician. Examples of these parameters are: the water vs. Lipiodol volume ratio [109], the pressure exerted on both fluids during the extemporaneous preparation of the emulsion, the number of times the emulsion is pushed through the three-way stopcock, or the nature and amount of excipients in the cytotoxic drug used. The importance of these parameters may be overlooked by clinicians, who may tend to believe that pushing the aqueous solution of cytotoxic agent into Lipiodol systematically results in a W/O emulsion. Furthermore, since an emulsion is not described by thermodynamic equilibrium laws, the type of emulsion (W/O or O/W) may not be reproducible even when it is prepared under similar conditions. This is particularly true when a 1:1 ratio is used to mix the aqueous phase and Lipiodol. Lastly, the ideal emulsion for TACE procedures has not been clearly defined. Most emulsions used in TACE, without the addition of surfactant, are characterized by highly polydispersed sizes that certainly evolve very rapidly in vivo because of their poor stability.

5.2.2. Type of emulsion

De Baere et al. [6] compared the stabilities of four types of Lipiodol and doxorubicin emulsions: small (10–40 μm) droplet water-in-oil (W/O); large (30–120 μm) droplet-W/O; small droplet O/W and large droplet O/W. Emulsion type and size were evaluated by light microscopy. The stability of all emulsions tested was considered to be good: no changes were observed in emulsion appearance, both in vitro (silicone model of the arterial tree) and in vivo (rat cremaster muscle model). The poorest embolic effect was achieved with small droplets of the O/W type. As expected, W/O emulsions had a similar, high embolic effect, close to that of pure Lipiodol. This effect can be attributed to their oily external phase [6]. Kan et al. [109] demonstrated that a W/O (1:2 ratio) emulsion in rats exhibited a higher doxorubicin carriage capacity (estimated on the basis of the water droplets in oil drops) and a longer release time than an O/W (2:1 ratio) emulsion.

5.2.3. Droplet size

In general, emulsification provides a polydispersed system in which large and small droplets coexist [110].
This is particularly true for extemporaneous emulsions of a cytotoxic agent with Lipiodol. Intuitively, it can be assumed that if Lipiodol-based emulsion droplets are too small, they will be trapped by both healthy and tumour tissues and will pass through the sinusoids to reach extrahepatic organs. Conversely, if they are too large, they will accumulate proximal to the tumour microvascular bed. This is also true for DEBs: DEB-induced biloma and necrosis of the liver parenchyma were recently attributed to the relatively large size of the beads used (500–700 μm) [75]. In fact, tumour vascular architecture probably influences droplet distribution. The distribution of six different Lipiodol + doxorubicin O/W mixtures (shaken for 5, 10, 15 and 30 min) within and around hypovascular liver metastases of human colorectal tumour was compared in mice following either intra-arterial or intra-portal injections. All droplets found within the centre of hypovascular tumours measured less than 20 μm. Larger droplets aggregated in and occluded microvessels surrounding the tumour, but did not enter the tumour. Interestingly, microdroplets first adhered to the vascular endothelium and then advanced towards the centre of the tumour where they accumulated [111]. However, because of their small size, these droplets can be easily entrapped by the lung, thereby resulting in pulmonary toxicity.

In rabbits bearing the VX2 liver tumour, the highest tumour/healthy liver parenchyma uptake 4 days after selective injection into the hepatic artery was achieved with a “large” (30–120 μm with >70% of droplet diameters in the range 70–100 μm) droplet W/O emulsion, compared with all other emulsions tested. The highest lung uptake was observed with pure Lipiodol and small droplets (10–40 μm with >70% of droplet diameters in the range 20–30 μm), O/W emulsion [100].

A clinical study investigating the influence of the droplet size of Lipiodol and epirubicin water-in-oil-in-water (W/O/W) emulsions (average diameter of 30 vs. 70 μm), concluded that tumour necrosis (estimated from the decline in serum α-fetoprotein (AFP) levels in the first week) was greater in patients who received the 70 μm emulsion than in those who received the 30 μm emulsion [112].

In summary, no clear conclusion can be drawn concerning this important issue. Further studies are definitely needed, with rigorous investigation of the in vivo behaviour of various types of well-characterized emulsions.

5.2.4. Viscosity

The type of emulsion (W/O or O/W) is the main parameter determining their viscosity. Droplets of the discontinuous phase can be considered to be particles in solution that increase the initial viscosity of the continuous phase. The viscosity of W/O emulsions was found to be higher than that of Lipiodol alone which, in turn, was higher than that of O/W emulsions [6].

Lipiodol exhibits Newtonian behaviour (i.e. viscosity does not depend on shear stress) [6]. In this situation, viscosity only depends on temperature and pressure, not on the forces acting on the emulsion. However, Lipiodol/doxorubicin O/W and W/O emulsions were found to be non-Newtonian (i.e. the viscosity alters with shear rate) below a shear stress value of 40 s⁻¹. Viscosity was, in descending order: W/O > Lipiodol > O/W. No correlation was observed between viscosity and the embolic effect of the emulsion in rats and rabbits [6].

However, based on the assumption that increased viscosity of Lipiodol would increase its tumour retention, the biodistribution of various formulations of radiolabelled Lipiodol mixed with stearic acid to increase viscosity (up to 67 mPa·s) was subsequently investigated in rats bearing N1S1 liver tumour. At 72 h, the optimum tumour uptake was observed with the formulation incorporating 0.8% stearic acid (54 mPa·s) [113].

5.3. Drug-delivery properties of Lipiodol

In vivo studies have demonstrated that the most distal release of doxorubicin from Lipiodol was observed for W/O emulsions [109], corresponding to the optimal site of release. This effect was actually observed when droplets reached arteries corresponding to their own diameter [6]. Water-in-oil type emulsions are superior to O/W type emulsions in terms of drug delivery capacity, as shown in both mice and rats bearing liver tumours. Furthermore, they result in a longer contact time than O/W emulsions following selective injection of various types of Lipiodol/doxorubicin emulsions [109]. Intuitively, these results make sense, as the aqueous solution which represents the dispersed, discontinuous phase in which the cytotoxic agent must be solubilized, can be delivered to a more distal and selective level than an O/W emulsion, in which the aqueous continuous phase containing the cytotoxic drug could be easily flushed away by local arterial blood flow.

An important issue is the distribution of droplets in the microvascular network. Various types of Lipiodol-doxorubicin emulsions (large droplet O/W or W/O and small droplet W/O) have a propensity to pass through the largest arteries in bifurcations (like pure Lipiodol), with virtually no passage through narrower arteries [6]. This phenomenon may facilitate the selectivity of oily droplets to the tumour, as it is generally accepted that tumour vessels are usually larger than healthy vessels at a similar level of bifurcation [6]. Two possible explanations can be proposed for this phenomenon: (a) when the artery diameter/particle diameter ratio is less than 30, particle distribution is not proportional to flow in microvascular bifurcations and distribution preferentially occurs through the vessel with the largest diameter [114]; (b) the combination of the high surface tension of Lipiodol-based droplets and the low shear rate in narrow microarteries [6].

The mechanism by which cytotoxic drugs are retained within the tumour tissue to allow sustained impregnation is unclear and most probably depends on the physicochemical properties of the molecule itself, notably its HLB, charge and molecular weight (MW). The drug-delivery potential of
iodinated poppy seed oil should therefore be characterized on a case-by-case basis.

The enhanced permeability and retention (EPR) effect is a universal phenomenon by which macromolecular agents are passively retained in the interstitial space of tumours as the consequence of high microvascular density and permeability and the absence of lymphatic drainage [23,115–117]. The EPR effect is applicable to macromolecules with MW higher than 40 kDa [117]. Despite its relatively low MW, SMANCS accumulates in HCC via the EPR effect because it binds to albumin and consequently reaches a MW value of about 80 kDa [23]. Arterial infusion of a SMANCS/Lipiodol mixture is therefore an excellent targeting method, as shown in the rabbit VX2 model of HCC [98].

Compared with Lipiodol-based emulsions, DEBs seem to release doxorubicin at a slower rate [118]. In a pig model, doxorubicin-loaded DEBs eluted 43% of their initial cytotoxic agent load 28 days after left lobe hepatic artery embolization, and this effect was not complete after 90 days (89% elution), regardless of the DEBs size [118].

Indeed, DEBs are less prone to inter- and intra-operator variability compared with conventional TACE [66]. However, these characteristics have not been shown to be associated with a significant, evidence-based clinical benefit (response rate or survival) [74]. It may be possible that the release of the drug from DEBs is too slow to achieve sufficiently high cytotoxic concentrations, as in the case of metronomic chemotherapy.

5.4. Role of Lipiodol in modulating the pharmacokinetics of associated chemotherapies

The primary objective of TACE is to locally deliver the drug and minimize systemic toxicity by decreasing drug levels in non-tumour tissue. Only two prospective, comparative clinical studies with conflicting results have investigated the effect of Lipiodol on circulating doxorubicin concentrations. Johnson et al. [119] did not observe any difference in the pharmacokinetic behaviour of doxorubicin or its principal metabolite doxorubicinol in 9 patients receiving two courses of a bolus injection of doxorubicin into the common hepatic artery, in one of which the drug was mixed with 10 mL of Lipiodol. Furthermore, mixing doxorubicin with Lipiodol had no significant effect in terms of toxicity. A potential drawback of this study may be the disproportionate volume of saline used to dissolve doxorubicin (25 mL) compared with that of Lipiodol (10 mL), making it an O/W emulsion [8]. In a subsequent study [120], the biodistribution of doxorubicin was investigated in 18 patients who received either doxorubicin alone into the hepatic artery (Group 1) or emulsified in Lipiodol without (Group 2) or with (Group 3) gelatin sponge particle embolization. In this study, a W/O emulsion was prepared (doxorubicin was dissolved in 2.5 mL of water-soluble contrast medium and the Lipiodol volume was 10 mL). The doxorubicin peak plasma concentration was significantly higher in Group 1 (no Lipiodol) than in Groups 2 or 3. The area under the curve was also higher for Group 1 compared with Groups 2 and 3. This study confirmed the preferential concentration of cytotoxic drug in the tumour measured after surgical resection observed with multiple cytotoxic agents in several studies [11,21,55,121]. However, no local correlation between iodine (used as a marker of Lipiodol uptake) and doxorubicin concentrations was recently reported in the VX2 tumour in rabbits [122]. Indeed, determination of this relationship would elucidate the controversial [37] value of Lipiodol as a drug delivery matrix and would distinguish between drug-induced cytotoxicity and Lipiodol-induced ischaemia. Noteworthy, an O/W emulsion was used in this study [122]. As mentioned above, it may be speculated that this type of emulsion may have had less efficient drug delivery properties than W/O emulsion.

6. Effects of Lipiodol per se on hepatocellular carcinoma

In vitro, both clonogenic assay and Trypan blue test demonstrated that Lipiodol increased the cytotoxicity of doxorubicin on HepG2 tumour cells [123]. Lipiodol-induced cytotoxicity was greater for tumour cell lines (HepG2 and MCF-7 [human breast cancer]) than for human hepatocytes in one study [124]. However, no toxic effects were found in other studies [86,87].

In vivo, Lipiodol may induce direct pro-necrotic effects in liver tumours, depending on the experimental model. This type of effect has been suggested for a long time.

Although rat models of HCC are widely used to investigate the pharmacodynamic effects of Lipiodol, either alone or associated with cytotoxic drugs, the size of the rat hepatic artery branches makes superselective embolization virtually impossible. Therefore, most authors injected the Lipiodol + cytotoxic drug by catheterization of the common hepatic artery via the gastroduodenal artery. Overall, a therapeutic effect for Lipiodol alone is controversial (two studies supported this effect, one in VX2 tumour-bearing rabbits [44] and one in rats [125], while two studies in rats did not show any significant effect [126,127]). If it exists, this therapeutic effect is probably modest.

Interestingly, in one study comparing the pharmacokinetics of doxorubicin after conventional or DEB-TACE in rabbits, a doxorubicin-independent necrotic effect was found in the conventional TACE group and ascribed to the combination of the cytotoxic effects of both Lipiodol and embolic material [70].

In patients, a therapeutic effect (dramatic decrease in blood AFP levels and tumour necrosis) has been reported with both 131I-Lipiodol and non-radiolabelled oil [85]. However, in another study, Lipiodol alone had practically no therapeutic effect [128].

Typically, HCC is a highly hypoxic tumour [90]. The transcriptional regulator factor HIF-1α (hypoxia-inducible-factor 1) regulates the adaptive response to lack of oxygen.
HIF-1α translocates to the nucleus and forms the HIF-1 complex that initiates vascular endothelial growth factor (VEGF) transcription. The pro-ischaemic effect of TACE may induce HCC cells to secrete more VEGF. This effect has been observed in patients [130]. In rabbits, selective injection of Lipiodol alone (without the addition of embolic agent) induced VEGF expression and an increase in microvessel density (MVD). MVD was significantly correlated with VEGF expression [131]. In patients, a transient increase in plasma VEGF levels was observed (day 1 post-TACE) and the change in plasma VEGF levels (1 month after TACE) was significantly associated with Lipiodol retention by the tumour [132]. In a prospective study in 71 patients undergoing TACE, below-median VEGF plasma levels were a significant predictor of a longer survival [133]. Taken together, these data support the potential value of the combination of anti-angiogenic drugs with TACE in HCC. However, the most appropriate time window for the administration of such agents, respective to the TACE procedure, has yet to be established. Investigations designed to improve the synergy between TACE and targeted therapies are a priority in the field of HCC treatment.

7. Perspectives

7.1. Pharmacological management

There is considerable ongoing research to improve the pharmacological management of unresectable HCC. The multikinase inhibitor sorafenib, is the only targeted therapy approved by health authorities (Europe, US, Japan, etc.) for the first-line treatment of unresectable HCC in patients who are not candidates for TACE, based on a 2.8-month survival advantage over best supportive care in the Sorafenib Hepatocellular carcinoma Assessment Randomized Protocol (SHARP) study [134]. However, sorafenib-induced side effects may seriously affect the patient’s quality of life [135]. Combination therapy associating sorafenib and TACE may be a promising approach in patients with advanced/intermediate HCC [136,137]. However, so far, it is unclear whether this agent combined with TACE has additive or synergistic effects compared with TACE alone [137]. Phase II and III clinical trials evaluating sorafenib combined with locoregional therapies are ongoing [137]. Although molecular targeted therapies in HCC are definitely an interesting and active area of research [138], a promising future can reasonably be expected for interventional procedures and combination of these approaches in selected patients. Combination of TACE and radiofrequency ablation seems also to be a promising approach for the control of HCC [139].

7.2. Thermochemoembolization

Thermochemoembolization is a technique based on targeted deposition of magnetic nanoparticles into the tumour, followed by application of an alternating current magnetic field inducing focused heating (39–49°C). Superparamagnetic iron oxide (SPIO) nanoparticles may sensitize the tumour to the effects of hyperthermia. The presence of both iodine and iron oxide may allow dual (CT and magnetic resonance imaging, MRI) imaging. Highly selective targeting of VX2 tumours in rabbits with therapeutic temperatures has been demonstrated using SPIO nanoparticles suspended in Lipiodol [140]. However, further investigation of the tissue reaction showed ischaemic necrosis of healthy liver parenchyma [141]. Furthermore, a suspension of SPIO (diameter 150 nm) in Lipiodol was found to be extensively phagocytosed in the liver of healthy pigs and was followed by extensive necrosis. No hepatic clearance of the suspension was observed 28 days after injection. Microvascular occlusions were also observed [142]. Despite these early negative results, thermochemoembolization remains a field of extensive research.

7.3. Immunoembolization

Immunoembolization is another interesting field of investigation. This technique refers to the injection of granulocyte-macrophage colony-stimulating factor (GM-CSF) emulsified with Lipiodol (associated with gelatin sponge particles) into the hepatic artery [2]. Promising results have been reported for immunoembolization with GM-CSF in patients with liver metastases from primary uveal melanoma [143].

7.4. Gene therapy

Interventional therapy procedures provide unique opportunities for the delivery of gene therapy vectors into HCC or other cancer. Several approaches can be anticipated [144]: (a) restoration of tumour suppressor genes. Promising results on tumour growth were obtained following selective administration of adenovirus (Ad)-p53 gene mixed with Lipiodol in rabbit VX2 tumour [145]; (b) inhibition of oncogenes (e.g. Ras, pituitary tumour transforming gene-1 or PTTG1, telomerase reverse transcriptase or TERT, etc.); (c) gene-directed enzyme/prodrug therapy (i.e. transfer of exogenous genes which convert a non-toxic prodrug into a cytotoxic molecule); (d) genetic immunotherapy; (e) antiangiogenic gene therapy (an approach which may be of particular interest in HCC); (f) delivery of oncolytic viruses.

8. Discussion

Selectivity of Lipiodol uptake by HCC nodules and its long-lasting capture is of great clinical relevance. The mechanism for tumour uptake and long-lasting stagnation remains debated. Several hypotheses (not mutually exclusive) have been proposed and are summarized in Table 4. Direct capture by tumour and endothelial cells is quantitatively limited.
Table 4

Current hypotheses for the uptake of Lipiodol by hepatocellular carcinoma.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Basis for the hypothesis</th>
</tr>
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<tbody>
<tr>
<td>Propensity of oily droplets to pass through the largest microvessel at each bifurcation</td>
<td>Larger diameter vessels preferentially supply the tumour [6]</td>
</tr>
<tr>
<td>Tumour vascularity improves Lipiodol uptake</td>
<td>HCC is one of the most hypervascular solid tumours [90,91]</td>
</tr>
<tr>
<td>Almost complete absence of a reticulo-endothelial system in the tumour: no degradation and absorption of the oil</td>
<td>Kupffer cells are responsible for eliminating Lipiodol from the healthy liver parenchyma [146]</td>
</tr>
<tr>
<td>Direct capture by tumour and endothelial cells</td>
<td>Demonstrated in numerous studies [85,87,123]</td>
</tr>
<tr>
<td>Lack of lymphatic system in HCC: no drainage</td>
<td>Suggested in [147]</td>
</tr>
<tr>
<td>Difference between normal liver tissue and HCC</td>
<td>Dual blood supply (hepatic artery and portal vein) probably induces more rapid washout from the normal parenchyma</td>
</tr>
</tbody>
</table>

The major advances in “omics”, medical imaging, nanotechnologies and pharmacology pave the way for a drastically new approach to treat patients. This new process is based on an in-depth knowledge of pathophysiological mechanisms of diseases and has led to the birth of a new, target-centred era based on a specific biological hypothesis for the identification and development of new molecular entities (NME) to interact with the target [148].

The concept of “theranostics” was coined in 1998 by the US consultant John Funkhouser to describe a material that allows the combined diagnosis, treatment and follow-up of a disease [149]. This approach allows the selection of a subpopulation of patients most likely to benefit from targeted therapy or, conversely, those at higher risk of adverse effects. From the clinician’s viewpoint, personalized medicine has obvious major consequences in terms of patient management. The revolutionary concept of theranostics has evolved over time, integrating two distinct approaches that both encompass all steps of patient management:

(a) The biomarker approach to diagnose the disease, determine the best course of treatment, follow the patient’s response and detect potential recurrence of the disease.
(b) Guidance and follow-up of therapeutic interventions (e.g. local drug delivery, medical device or cell therapy). Medical imaging is the prerequisite for such approaches.

The concept of theranostics perfectly applies to Lipiodol-based chemoeMBOLization and this technique is probably the first example of the validity of theranostics [117]. Given the radiopacity and drug delivery capacity of Lipiodol, several perspectives (summarized above) can now be considered. These properties are consistent with the two conceptual approaches of theranostics.

The prognostic benefit associated with early tumour labelling by Lipiodol [105–108] confirms its potential value as a biomarker, while the relationship with the behaviour of Lipiodol in the stroma and cell compartments requires further investigation.

DEBs are an interesting approach to standardize HCC embolization. Table 5 compares and summarizes the main properties of Lipiodol and DEBs for TACE.

DEBs cannot be directly imaged by fluoroscopy during the procedure and must be mixed with a water-soluble iodinated CM to indirectly monitor delivery of the embolic material. The dilution ratio is empirical. Furthermore, visualization of the CM does not provide any information on the site of beads in the lesion. Over recent years, extensive research has been conducted to develop imageable beads for MRI, logically based on iron oxide nanoparticles [151,152], fluoroscopy based on Lipiodol [150,153] or both techniques (i.e. multimodal approach) [154]. Signal intensity as a surrogate biomarker for local drug delivery would be clinically and pharmacologically meaningful. This goal has not yet been achieved with either DEBs or Lipiodol.

The stability of Lipiodol-based emulsions and hence their drug-delivery capacities is greatly influenced by the ratio between Lipiodol and saline or water-soluble contrast medium used to solubilize the cytotoxic drug. This ratio determines the O/W or W/O type of the emulsion. W/O emulsions of adequate size will probably allow the cytotoxic drug to enter the portal compartment before phase separation [11], as deemed desirable based on non-clinical studies [6,48]. It has been suggested that a Lipiodol/doxorubicin volume

Table 5

Conventional (Lipiodol-based) TACE vs. drug-eluting bead-based TACE.

<table>
<thead>
<tr>
<th></th>
<th>Conventional TACE</th>
<th>Drug eluting bead-based TACE</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Proven benefit on survival</td>
<td>Yes</td>
<td>Yes</td>
<td>[3,5,73]</td>
</tr>
<tr>
<td>Real-time fluoroscopy-guided delivery</td>
<td>Yes</td>
<td>No</td>
<td>[7,21,149]</td>
</tr>
<tr>
<td>Tumour labelling on CT (prognostic value)</td>
<td>Yes</td>
<td>No</td>
<td>[105–108]</td>
</tr>
<tr>
<td>Embolization effect</td>
<td>Transient plastic embolization</td>
<td>Non-plastic embolization</td>
<td>[7,49,66,69,81,96,118]</td>
</tr>
<tr>
<td>Vascular selectivity for the tumour</td>
<td>Yes (depends on vessel size)</td>
<td>Yes if small (&lt;300 μm)</td>
<td>[6,100,118]</td>
</tr>
<tr>
<td>Release of cytotoxic agent</td>
<td>Fast</td>
<td>Slow</td>
<td>[118]</td>
</tr>
<tr>
<td>Systemic release of the cytotoxic drug</td>
<td>Moderate</td>
<td>Low</td>
<td>[66,69,71,120]</td>
</tr>
<tr>
<td>Incidence of biloma and liver infarct</td>
<td>Low</td>
<td>High</td>
<td>[75,76]</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>High</td>
<td>–</td>
</tr>
</tbody>
</table>
2–3:1 ratio would optimize the local bioavailability of the drug [8].

Many preclinical and clinical studies have compared various types of emulsions or suspensions comprising cytotoxic drugs and Lipiodol. Unfortunately, these emulsions/suspensions are often inadequately characterized, thereby making it hazardous to define any clear conclusions. Tailored emulsion technology is a rapidly evolving field in drug-delivery technologies, notably for anti-cancer drugs [154]. A growing knowledge in the field of formulation (selection of emulsifiers, additives, and drug incorporation methods) and the basic knowledge of lipid chemistry and cytotoxic drug solubility in emulsion systems suggest a bright future for Lipiodol-based emulsions. However, the small number of approved and marketed cytotoxic or cytostatic molecules remains a major constraint in linking physicochemical parameters of these drugs to stability, formulability, clinical efficacy and tolerability of lipid-based emulsions [155]. This issue cannot be resolved without close collaboration between drug companies, clinicians and health authorities.

9. Conclusion

The benefits associated with the use of Lipiodol for TACE are widely acknowledged and have led to a huge number of preclinical and clinical studies over the last 25 years, with continuing interest in this field. Its unique combination of properties as a tumour-seeking, drug-delivery, embolic and X-ray opaque agent explains why it is used worldwide for chemoembolization procedures for many clinical indications, beyond hepatic malignancies. The use of Lipiodol is acknowledged in international and national guidelines [2,3]. As it is performed today, TACE has the drawback of being a heterogeneous technique (type of cytotoxic drug, type of embolic agent, emulsion, timing, selectivity, schedule, etc.), making it a field of intense and challenging research. Two additional drawbacks are worth mentioning: firstly, no cytotoxic agents are specifically authorized for TACE procedures in Western countries; secondly, shortages of many cytotoxic drugs, primarily generic drugs, are becoming common and raise a serious problem for clinicians [156].

In addition to its radiopaque properties, Lipiodol has the unique property of transiently reducing intra-tumour portal perfusion and locally delivering the drug of interest. Future research is expected to improve the efficacy of Lipiodol as a versatile drug delivery system. Despite its venerable age, Lipiodol remains the topic of a very active research associated with promising perspectives.

Conflict of interest

Jean-Marc Idée is employee of Guerbet.

Boris Guiu declared no conflict of interest.

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