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Review Article

Enhancement of treatment efficacy of hepatic tumours using Trans-arterial-chemoembolization

Krishna Pillai, Kevin Ke, Ahmed Mekkawy, Javed Akhter, David L Morris

Department of Surgery, UNSW, St. George Hospital, Kogarah, NSW, Australia

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Abstract: This review article examines the basic principle underlying trans-arterial chemoembolization (TACE) used for treating unrespectable liver cancer with discussion on the barriers that are present for efficient drug delivery with suggestions on methods that may be used to overcome these barriers and hence enhance the efficacy of the technique. Current drugs used with TACE along with inhibitors of neovascularisation are briefly discussed. It also compares the conventional method of chemoembolization with TACE and rationalizes why there is not much of a difference between the two methods on treatment efficacy. Further it also suggests alternative methods of drug delivery that may be used instead of TACE. Additionally, it discusses the disadvantages on using non degradable microspheres with recommendations for degradable microspheres within 24 hours to overcome rebound neovascularisation owing to hypoxia. Finally, the review examines some of the biomarkers that are used to assess treatment efficacy with indication that non-invasive and sensitive biomarkers should be identified for routine screening and early detection. The review concludes that, if the current barriers present in TACE can be overcome along with the use of degradable microspheres and efficient biomarkers for monitoring efficacy, then a more robust treatment would emerge that may even serve as a cure.

Keywords: Transarterial-chemoembolization, hepatic cellular carcinoma, neo-vascularisation, Sorafenib, biomarkers, microspheres

Introduction

One third of world's cancer related death has been reported to be caused by hepatic cellular carcinoma (HCC) [1, 2]. HCC is distinctively prevalent in South East Asia, amongst which China leads with 55% of the total world cases [3, 4] suggesting a clear ethnic difference. This difference may be due to a number of factors such as genetic susceptibility, food habits, exposure to liver toxins, liver infection etc. [5, 6]. In California (USA), there is a much higher incidence of HCC in pacific islanders and Latin American [7] and those residing near ethnic neighbourhood indicating that life style factors may be involved. In Europe, there is a wide distribution of HCC with countries in Eastern Europe having a higher incidence. Incidence of HCC in USA has been reported to peak between the age of 50-54 with sex disparity suggesting that estrogens may play a protective role [8, 9].

HCC is the end result of chronic liver infection owing to exposure to liver toxins or viral infection such as hepatitis virus B & C (HBV & HCV) [10, 11]. High body mass index, fatty diets, alcohol and tobacco use may also predispose subjects to HCC [6, 12] whilst a higher incidence also exists in diabetic patients. The pathogenesis by which these agents cause HCC has been reviewed recently by Singh et al. [13]. The selection of treatment modality for HCC depends largely on tumour size, multiplicity and the status of liver [14, 15]. Various treatment methods have been developed such as surgical resection, thermo/cryo ablation, radiotherapy, and chemotherapy [16]. For non resectable tumours, trans-arterial chemo-embolisation (TACE) using conventional methods (c-TACE) with drug delivery in an emulsion (lipiodol) along with arterial plugging with gelatine has been frequently used although more recently drug eluting microspheres has become very popular [17, 18]. Owing to rebound development of tumour micro-

vasculature after TACE due to hypoxic conditions, anti-angiogenic agents such as tyrosine kinase inhibitor, sorafenib, brivanib, sunitinib, regorafenib etc. are used in therapy to inhibit neo-vascularisation of embolised tumours [19, 20].

Drugs commonly used for treating HCC

A selection of chemotherapeutic agents have been used for the treatment of HCC either as single gents or in combination for systemic or loco regional delivery [21]. These agents are either hormonal (ostreotide, tamoxifen), biologic (Thalidomide, interferon), chemotherapy (Sorafenib, 5-fluorouracil, cisplatin, gemcitabine, doxorubicin, capecitabine, mitoxantrone, epirubicin, etopside) or bevacizumab as targeted therapeutic agent [22]. In particular, most of the chemotherapy agents are nucleosides that disrupt the synthesis of DNA and their mode of action along with the other agents have also been fully reviewed recently [23]. Further, the efficacy of these agents either singly or in combination has also been discussed by several authors [21, 24]. Since rebound neo-vascular development in tumours is a common occurrence with TACE, methods to suppress this phenomena using vascular epidermal growth factor inhibitors (VEGF) of which amongst the existing tyrosine kinase inhibitors, sorafenib proved superior in several clinical studies owing to its multiplicity in action. Hence, sorafenib or in combination with other chemotoxic agents are used for treating non-resectable liver tumours [25].

Strategic principals of TACE

The fundamental principles underlying TACE is to disrupt (embolize) the proximal blood supply to the tumour with deprivation of nutrients and oxygen supply whilst delivering a sustained supply of chemotherapeutic drugs to the tumour cells. The chemotherapeutic agents are either delivered lodged within the embolus material in drug eluting microspheres (dem-TACE) or suspended in lipiodol emulsion that ascertains a slow and sustained release locally known as conventional TACE (c-TACE). The main advantage being a substantial and sustained exposure of the agents to tumour cells while reducing systemic exposure to a minimum [26]. This principal and efficacy of the system has been summarised in an equation as given by Collins et al. [27].

$$R_d = \frac{\frac{AUC_{target\,(locoregional)}}{AUC_{systemic\,(locoregional)}}}{\frac{AUC_{target\,(IV)}}{AUC_{systemic\,(IV)}}} = 1 + \frac{CL_{total}}{Q(1 - E)}$$

R_D = Overall selectivity; AUC = area under the curve (total amount of drug delivered); IV = intravenous; CL_{total} = total body clearance of drug; Q = blood flow through target organ; E = organ extraction ratio.

TACE method of drug delivery seems to be an ideal method that targets the tumour specifically and hence capable of producing effective tumour regression with minimal systemic exposure. However, the hypoxic environment created by the embolus induces the tumour cells to over express angiogenic factors (VEGF, PDGF, etc) that is responsible for neo-vascularisation and hence rebound development of tumour [28, 29], although initial response is regional tumour necrosis. Further, neo-vascularisation (poorly developed and leaky blood vessels) of tumour may also lead to novel blood supply that may in theory enhance clearance of the drugs from the tumour since there is a negative drug gradient present in the new blood vessels [30]. How significant this loss would make to tumour regression may need investigation. This drawback is particularly applicable to very slow drug eluting non-biodegradable microspheres with embolus that are long lasting such as DC beads that do not degrade [31]. This principal has been illustrated in **Figure 1**. Since, tumoral capillary beds that are developed are leaky; it may also increase the intra tumoral fluid pressure (ITFP) further in the cellular matrix and hence interfere with drug transfer. Hence, further research may be necessary to determine how neo-vascularisation effect drug concentration and drug dispersion in the tumour. In animal models, neo-vascularisation takes place within 3 days [32] and in human it has been reported to be in 36 hours [33].

In systemic delivery, the drugs are prone to dilution in the general circulation with side effects on all the organ systems. Hence, the intra venous (IV) dosage is generally calculated at a higher dose to account for the systemic

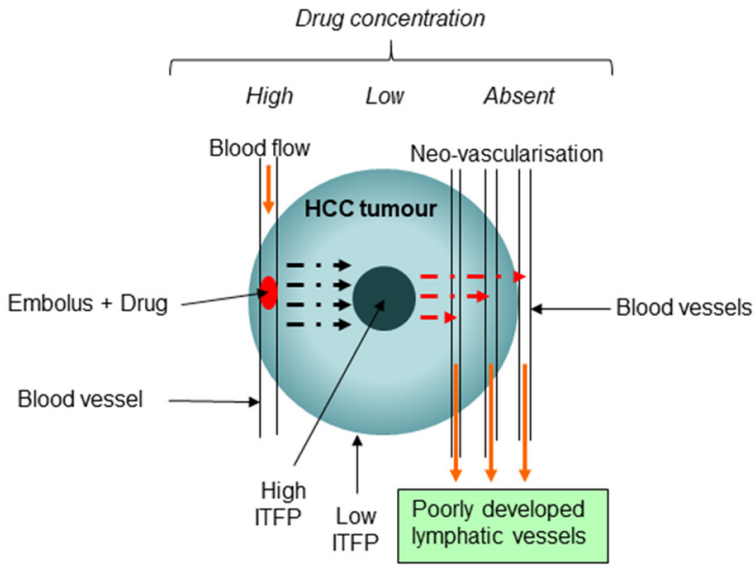

Figure 1. It is a diagrammatic representation of enhanced clearance with build-up of Intra-tumoral fluid pressure. ⟶ Indicates direction of blood flow in the blood vessels; ⟶ Direction of drug diffusion; ⟶ drugs escaping to newly formed blood capillaries. ITFP = Intra-tumoral fluid pressure.

dilution factor. However, in the case of TACE, since drugs are delivered locally to the tumour, the dosage used may be considerably reduced since the dilution factor is minimal [34], with a considerable reduction of systemic exposure and hence the ensuing side effects. This is a major advantage, besides delivering the drugs in situ to the tumour in a sustained manner.

The liver is a highly perfused organ with arterial blood supply accounting for one third of the total blood received whilst venous supply is about two third and hence clearance may be much higher as compared to other organs [35]. Therefore, drug loaded embolizing agents need a much higher content of chemotherapeutic drugs in order to account for the higher clearance [36]. Higher clearance may also predispose to a slight elevated level of chemo-agents systemically and hence side effects when treating HCC using TACE [37], although this elevated level is considerably less compared to systemic delivery.

Marginal differences in efficacy between transarterial embolisation (TAE) and transarterial chemoembolisation (TACE)

The principal underlying TAE is to create a nutritional deficiency as well as a hypoxic environment by introducing an embolus within the microvasculature of the tumour. Cancer cells undergo apoptosis initially owing to deprivation of nutrients and oxygen, although cancer cells rely on aerobic metabolism (Warburg effect) mostly [38]. However, within a very short duration of time, tumour cells undergo changes owing to generous induction of hypoxic factors HIF alpha and beta [39] and become resistant owing to expression of several other survival proteins that induces, anti-apoptosis, replication, angiogenesis and metastasis [40, 41], thereby preserving the tumour population. Hence, the end results, although tumour shrinkage is observed initially, residual tumour cells are preserved with subsequent recurrence of the disease.

Introducing a cytotoxic agent to the hypoxic condition should in principal increase the efficacy of the treatment (a double-edged sword) but, many of the cytotoxic agents are more effective in a fast replicating cell population [42]. In a hypoxic situation, tumour cell replication is much slower and hence may not allow the cytotoxic to have its full effect. Hence, several clinical studies have shown that there is not much of a difference in efficacy between TAE and TACE [43, 44] since hypoxia may act as a barrier.

Taken together, the introduction of microspheres that disintegrate shortly <24 hrs, with the liberation of its drug load at the tumour site may be a better form of therapy since the rebound hyper-vascularisation may be avoided since neo-vascularisation takes 36 hours in human. In addition, if the chemo-agent delivered by the short lived microspheres can be tethered to a suitable agent that has the potential of binding to the tumour matrix but with ability to release the active agent slowly would be an advantage since the cells will be exposed to the agent continually over a long period of time without long term induced hypoxia.

Clinical evidence for TACE

Currently TACE is recommended as first line therapy for patients categorised as moderate

under the Barcelona Liver Cancer Clinic (BLCC) staging system [45]. However, a 2011 Cochrane review analysing 9 trials involving 645 patients showed that there was insufficient evidence to determine whether TACE or TAE showed benefits to survival [46]. Critics of the Cochrane review assert that TACE methods are continually improving, and the Cochrane review was too strict in its inclusion of trials [47]. It will be difficult to conclusively demonstrate whether TACE offers survival benefit due to the substantial time and cost of conducting randomised controlled trials, despite there being still a need to do so. Nonetheless, as it is currently in widespread use across the world, efforts to improve TACE methods such as those described by this paper will still be of practical benefit to clinicians.

Methods to enhance the efficacy of TACE

Currently, a number of chemotherapeutic drugs are available for treating non-resectable HCC tumours, more recently several tyrosine kinase receptors have been tested clinically and of which sorafenib a multi-receptor inhibitor with anti VEGF, PDGF, etc outperformed the others [48, 49]. Hence, a combination of anti-tumour drugs and sorafenib are used in treatment in order to derive maximum benefit [50, 51]. There are also other new tyrosine kinase inhibitors that can be used as a second line therapy if the tumour is resistant to sorafenib [52]. Further, a number of new tyrosine kinase such as Lenvatinib, imatinib etc have been tested in clinical trials with equal efficacy as sorafenib [53]. The delivery of tyrosine kinase inhibitors and other anti-angiogenic agents, either oral or systemic has shown numerous side effects and patient non-compliance [54, 55] and hence the delivery at the tumour site using TACE may be a better option since local delivery may be more effective without undue systemic toxicity with additional benefit of continuous delivery.

When using doxorubicin, mitoxantrone or other drugs which are weak bases, owing to their interaction with the tumoral weakly acidic pH (formation of salt) their uptake through cellular membrane is often reduced [56, 57]. Hence, methods to raise the tumoral pH to weakly basic condition may be necessary to enhance the cytotoxic effect of the drug. This may be in the form of additives that would raise the pH of the tumour environment or on the other hand increase the concentration of doxorubicin to account for the poor absorption. This paradigm needs further in vivo and in vitro studies. On the contrary, selection of suitable cytotoxic which are weakly acidic may be more compatible with the tumour acidic environment. On the other hand, alkalization of tumour environment may be another option using proton pump inhibitors [58].

In the case of mucin producing HCC tumours that enable them to form a protective barrier against drug penetration, whilst also enhancing survival pathways [59], suitable agents that break down this barrier may provide better penetration of chemotherapeutic agents thus increasing efficacy. Mucolytics such as N-acetylcysteine, bromelain or other glycolytic and reducing agents [60, 61] may be incorporated to disintegrate this barrier and allow better penetration of drugs. Numerous studies on mucin producing cancers showed that the efficacy of cytotoxic was increased in the presence of N-acetyl cysteine and bromelain [62, 63], whilst synergistic combinations enabled a dramatic reduction of cytotoxic (paper under review).

Owing to the dense ECM present in the tumour environment, drug transfer is often compromised and hence suitable agents such as bromelain, collagenase etc. and other proteolytic enzymes should be incorporated into the TACE system to breakdown this barrier to enable a better drug transfer [64]. The suppression of collagen I synthesis by losartan in a dose dependent manner has been demonstrated in preclinical models [65]. Further, hyperthermia and ultrasound have also been suggested as a method of softening the dense tumour matrix [66, 67].

Since the Intra-Tumoral-Fluid Pressure (ITFP) within the tumour is higher than the surrounding [68], drug passage through the tumour matrix may be difficult resulting in reduced efficacy. Hence, suitable methods such as ultrasound, hyperthermia or a combination of both may enable to reduce the intra tumoral pressure [69, 70]. Other methods such as using vasodilators, small molecular weight chemotherapeutics and drugs that increase vascular permeability have been discussed in a recent review [71]. On the other hand, delivery of suitable chemical agents to reduce the ITFP may

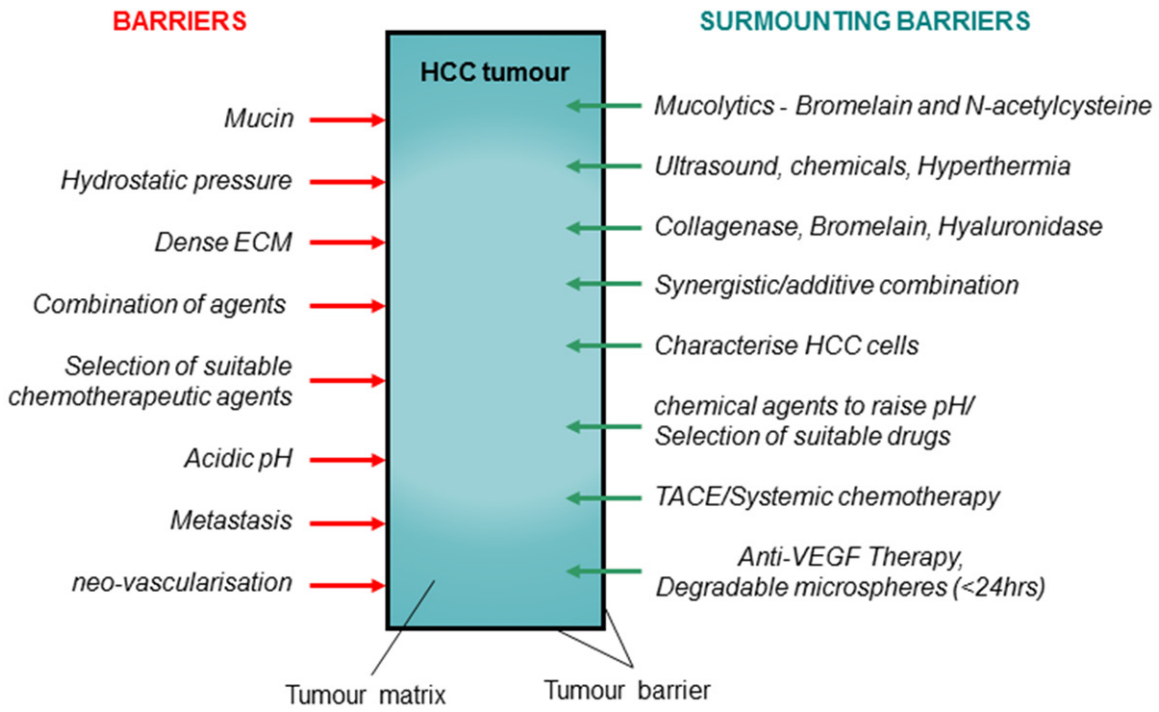

Figure 2. Barriers to drug penetration and efficacy with possible methods to surmount them to increase the efficacy of TACE. ECM: extracellular matrix; HCC: Hepatic cellular carcinoma; TACE: trans-arterial-chemoembolization; VEGF: vascular epidermal growth factors.

be necessary to ensure a better absorption of cytotoxic. High ITFP in the tumour is mainly contributed by the stiffening of the cellular matrix along with compromised fluid extraction since the lymphatic out flow is poorly developed. Treatment efficacy is mainly dependent on response to a particular chemotherapeutic agent and at the same time on attaining suitable concentration at the treatment site for sufficient time. Hence, reducing the ITFP or normalising it may greatly enhance drug transfer into cancer cells with greater efficacy. The barriers and possible solutions for increasing the efficacy of TACE may be summarised as shown in **Figure 2**.

Recent classification of HCC has divided them broadly into two groups such as proliferators and non-proliferators that correlate with clinical pathological features, aetiology, and prognosis [72]. Tumours from the proliferator class are highly heterogenous with enhancement replication pathways such as Insulin-like Growth Factor-1 (IGF1), mechanistic target of rapamycin (MTOR) and stem cell feature (NOTCH) [73, 74]. Further this class display numerous gene expression associated with tumour recurrence and poor prognosis [75]. Currently, extensive work is undergoing to target some of the new oncogenes that have been identified [76] and with future clinical trials, molecular classification with specific selection of chemotherapeutic drugs may enable a more effective treatment.

New development in drug therapy targeting both the tumour cells as well as angiogenesis using a number of different chemotherapeutic drugs together with tyrosine kinase inhibitors has shown plausible results in increasing patient survival [20, 77]. However, at the current therapeutic dosage, these agents have numerous undesirable side effects [78]. Hence, chemotherapy is generally given in four cycles over a month with 7 days rest between cycles. However, if these agents can be combined synergistically, then, the effective dosage of both the agents may be dramatically reduced and hence, therapy may be given more frequently with possible better tumour ablation. Recent in vitro study using doxorubicin + lonafarnib or sorafenib + lonafarnib has shown tremendous synergistic efficacy in tumour cell reduction as compared to doxorubicin + sorafenib [79].

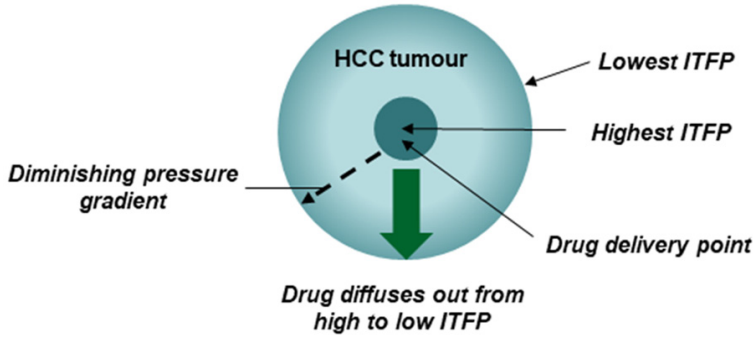

Figure 3. Demonstrates the principals involved in intra-tumoral drug delivery system. ITFP: intratumoural fluid pressure.

Intra-tumoral drug delivery to overcome the disadvantages posed by TACE

Recent work has indicated that intra-tumoural drug delivery using liposomes conjugated to drugs is an efficient way of delivering chemotherapeutic agents for treating malignant tumours and the various methods that may be employed to surpass some of the barriers for efficient penetration of chemo-agents has been reviewed by Goins et al. [80]. Further, slow releasing paclitaxel containing microspheres have been successfully delivered by intratumoral delivery with great efficacy compared to free drug intra-tumoural delivery in in vitro and in vivo studies [81]. Other studies using intratumoural injection of gels containing losartan microspheres and PLG-g-mPEG-cisplatin nanoparticles showed improved drug penetration, retention and anti-tumor activity [82]. In essence, this delivery method avoids embolisation of the blood vessels and hence may avoid neo-vascularisation and rebound tumour development that is encountered using the classical TACE method. Although, the drugs are delivered intra-tumourally, the drugs still have to overcome some of the barriers that are found within the tumour such as high ITFP, abnormal tumour vasculature, stiff extracellular matrix, poor lymphatics acidic pH etc.

However, one of the major advantage of this method is that the drugs do not have to cross from low to high ITFP as in the TACE method of drug delivery [83], since the tumoral pressure is highest within the central core of the tumour where the drug is delivered and the passage is from high to low pressure (diminishing pressure gradient) at the circumference of the tumour [84] as illustrated in **Figure 3**.

Drugs are transported from the intravascular space into the interstitial space in two main ways - diffusion and convection. Diffusion occurs at a faster rate for lower molecular size substances due to faster drift velocities. As derived by Einstein in 1905, the average drift displacement of a suspended sphere in a liquid is inversely proportional to its radius [85]. Therefore, as the molecular size of the drug increases, diffusion becomes less significant, and convection becomes the predominating process for drug transport. The tumours have higher ITFP values centrally with decreasing net fluid pressure away from the tumour, into the intravascular space [86]. This means that drugs have less ability to penetrate high ITFP tumours from the vascular space, especially higher molecular weight drugs that depend on convection as the primary means of drug transport. Drugs with low molecular weight will be less affected by this because they are more easily able to use diffusion to travel into the tumour.

Further, introducing drug carrying microspheres at the central point of tumour will overcome this as the drug will be present at the site of the tumour, in close proximity to tumour cells, hence can be a way to overcome the barrier of high ITFP.

In principal one of the major obstacles in this method of drug delivery is the high ITFP within the central core of the tumour where the drug is delivered and this high ITFP may oppose drug elution from the microspheres. A study shows that drugs are eluted freely and hence the high central ITFP is no obstacle [87]. This may be due to diffusion of drugs from high concentration within the sphere to low concentration (tumour matrix) following Fickian law of diffusion [88].

Further, introducing nano-conjugated drugs or drug carrying microspheres at the central point of tumour where ITFP is greatest may oppose free delivery of medicament. Piercing may in fact lead to efflux of tumoural fluid with metastatic cells leading to cancer spread. Other challenges that are presented to TACE, such as

low tumoral pH, poor lymphatic drainage, etc. are also applicable to this method of delivery, although similar remedial techniques may be adopted. Therefore, using the intra-tumoral delivery system (ITD), single drugs such as doxorubicin, mitoxantrone, cisplatin etc. may be an efficient therapy since rebound tumour development owing to neo-angiogenesis that is common in TACE is absent.

Hepatic arterial infusion chemotherapy

Hepatic arterial infusion chemotherapy (HAIC) is the treatment of hepatic tumors using an infusion of chemotherapeutic agents through the hepatic artery and its downstream branches. Currently, while it is not used as standard of care under the AASLD, it is widely used in Japan and endorsed for the treatment of advanced cases of HCC [89]. As opposed to TACE there is no embolization involved, although it shares a similar characteristic with TACE in the locoregional delivery of drug. As such, HAIC displays a pharmacokinetic advantage compared to systemic drug delivery, following Collins' model described previously. This has been confirmed for the drug (FUdR) (floxuridine), with which tumor concentrations 14 times higher were able to be achieved as measured through radiolabelling [90]. A review by Ensminger (2002) describes how pharmacokinetic as well as pharmacodynamic parameters must be considered when choosing drugs for HAIC [91]. For a drug to be appropriate for HAIC, it must be dosed at a rate that does not saturate the tumor cells, otherwise the incremental benefit of delivering through infusion will be small. Drugs also must have relatively high hepatic extraction ratios otherwise there will be no advantage over intravenous delivery [91].

There are many theoretical advantages with HAIC over TACE. Firstly, higher amounts of drug can be delivered for longer periods of time, as HAIC is not limited by the release kinetics of TACE, whether it be through microspheres, or lipiodol injections. By performing an infusion into the tumor over potentially several hours or even days, we can maintain tumor concentrations at a high equilibrium which is difficult to achieve through TACE. Dynamic control of drug infusion is possible with HAIC through simply adjusting drug concentrations and infusion rates in the implantable port system whereas in TACE, there is no way to control the delivery of drug once injection is complete. Moreover, there will be no rebound angiogenesis using HAIC because there is no embolization, which means drug can be delivered indefinitely whereas with TACE, rebound angiogenesis will quickly cause drug washout through increased tumor clearance of drug.

Experimental evidence that supports the use of HAIC largely comes from high observed rates of tumor response as described through RECIST criteria and mRECIST criteria, as well as a collection of trials comparing HAIC to systemic chemotherapy. Notably, there are no randomised trials comparing TACE with HAIC, representing an area of potential future research. One of the only trials to compare TACE and HAIC came from Kim et al., who compared 36 patients prospectively given HAIC with a retrospectively matched group of patients undergoing TACE with similar patient and tumor characteristics [92]. This study demonstrated a higher rate of objective response according to mRECIST in the HAIC group compared to TACE group (16.7% vs 0%, P = 0.03), as well as higher median survival (193 vs 119 days, P = 0.026). However, the study is limited by the fact that different drugs were used - the HAIC group was treated using 5FU and cisplatin, while the TACE group was treated using doxorubicin. Nevertheless, it is promising evidence that HAIC can be considered as a potential alternative treatment to TACE. Daniels and Wallman also have described significantly lower complication rates through their use of HAIC as opposed to reported complication rates of TACE therapy (1.4% vs 31%) [93]. Another recent trial showed a major survival advantage using HAIC with sorafenib over only sorafenib in a cohort of 247 HCC patients with portal vein invasion randomised to one of two groups (13.37 vs 7.13 months) [94]. The trial also showed a benefit in the time to progression (7 vs 2 months), as well as in response rate (41 vs 2%), however, there were more instances of vomiting (6 vs 1%), neutropenia (10 vs 2%), and thrombocytopenia (13 vs 5%) in the sorafenib and HAIC group. This trial presents substantial evidence that a change in the standard of care is necessary for HCC patients with portal vein invasion and suggests that further research into HAIC in different patient groups is required.

Development of degradable microspheres for TACE

Degradable microspheres are designed to provide embolisation on a transient basis depending on duration of treatment time required after which they disintegrate within the vessels without having any deleterious effect on other organs such as cytotoxicity or subsequent embolization of smaller capillaries with their residual material. Presently, a few different degradable microspheres have been developed using polymerised polylactic glycolic acid (PLGA), PLGA-polyethylene glycol (PEG)-PLGA, carboxymethylcellulose-chitosan (CMC-CCN), chitosan, hydroxyethyl acrylate (HEA) and degradable starch microspheres (DSM) [95].

PLGA microspheres degrades in vivo by hydrolysis of the ester bonds that are found between polylactic acid and polyglycolic, with the former degrading further into lactic acid that is excreted or converted into glucose to form adenosine triphosphate [96] whilst the latter also undergoes further hydrolysis into the monomers which is excreted through the kidneys or used in the tricarboxylic acid cycle [97]. The safety and efficacy of these PLGA microspheres (Occlusin500 from IMBiotechnologies Ltd, Edmonton, AB, Canada) have been successfully tested in sheep model with 150-212 um spheres [98]. Although technically PLGA microspheres degrade in 6 months Occlusin500 took up to 9 months owing to the development fibrous growth within the blood vessel and hence it was disqualified as degradable microspheres since it fell short of the standard set by ISO 10993-1 (international standard for device) which is <30 days [99].

The in vivo degradation of PLGA-PEG-PLGA begins with the degradation of PLGA by similar mechanism as above whilst PEG (polyethylene oxide) is excreted unchanged in urine with limited toxicity [100]. However, if metabolised in the kidneys, PEG could form ethylene glycol metabolite i.e. calcium oxalate and carbon dioxide which may pose toxicity. In vivo, these microspheres were reported to degrade in less than 7 days using 300-500 and 700-900 um spheres in sheep [101]. However, decreased particle size showed more distal occlusion, greater necrosis and lower recanalization rate [102]. Further the microspheres produced less ischemic damage relative to the controls (trisacryl-gelatine) owing to its short half-life. Given its short embolic life, there will be no fibrotic tissue formation; however, the risk of migration with embolisation in non-target tissues may occur.

The disintegration of CMC-CNN microspheres is determined by the percentage oxidation of carboxymethylcellulose (CMC), 10% oxidised form disintegrates within 14 days whilst 25% oxidised in 30 days [103, 104]. In vivo, lysozyme separates the two components by cleaving the Schiff's base. CMC is non-toxic with limited degradation into glucose by hydrolysis of the 1-4 glycosidic linkages. Chitosan is regarded as non-toxic and it undergoes lyzozymic degradation. These spheres can be produced in a variety of sizes (100-1550 um) [104]. The safety of these microspheres was tested using the renal artery of Rabbit model; however, migration was not addressed [105].

Chitosan microspheres are polymer of glucosamine with N-acetylglucosamine, linked together by 1-4 glycosidic bonds that are hydrolysed by lysozyme into glucosamine [106]. Glucosamine is then converted into glycosaminoglycans, proteoglycans and glycolipids [107] with low systemic toxicity. The safety of chitosan microspheres (150-250 um) were tested in rabbits and the first sign of degradation was observed at 24 weeks (6 months) with complete absence at 32 weeks (8 months). Inflammatory response persisted for 32 weeks, however with low eosinophil count suggesting that allergic reaction may not occur [108].

Degradable starch microspheres (DSM) consists of polymerised partly hydrolysed starch molecules that are linked using glycerol ether groups and are easily degraded by blood α-amylase [109]. EmboCept® S DSM 35/50 (PharmaCept GmbH, Berlin, and Germany) manufactures DSM with an average diameter of 50 um with a half-life being 35-50 minutes both in vitro and in vivo [110]. Hence, repeated treatment is required to achieve optimal results. Degraded smaller fragments of microspheres may lodge non-specifically in other organ system resulting in ischemia and severe pain although this is only very temporary and blood flow is normally resumed in a matter of minutes (10-15 mins) [111]. Recent study has indicated that overall survival of patients can be improv-

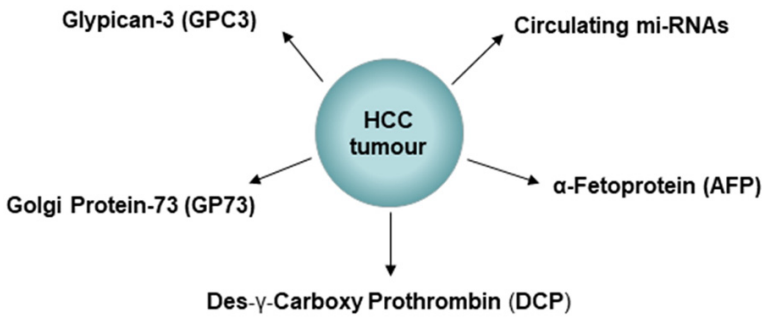

Figure 4. Shows some of the common biomarkers that are detected in HCC for diagnostic and therapeutic purpose.

ed using a combination of cytostatic drugs and DSM when compared to that of IV delivery of cytostatic drugs [112].

Biomarkers to assess the efficacy of TACE treatment

Specific and sensitive tumour biomarkers will enable not only early detection of the disease but also assess treatment efficacy in HCC [13]. Currently, several tumour markers have been identified for the detection of HCC such as α-fetoprotein (AFP), des-γ-carboxyprothrombin (DCP), Glypican-3 (GPC3), Golgi protein-73 (GP-73), and circulating mi-RNAs (**Figure 4**). There are also several reviews addressing other tumour markers for the detection of HCC [113-115].

Glypican-3 (GPC3) is a heparin sulfate proteoglycan and plays a vital role in regulating the growth of cells particularly through the wnt and hedgehog signalling pathway [116]. Numerous studies have found the absence or very low expression of GPC3 in normal liver, focal nodular hyperplasia and hepatocellular adenoma but highly expressed in HCC [117]. Although GPC3 can sometimes be expressed in other cancers such as liposarcoma, lung squamous cell carcinoma and testicular non-seminomatous germ cell tumour, studies have indicated its diagnostic value as a serum marker in HCC [118]. Notably, GPC3 has a higher specificity and sensitivity than human cervical cancer oncogene (HCCR) and Alfa fetoprotein (AFP) in the diagnosis of HCC and the combination of the three markers have indicated a much higher sensitivity to the detection of HCC than any other markers [119].

Alpha-fetoprotein (AFP) is primarily produced in the liver by the foetus during development, however it subsides after birth. The glycosylated form of AFP designated as AFP-L3 is closely associated with HCC and the simultaneous determination of AFP-L3, AFP with p53 antigen or with des-γ-carboxyprothrombin (DCP) has shown great diagnostic accuracy and sensitivity than any of the markers individually [120, 121].

Des-γ-carboxyprothrombin (DCP) is a non-functional precursor of prothrombin that is excessively secreted by HCC cells which raises the level of DCP. The level of blood DCP has been well correlated with tumour diameter, disease progression [122]; portal vein invasion with correlation to survival [123]. It has also been suggested that DCP may promote angiogenesis through activation of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) [124]. Compared to other tumour markers such as AFP, AFP-L3 and Golgi protein 73 (GP73), DCP has 60% sensitivity and 64.5% specificity for early stage diagnosis and 62.5% and 85.5% for all stages [125].

Golgi protein-73 (GP73) is a 73 kDa transmembrane glycoprotein is highly expressed in liver tumours and it promotes HCC cell invasion through the (CREB) mediated pathway [125]. It is a highly sensitive and specific serum biomarker for HCC as indicated by several studies [126] with better scores compared to AFP. Sixty percent of AFP negative HCC patients tested positive for GP73 in a recent study [127] although the sensitivity and diagnostic accuracy was lower. Hence, a combination of AFP and GP73 may serve as a useful diagnostic as well as treatment efficacy markers [128].

Micro RNAs have vital role in the development of HCC since healthy livers express high levels of miRNA 122 compared to HCC [129]. Studies have also shown that miRNA-122 may act as a tumour suppressor [130]. Further miRNA-23a was found to be associated with multiple local hepatic lesions [131] and a cut of value of > 2^{10} miRNA-23a was comparatively more accurate for diagnosis of HCC since it was significantly specific and sensitive as compared to AFP with a cut off value of > 200 ng/ml [132]. Hence, miRNA-23a may have an important role in diag-

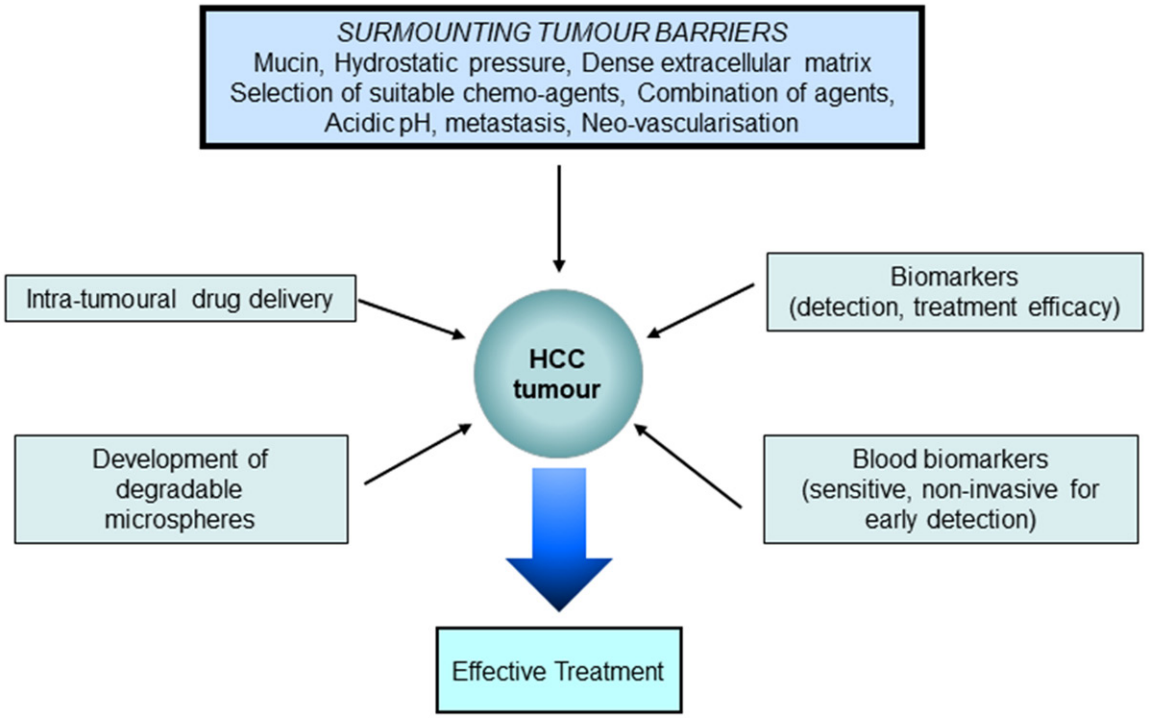

Figure 5. TACE as an ideal treatment for non-resectable HCC.

nosis and prognosis. Similarly, miRNA-494 has been proposed for both diagnosis and as a prognostic agent [133].

There are several reviews examining both diagnostic and prognostic biomarkers that can be detected in both histo-pathological and blood samples [13, 113-115], with emphasis that a number of biomarkers should be used for diagnostic and prognostic screening since there is a inter patient variation of biomarker expression. Hence, the judicious use of biomarkers on an individual basis may enable a more accurate diagnostic and prognostic evaluation in HCC patients.

Conclusion

TACE appears to be an ideal method for drug delivery, although the use of non-degradable or that degrade very slowly may work against the efficient performance of the cytotoxic drugs since rebound vascular development that supports tumour regrowth takes place within 36 hours in human. The use of oral anti VEGF and tyrosine kinase inhibitors to overcome neovascularisation carry inherent disadvantage such as non-patient compliance owing to severe side effects. Further, these anti angiogenic therapy cannot be used on a long-term basis to overcome neovascularisation since they affect other organ systems [134]. Incorporating the tyrosine kinase inhibitors or anti VEGF therapy within the microspheres may reduce the side effects since the delivery is locoregional with minimal systemic exposure. However, the non-biodegradable spheres create permanent embolism that are very long lasting with lasting neovascularisation. Hence, the use of degradable microspheres may overcome neovascularisation although one major disadvantage is the embolization of non-target organs with degraded fragments, that are however relatively short lived. Other inbuilt barriers within the tumour will still prevail and has to be overcome in order to attain good efficacy. In the case of intra-tumoral drug delivery, although the drug is delivered within the tumour, factors such as low pH, dense tumour matrix and other barriers will still prevail, and they have to be overcome as discussed earlier. After drug delivery by TACE, the efficacy of treatment can be monitored using either dual biomarkers such as AFP-L3 and DCP or the use of several biomarkers that may be specific in certain patients. Since there are several variabilities within the

tumour environment that is patient dependant, it would be difficult to attain uniform treatment response amongst patients although certain parameters such as selection of cytotoxic depending on tumour characteristics, type of microspheres, size etc, can be controlled. In an ideal situation where the existing barriers to drug transfer and other inherent disadvantages in microspheres can be overcome, then TACE may serve as an ideal treatment with probable better outcome and even serve as a cure for non-resectable HCC (**Figure 5**).

Disclosure of conflict of interest

None.

Address correspondence to: Krishna Pillai, Department of Surgery, University of New South Wales, St. George Hospital, Kogarah, NSW, Australia. Tel: 61 (2) 91132975; E-mail: panthera6444@yahoo.com.au

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