Screening of anticancer drugs for chemoembolization of hepatocellular carcinoma

Mathieu Boulin[a,e], Séverine Guiu[e,f], Bruno Chauffert[e,f], Serge Aho[b], Jean-Pierre Cercueil[c,e], François Ghiringhelli[e,f], Denis Krause[c], Philippe Fagnoni[a,e], Patrick Hillon[d,e], Laurent Bedenne[d,e] and Boris Guiu[c,e]

The aim of this study was to select the best candidate drug for transarterial chemoembolization by in-vitro cytotoxic evaluations of 11 anticancer drugs on three human hepatocellular carcinoma (HCC) cell lines. The SNU-398, HepG2, and SNU-449 human HCC cell lines were exposed for 30 min to 11 concentrations of doxorubicin, epirubicin, idarubicin, mitoxantrone, carboplatin, cisplatin, oxaliplatin, 5-fluorouracil, gemcitabine, mitomycin C, or paclitaxel. Cytotoxicity was measured using a quantitative colorimetric assay. For each drug and cell line, we calculated the drug concentration that caused 90% cell death (IC_{90}). To enable comparisons of drugs with different concentration ranges, we computed the cytotoxic index (C_yI) as the ratio of maximal drug concentration of more than IC_{90}. Parameters were estimated using nonlinear regression models. Idarubicin was the most active drug on all three cell lines. With SNU-398 cells, the idarubicin C_yI was 2.4-fold, 2.5-fold, 57-fold, 148-fold, and more than 58 748-fold higher than the C_yIs of mitoxantrone, epirubicin, doxorubicin, gemcitabine, and other drugs, respectively. With HepG2 cells, the idarubicin C_yI was 27-fold, 28-fold, 51-fold, and more than 1343-fold higher than the C_yIs of doxorubicin, epirubicin, mitoxantrone, and other drugs, respectively. On the resistant SNU-449 cell line, the idarubicin C_yI was 2.9-fold and 14-fold higher than the C_yIs of paclitaxel and gemcitabine, respectively, the only other drugs effective on this cell line. Among 11 chemotherapeutic agents including doxorubicin, cisplatin, and epirubicin, the most effective on three HCC cell lines was idarubicin. Further clinical investigations are needed to evaluate the safety and efficacy of idarubicin for transarterial chemoembolization in HCC. *Anti-Cancer Drugs* 22:741–748 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2011, **22**:741–748

Keywords: anticancer drugs, chemoembolization, hepatocellular carcinoma

Departments of [a]Pharmacy, [b]Epidemiology, [c]Radiology, [d]Hepatogastroenterology, CHU (University Hospital), [e]University of Burgundy, INSERM U866 and [f]Department of Oncology, Georges Francois Leclerc Anticancer Center, Dijon, France

Correspondence to Dr Mathieu Boulin, Department of Pharmacy, CHU (University Hospital), 2 Boulevard Maréchal de Lattre de Tassigny, BP 77908, Dijon F-21079, France
Tel: +33 380 293 796; fax: +33 380 293 275;
e-mail: mathieu.boulin@chu-dijon.fr
and
Boris Guiu, Department of Radiology, CHU (University Hospital), 2 Boulevard Maréchal de Lattre de Tassigny, BP 77908, Dijon F-21079, France
Tel: +33 380 293 686; fax: +33 380 295 455;
e-mail: boris.guiu@chu-dijon.fr

Received 20 December 2010 **Revised form accepted** 10 March 2011

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third most common cause of cancer-related death worldwide [1,2]. Only 30% of patients can receive potentially curative treatments such as liver transplantation, resection, or percutaneous ablation [3]. Among palliative treatments, transarterial chemoembolization (TACE) has a central role. More specifically, TACE is the mainstay of the treatment of patients with intermediate-stage HCC (multinodular asymptomatic tumors without vascular invasion or extrahepatic spread) [4].

Two randomized controlled trials (RCTs) and two meta-analyses showed statistically significant survival gains with TACE in patients with unresectable HCC, compared with supportive care or systemic chemotherapy [5–8]. TACE has a favorable long-term toxicity profile [9], and has been used worldwide for several years. However, the TACE procedure varies widely across centers and interventional radiologists, especially regarding anticancer drugs, doses, embolic agents, methods of delivery, and schedules [10]. One of the key theoretic advantages of TACE is tumor exposure to high concentrations of the chemotherapeutic agent. Therefore, the choice of the drug is probably of major importance, especially as HCC is considered one of the most resistant tumors to pharmacological treatment [11]. Resistance to anticancer drugs in HCC is, similar to in other malignancies, partly related to multidrug resistance (MDR), an intrinsic or acquired cross-resistance to a variety of structurally and functionally unrelated drugs [12]. MDR might be caused by an increased ATP-dependent efflux of drugs from within to outside the cells, mediated by MDR proteins belonging to the ATP-binding cassette transport family. In HCC cell lines, resistance to doxorubicin and cisplatin, attributed to the overexpression of MDR proteins, has been previously

0959-4973 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e328346a0c5

explored [13,14]. Doxorubicin and cisplatin are the most widely used drugs for TACE [10], but no preclinical screening studies or RCTs have been published to support their use rather than that of other agents. Furthermore, in the literature on TACE, there is no consensus about the best chemotherapeutic agent or optimal treatment regimen [10] as survival rates vary widely. Recently, many studies focused on drug-eluting microspheres using doxorubicin [15–18], although loading other drugs might be associated with greater improvements in the treatment efficacy.

Therefore, we designed an in-vitro study to compare the cytotoxicity of multiple anticancer drugs on human HCC cell lines with the goal of selecting the best candidate for TACE in HCC.

Materials and methods

Cell culture

SNU-398, HepG2, and SNU-449 human HCC cell lines were purchased from the American Type Culture Collection (Manassas, Virginia, USA). They were all derived from hepatitis B virus-induced tumors, even though there is no evidence of a hepatitis B virus genome in HepG2 cells [19]. The HepG2 cell line was taken before chemotherapy from a 15-year-old Caucasian boy. It is widely used for in-vitro studies on HCC and constitutes the standard cell line in our experiment. The SNU-398 cell line was obtained from a 42-year Asian man previously treated with TACE with ethiodized oil plus a combination of doxorubicin and mitomycin C [19]. In brief, the original tumor was single nodule with perinodular extensions, and histologically a trabecular type. SNU-398 cells were selected as the model of doxorubicin- pretreated cells. The SNU-449 cell line was taken before chemotherapy from a 52-year-old Asian man [19]. SNU-449 cells were derived from a tumor with predominantly compact histology. The main characteristic of SNU-449 cells is their resistance to various anticancer drugs [13].

The cell lines were cultured in a CO_2 incubator at 37°C in RPMI 1640 (Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum. For experiments, cells were detached with a mixture of trypsin (1 g/l) and EDTA (0.4 g/l) in Hanks Balanced Saline Solution (Lonza).

Drugs and concentrations

We tested anthracyclines (doxorubicin, epirubicin, idarubicin, and the related drug mitoxantrone), platinum derivatives (carboplatin, cisplatin, and oxaliplatin), antimetabolites (5-fluorouracil and gemcitabine), the alkylating antibiotic mitomycin C, and the taxane paclitaxel. For each drug, we chose the available form (solution or powder) that produced the highest concentration. Five drugs were used as solutions: mitoxantrone [Mitoxantrone (2 mg/ml); Mylan, Canonsburg, Pennsylvania, USA], carboplatin [Carboplatine (10 mg/ml); Mylan], cisplatin [Cisplatine (1 mg/ml); Mylan], 5-fluorouracil [Fluorouracile (50 mg/ml); Teva Classics, Petah Tikva, Israel], and paclitaxel [Taxol (6 mg/ml); Bristol-Myers Squibb, New York, USA]. Six drugs were used as powder reconstituted in sterile water: doxorubicin (Doxorubicine; Sanofi Aventis, Paris, France), epirubicin (Farmorubicine; Pfizer), idarubicin (Zavedos; Pfizer), gemcitabine (Gemzar; Lilly, Indianapolis, Indiana, USA), oxaliplatin (Oxaliplatine; Mylan), and mitomycin C (Ametycine; Sanofi Aventis).

For each drug, we prepared 11 concentrations from the highest achievable concentration to the lowest concentration obtained by $1:3^n$ ($n = 0$–11) serial dilutions (i.e. 1; 1 : 3; 1 : 9–1 : 59 049) in Ham's F10 medium (Lonza). For solutions, the highest concentration was that of the undiluted solution. For powders, the highest concentration was the maximal reconstitution concentration recommended by the manufacturers: 37 500 μg/ml for doxorubicin, 2000 μg/ml for epirubicin, 1000 μg/ml for epirubicin, 40 000 μg/ml for gemcitabine, 5000 μg/ml for oxaliplatin, and 1000 μg/ml for mitomycin C.

Cytotoxicity assay

Cells were seeded onto 96-well tissue culture plates (20 000 cells/well) and cultured for 72 h until confluence. After exposure to drugs at various concentrations for 30 min at 37°C, the cells were washed twice with Ham's F10 medium and incubated for 4 additional days in a drug-free fresh culture medium. Cell survival was assessed using a quantitative colorimetric assay as previously described [20]. In brief, adherent surviving cells were fixed with 200 μl of pure ethanol for 10 min and stained with 1% crystal violet in distilled water for 5 min. Excess dye was flushed out with water and cell-fixed dye was then eluted using 33% acetic acid. The optical density (OD) was read at 555-nm wavelength using an automatic spectrophotometer (Asys Hitech GmbH, Vienna, Austria). Untreated cells were subjected to the same experimental protocol without drug exposure and served as controls (i.e. 100% survival). To maintain strict comparability, all manipulations were carried out simultaneously by three operators who had 3, 4, and 3 years of experience, respectively, with cell cultures and experimental studies.

Data analysis

Each point of the concentration–survival curves was the mean ± standard deviation of the ratio of treated-cell OD over corresponding control-cell OD, obtained from triplicate measurements.

IC_{50} and IC_{90} were defined as the drug concentrations that caused 50 and 90% cell death, respectively, compared with the control. To determine IC_{50} and IC_{90}, 11 models were assessed for each drug on each of the three cell

lines. The initial model was chosen based on the shape of the concentration–survival curves. It was then compared with the following models: logistic models (3–5 parameters), log logistic models (2–5 parameters), and four Weibull models (depending on the number of parameters) [21]. The Gompertz model, which resembles the Weibull model, was not tested. The final model was selected based on the Akaike's information criterion and of residual variance. IC_{50}, IC_{90}, and their standard deviations were calculated using the best model. In this model, 95% confidence intervals (95% CIs) of IC_{90} were estimated using the δ method.

We also computed the cytotoxic index (C_yI) as the ratio of maximal drug concentration over IC_{90} for each drug and each cell line. As an example, a C_yI of 100 indicates that the drug kills 90% of the cells even when diluted (1 : 100).

All models were estimated by nonlinear least squares regression. Computations were performed using the Stata 10 software (StataCorp, College Station, Texas, USA) and the R 2.10 software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Cytotoxicity curves, IC_{50}, and IC_{90}

Cell survival was plotted according to the drug concentration (Fig. 1). IC_{50} and IC_{90} values are reported in Table 1. The SNU-449 cell line was the most resistant to anthracyclines (IC_{50} not achieved for doxorubicin, epirubicin, or the related drug mitoxantrone), except idarubicin, which was nevertheless less active on SNU-449 than on the other two cell lines. SNU-449 cells showed incomplete sensitivity to platinum derivatives, even at the highest concentration (IC_{90} not reached). Apart from idarubicin, the most active agents on SNU-449 cells were paclitaxel and gemcitabine, which were completely cytotoxic in high concentrations. In contrast, anthracyclines and mitoxantrone were active in low concentrations on SNU-398 and HepG2 cells. The HepG2 cell line was the most sensitive to platinum derivatives and mitomycin C. SNU-398 was the most sensitive cell line to gemcitabine. 5-Fluorouracil was almost inactive on the three cell lines, except when undiluted.

Finally, idarubicin was the only drug that was active in relatively low concentrations on the three HCC cell lines. The idarubicin IC_{90} was 9.9 ± 1.0 μg/ml for the resistant SNU-449 cell line and was already achieved at the lowest concentration (0.017 μg/ml) for the SNU-398 and HepG2 cell lines.

Cytotoxic index

Cytotoxicities with the various drug–cell line combinations were readily compared based on the CyI (Table 2). Idarubicin was the only drug for which all three C_yIs were greater than 1 and had the highest C_yI for each cell line. With SNU-398, the idarubicin C_yI was 2.4-fold (80 900/33 438) higher than that of mitoxantrone, 2.5-fold (80 900/32 102) higher than that of epirubicin, 57-fold (80 900/1412) higher than that of doxorubicin, 148-fold (80 900/548) higher than that of gemcitabine, and more than 58 748-fold higher than that of the other drugs. With HepG2, the idarubicin C_yI was 27-fold (68 488/2574) higher than that of doxorubicin, 28-fold (68 488/2421) higher than that of epirubicin, 51-fold (68 488/1340) higher than that of mitoxantrone, 1343-fold (68 488/51) higher than that of mitomycin C, and more than 5205-fold higher than that of the other drugs. With the resistant SNU-449 cell line, only two other drugs, paclitaxel and gemcitabine, had C_yIs greater than 1. The idarubicin C_yI with these cells was 2.9-fold (101/35) higher than that of paclitaxel and 14-fold (101/7) higher than that of gemcitabine.

Discussion

A recent systematic review of chemotherapeutic regimens in 52 studies of TACE showed that the most widely used anticancer drugs were doxorubicin (36%), cisplatin (31%), epirubicin (12%), mitoxantrone (8%), and mitomycin C (8%) [10]. Carboplatin and 5-fluorouracil are used as well [10]. This review did not suggest that one drug might be superior over the others. The optimal drug for TACE in HCC remains unclear, for several reasons. First, to our knowledge, no preclinical screening study has compared the efficacy of the drugs currently used for TACE in HCC. Second, although systemic chemotherapy has been extensively investigated over the past 30 years in patients with unresectable HCC, it is widely considered ineffective, regardless of the drug used [3,11]. Third, the considerable heterogeneity in the design of TACE RCTs regarding patients' characteristics, procedure modalities, embolizing agents, methods of delivery, doses of anticancer drug(s), and control arms, is a major obstacle to comparisons of drugs used in these studies. Finally, even if two TACE RCTs have specifically compared the efficacy of two drugs (doxorubicin and epirubicin) injected under the same conditions, they failed to demonstrate significant survival difference between them [22,23]. Therefore, we designed this intrinsically unbiased in-vitro study, in which observed cytotoxic effects were related only to the drugs, with no influence of confounding factors such as administration modalities. Moreover, the identical and simultaneous experimental conditions allowed reliable comparisons of the chemotherapeutic agents.

Few studies [24–26] have evaluated the cytotoxicity of anticancer agents on HCC cell lines, and none screened more than four agents. Their results are not relevant to TACE in HCC, for several reasons. First, the drugs

commonly used for TACE were not tested in these studies. Second, the main calculated parameter was IC_{50}, although 50% cytotoxicity is probably not relevant to the goal of achieving a complete response after one or a few TACE sessions. Finally, HCC cell lines were exposed to the drugs for 3–96 h [24–26], whereas Varela *et al.* [15] recently showed that doxorubicin release into the systemic circulation peaked only 20–40 min after the injection, whether TACE was performed using the conventional procedure or the DC Bead (Biocompatibles, Surrey, UK).

In our preclinical study, we tested a large variety of drugs with different action mechanisms. We selected not only the drugs commonly used for TACE of HCC [10] but also other anticancer agents for the following reasons: idarubicin because of its high hepatic penetration compared with other anthracyclines [27], and its ability

Fig. 1

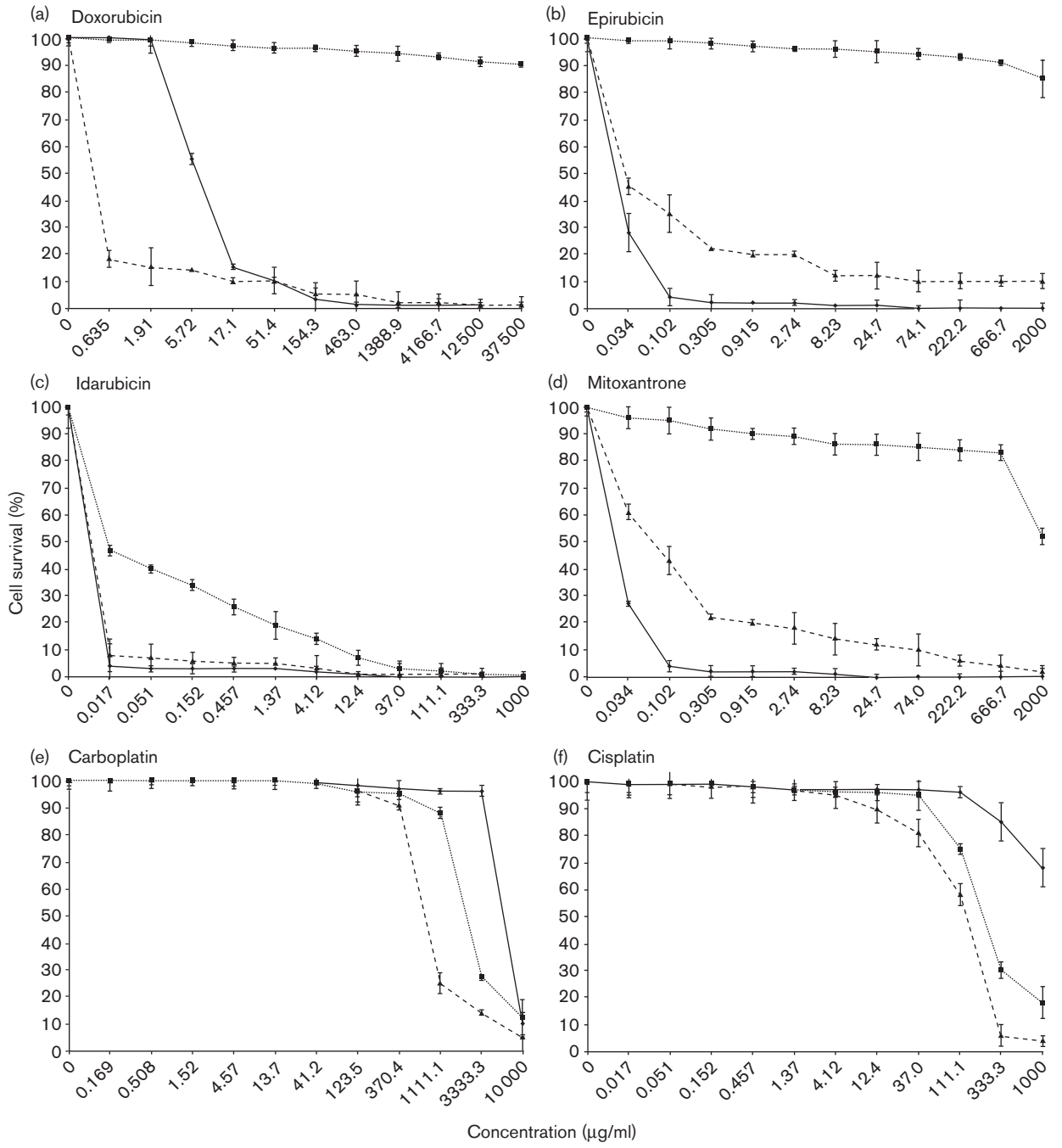
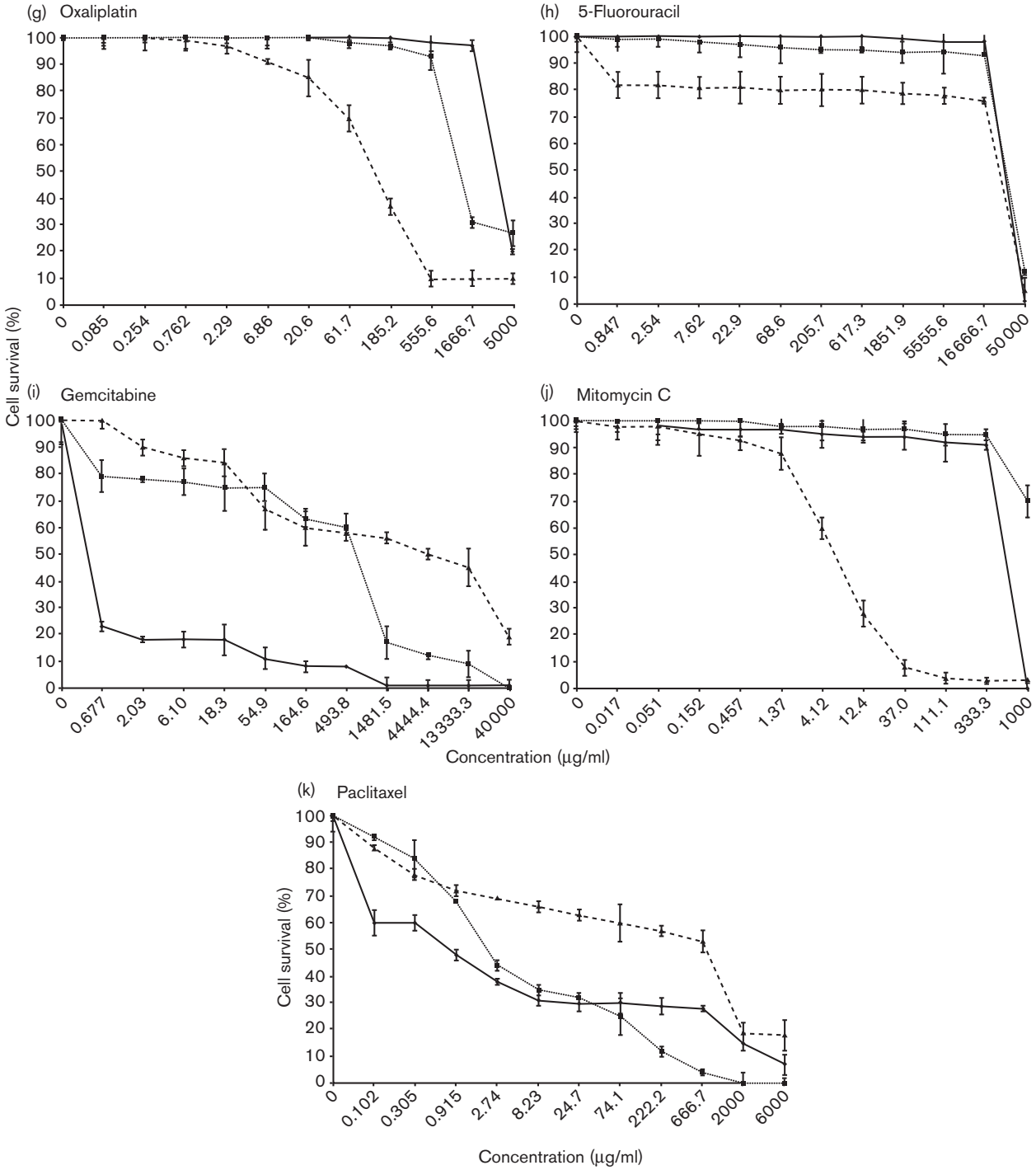


Fig. 1 (*continued*)



Comparative cytotoxicity of (a) doxorubicin, (b) epirubicin, (c) idarubicin, (d) mitoxantrone, (e) carboplatin, (f) cisplatin, (g) oxaliplatin, (h) 5-fluorouracil, (i) gemcitabine, (j) mitomycin C, and (k) paclitaxel on SNU-398, HepG2, and SNU-449 hepatocellular carcinoma cell lines. Cells were exposed to 11 concentrations of each drug for 30 min, and cytotoxicity was measured by a quantitative colorimetric assay as described in Materials and methods section. Cell survival was expressed as a percent of control-untreated cells. Each point was the mean ± standard deviation obtained from triplicate measurements. SNU-398: continuous black line; HepG2: dashed black line; SNU-449: dotted black line.

to overcome glycoprotein (PgP)-related MDR [28]; paclitaxel, to test one microtubule-stabilizing taxane, and because this drug has proved to be more potent than doxorubicin in terms of antiproliferative activity and in the induction of cell death in histocultures of HCC [25]; and gemcitabine and oxaliplatin because the

Table 1 IC_{50} and IC_{90} of 11 drugs on the SNU-398, HepG2, and SNU-449 HCC cell lines

	SNU-398		HepG2		SNU-449	
Drug	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Doxorubicin	6.2 ± 0.1	27 ± 3	0.00005 ± 0.00009	15 ± 23	NA	NA
Epirubicin	0.022 ± 0.002	0.062 ± 0.007	0.019 ± 0.002	0.82 ± 0.21	NA	NA
Idarubicin	0.008 ± 0.0003	0.012 ± 0.001	0.009 ± 0.0002	0.014 ± 0.001	0.019 ± 0.004	9.9 ± 1.0
Mitoxantrone	0.021 ± 0.006	0.060 ± 0.020	0.047 ± 0.011	1.5 ± 0.9	NA	NA
Carboplatin	5890 ± 524	7555 ± 541	649 ± 34	1539 ± 179	2261 ± 92	NA
Cisplatin	NA	NA	126 ± 5	282 ± 10	166 ± 9	NA
Oxaliplatin	3761 ± 457	NA	104 ± 6	380 ± 34	1043 ± 50	NA
5-Fluorouracil	29 351 ± 7813	36 309 ± 7827	35 369 ± 242	NA	38 664 ± 1580	NA
Gemcitabine	0.0006 ± 0.002	73 ± 77	1854 ± 1005	NA	662 ± 207	5668 ± 317
Mitomycin C	606 ± 86	804 ± 159	6.1 ± 0.4	19 ± 1	NA	NA
Paclitaxel	0.66 ± 0.40	NA	206 ± 142	NA	3.8 ± 1.5	171 ± 8

IC_{50} and IC_{90} were defined as the drug concentrations associated with 50 and 90% cell death, respectively. Data are the mean ± standard deviation. Values (in μg/ml) were calculated from the transformation of concentration–survival curves using nonlinear regression models as described in the Materials and methods section.
HCC, hepatocellular carcinoma; IC_{50} and IC_{90}, 50 and 90% cell death; NA, not achieved.

Table 2 Cytotoxic indexes of 11 drugs on SNU-398, HepG2, and SNU-449 HCC cell lines

Drug	SNU-398	HepG2	SNU-449
Doxorubicin	1412 (1155–1815)	2574 (584–NC)	ND
Epirubicin	32 102 (25 806–42 485)	2421 (1568–5309)	ND
Idarubicin	80 900 (68 027–99 602)	68 488 (60 240–79 063)	101 (83–129)
Mitoxantrone	33 438 (19 980–102 364)	1340 (561–6536)	ND
Carboplatin	1.3 (1.2–1.6)	6.5 (5.1–8.9)	ND
Cisplatin	ND	3.6 (3.3–3.8)	ND
Oxaliplatin	ND	13 (11–17)	ND
5-Fluorouracil	1.4 (1.0–2.5)	ND	ND
Gemcitabine	548 (172–NC)	ND	7 (3–27)
Mitomycin C	1.2 (1.0–2.2)	51 (44–61)	ND
Paclitaxel	ND	ND	35 (18–69)

Data are cytotoxic indexes (C_yIs), with 95% confidence intervals in parentheses. C_yI is the ratio of the maximal drug concentration over corresponding IC_{90}.
HCC, hepatocellular carcinoma; IC_{50} and IC_{90}, 50 and 90% cell death; NC, not calculable (IC_{90} 95% confidence interval lower boundary = 0); ND, not determined (IC_{90} not achieved).

combination of the two drugs, widely tested in oncology during the past years, has also demonstrated its interest when administered by the intravenous route in patients with advanced HCC [29].

We determined not only IC_{50} but also IC_{90} to identify the most potent agents, and we treated the cells for only 30 min to take into account the short drug-residence time in the liver and for a more strict selection of the most cytotoxic drug. Finally, we calculated C_yI to approach in-vivo conditions, as objectively comparing efficacy across drugs having different concentrations is challenging. The C_yI, which reflects the highest dilution having 90% cytotoxicity, is useful for comparing drugs expected to undergo gradual dilution in the hepatic arterial blood flow during TACE.

One of our most striking findings concerns doxorubicin. Although doxorubicin is the most widely used drug worldwide for TACE in HCC [10], it fell far short of being the most effective drug in our study. Interestingly, we found considerable heterogeneity in the drug activity across cell lines, although all three cell lines tested were induced by viral infection [19]. This heterogeneity may partly be explained by the short 30-min exposure time in our experiment. With longer exposure time, cytotoxicity would probably be higher for some drugs, especially those with a known time-dependent activity. As an example, it has been demonstrated that gemcitabine significantly inhibits the growth of HCC cells (HepG2, Hep3B, HLF, and PLC/PRF/5) in a dose-dependent and time-dependent manner [30]. Moreover, the investigators of this study have shown that gemcitabine inhibits the growth of these cells by cell cycle arrest without apoptosis and that the ERK/Chk1/2 signaling pathway was in part responsible for the resistance to the drug [30]. In our experiment, the HepG2 cell line was effectively resistant to gemcitabine (IC_{90} not achieved).

This variability in cell sensitivity to anticancer agents may help to explain why partial response is reported in up to 62% of patients treated with TACE [10], and that corresponding median survivals rarely exceed 2 years [8]. To increase the probability of a complete response, the drug used for TACE in HCC must have high cytotoxicity at low concentrations. The only drug that met this criterion with all three cell lines in our screening study was idarubicin, which had high C_yIs (101–80 900). The superiority of idarubicin in terms of cytotoxicity

was observed most notably on the SNU-449 cell line, which we selected for its known resistance to various chemotherapeutic agents [13]. The SNU-449 cell line was effectively the most resistant cell line in our experiment.

Idarubicin is an anthracycline that is widely used to treat acute leukemia [31]. It is administered through the intravenous route, for which the dose-limiting toxicity is hematological toxicity, manifesting chiefly as neutropenia [32]. Idarubicin has never been used through the intra-arterial route but has been tested orally in 45 patients with cirrhosis and unresectable HCC who received 5 mg dose daily for 21-day periods separated by 7-day intervals [33]. With a median number of four treatment periods (1–14) and a median time to progression of 4 months (2–17), the investigators concluded that idarubicin was safe and active in patients with cirrhosis. In addition to its powerful cytotoxic effect, other advantages of idarubicin for TACE in HCC may include an ability to overcome MDR. MDR is mainly attributed to the action of two proteins, the 170-kDa Pgp and the 190-kDa MDR-associated protein (MRP1), which pump drugs out of MDR cells [34–36]. Although Pgp-mediated and MRP1-mediated anthracycline efflux may not differ considerably across drugs, drug-uptake kinetics varies widely [37]. Thus, an enhanced uptake of a lipophilic drug such as idarubicin [38] leads to high intracellular concentrations, which are necessary to achieve cytotoxicity against MDR cells. Thus, idarubicin was more effective *in vitro* than other anthracyclines on MDR leukemia cell lines [28,39]. This property of idarubicin is of major importance, as *MDR1* and *MRP1* genes, and their products, Pgp and MRP1, are intrinsically expressed by HCC cells [14,40]. Known Pgp overexpression in SNU-449 cells [13,24] may explain the high resistance to anthracyclines (except idarubicin) that we observed in our experiment. This finding is in line with the results of Park *et al.* [13], which showed a close association between MDR1 gene expression in nine HCC cell lines and their resistance to doxorubicin. Moreover, high MRP1 gene expression in HCC is associated with a growth advantage and a more aggressive phenotype by pumping toxic substances out of the tumor cells [40]. Finally, another interesting property of idarubicin is its ability to be loaded in microspheres. The positively charged protonated amine group of idarubicin hydrochloride can interact with the negatively charged sulfonate of the microspheres, as occurs with doxorubicin hydrochloride as well.

Our study has several limitations. First, for technical reasons and to keep the experimental conditions strictly identical for all drugs, we limited the number of cell lines to three and the number of drugs to 11. Second, we used HCC cell lines of viral origin only, as no HCC cell lines related to alcohol abuse are available. Consequently, our results need to be confirmed in HCC related to alcohol abuse. We studied cytotoxicity after exposure to a single drug. The cytotoxic effect may be greater when several drugs are used, including molecular-targeted therapies. Given the large number of possible combinations, our screening study may be of assistance for designing investigations of combined therapies. Finally, our study was not designed to explore the mechanisms, which could explain the highest cytotoxicity of idarubicin.

In conclusion, our experimental screening study of 11 chemotherapeutic agents including the most commonly used drugs for TACE (doxorubicin, cisplatin, and epirubicin) demonstrated that idarubicin was the most effective drug *in vitro*, especially on the resistant SNU-449 cell line. On the basis of this encouraging result, a dose-escalation phase I trial of TACE in patients with unresectable HCC is ongoing with idarubicin-loaded microspheres.

Acknowledgements

Conflicts of interest

Grant support: this paper was supported by grants from the French National League against Cancer and from the Clinical Research Unit of Dijon University Hospital, Dijon, France. The authors thank Pr T. De Baere for his help in revising the manuscript.

References

1 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **32**: 2257–2276.
2 Altekruse SF, McGlynn KA, Reichmann ME. Hepatocellular carcinoma incidence, mortality and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009; **27**:485–491.
3 Bruix J, Llovet JM. Major achievements in hepatocellular carcinoma. *Lancet* 2009; **373**:614–616.
4 Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, *et al.* Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**:698–711.
5 Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, *et al.* Randomized controlled trial of transarterial ethiodized oil chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**:1164–1171.
6 Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, *et al.* Arterial embolization or chemoembolization versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**:1734–1739.
7 Camma C, Schepis F, Orlando A, Albanese M, Shahied L, Trevisani F, *et al.* Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized control trials. *Radiology* 2002; **224**:47–54.
8 Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003; **37**:429–442.
9 Buijs M, Vossen JA, Frangakis C, Hong K, Georgiades CS, Chen Y, *et al.* Nonresectable hepatocellular carcinoma: long-term toxicity in patients treated with transarterial chemoembolization–single-center experience. *Radiology* 2008; **249**:346–354.
10 Marelli L, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, *et al.* Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. *Cardiovasc Intervent Radiol* 2007; **30**:6–25.
11 Burroughs A, Hochhauser D, Meyer T. Systemic treatment and liver transplantation for hepatocellular carcinoma: two ends of the therapeutic spectrum. *Lancet Oncol* 2004; **5**:409–418.
12 Petraccia L, Onori P, Sferra R, Lucchetta MC, Liberati G, Grassi M, *et al.* MDR (multidrug resistance) in clinical therapeutic implications. *Clin Ter* 2003; **154**:325–335.

13 Park JG, Lee SK, Hong IG, Kim HS, Lim KH, Choe KJ, *et al.* MDR1 gene expression: its effect on drug resistance to doxorubicin in human hepatocellular carcinoma cell lines. *J Natl Cancer Inst* 1994; **86**:700–705.
14 Minemura M, Tanimura H, Tabor E. Overexpression of multidrug resistance genes MDR1 and Cmoat in human hepatocellular carcinoma and hepatoblastoma cell lines. *Int J Oncol* 1999; **15**:559–563.
15 Varela M, Real MI, Burrel M, Forner A, Sala M, Brunet M, *et al.* Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; **46**:474–481.
16 Malagari K, Chatzimichail K, Alexopoulou E, Kelekis A, Hall B, Dourakis S, *et al.* Transarterial chemoembolisation of unresectable hepatocellular carcinoma with drug eluting beads: results of an open-label study of 62 patients. *Cardiovasc Interv Radiol* 2008; **31**:269–280.
17 Malagari K, Alexopoulou E, Chatzimichail K, Hall B, Koskinas J, Ryan S, *et al.* Transcatheter chemoembolization in the treatment of HCC in patients not eligible for curative treatments: midterm results of doxorubicin-loaded DC bead. *Abdom Imaging* 2008; **33**:512–519.
18 Lammer J, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, *et al.* Prospective randomized study of doxorubicin-eluting bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Interv Radiol* 2010; **33**:41–52.
19 Park JG, Lee JH, Kang MS, Park KJ, Jeon YM, Lee HJ, *et al.* Characterization of cell lines established from human hepatocellular carcinoma. *Int J Cancer* 1995; **62**:276–282.
20 Royer B, Guardiola E, Polycarpe E, Hoizey G, Delroeux D, Combe M, *et al.* Serum and intraperitoneal pharmacokinetics of cisplatin within intraoperative intraperitoneal chemotherapy: influence of protein binding. *Anticancer Drugs* 2005; **16**:1009–1016.
21 Seber GAF, Wild CJ. *Nonlinear regression*. New York: John Wiley & Sons Inc.; 1989.
22 Watanabe S, Nishioka M, Ohta Y, Ogawa N, Ito S, Yamamoto Y. Prospective and randomized controlled study of chemoembolization therapy in patients with advanced hepatocellular carcinoma: cooperative study group for liver cancer treatment in Shikoku area. *Cancer Chemother Pharmacol* 1994; **33** (**Suppl**):S93–S96.
23 Kawai S, Tani M, Okamura J, Ogawa M, Oshashi Y, Monden M, *et al.* Prospective and randomized trial of lipiodol-transcatheter arterial chemoembolization for treatment of hepatocellular carcinoma: a comparison of epirubicin and doxorubicin (second cooperative study). The cooperative study group for liver cancer treatment of Japan. *Semin Oncol* 1997; **24** (**Suppl**):S6–S45.
24 Mok TS, Choi E, Yau D, Johri A, Yeo W, Chan AT, *et al.* Effects of patupilone (epotheline B; EPO906), a novel chemotherapeutic agent, in hepatocellular carcinoma: an in vitro study. *Oncology* 2006; **71**:292–296.
25 Chuu JJ, Liu JM, Tsou MH, Huang CL, Chen CP, Wang HS, *et al.* Effects of paclitaxel and doxorubicin in histocultures of hepatocellular carcinomas. *J Biomed Sci* 2007; **14**:233–244.
26 Chunhu Z, Suiyu H, Meiqun C, Guilin X, Yunhui L. Antiproliferative and apoptotic effects of paeonol on human hepatocellular carcinoma cells. *Anticancer Drugs* 2008; **19**:401–409.
27 Broggini M, Italia C, Colombo T, Marmonti L, Donellim MG. Activity and distribution of i.v. and oral 4-demethoxydaunorubicin in murine experimental tumors. *Cancer Treat Rep* 1984; **68**:739–747.
28 Roovers DJ, Van Vliet M, Bloem AC, Lokhorst HM. Idarubicin overcomes P-glycoprotein-related multidrug resistance: comparison with doxorubicin and daunorubicin in human multiple myeloma cell lines. *Leuk Res* 1999; **23**:539–548.
29 Louafi S, Boige V, Ducreux M, Bonyhay L, Mansourbakht T, de Baere T, *et al.* Gemcitabine plus oxaliplatin (GEMOX) in patients with advanced hepatocellular carcinoma (HCC): results of a phase II study. *Cancer* 2007; **109**:1384–1390.
30 Matsumoto K, Nagahara T, Okano J, Murawaki Y. The growth inhibition of hepatocellular and cholangiocellular carcinoma cells by gemcitabine and the roles of extracellular signal-regulated and checkpoint kinases. *Oncol Rep* 2008; **20**:863–872.
31 Berman E, Heller G, Santorsa JA, McKenzie S, Gee T, Kempin S, *et al.* Results of a randomized trial comparing idarubicin and cytosine arabinoside with daunorubicin and cytosine arabinoside in adult patients with newly diagnosed acute myelogenous leukemia. *Blood* 1991; **77**: 1666–1674.
32 Weiss MA, Drullinsky P, Maslak P, Scheinberg D, Golde DW. A phase I trial of a single high dose of idarubicin combined with high-dose cytarabine as induction therapy in relapsed or refractory adult patients with acute lymphoblastic leukemia. *Leukemia* 1998; **12**:865–868.
33 Tumolo S, Toffoli G, Baerz A. Oral idarubicin for the treatment of hepatocellular carcinoma. *Proc Am Soci Clin Oncol* 2002; **21** (**Suppl**):S635.
34 Chauffert B, Martin M, Hammann A, Michel MF, Martin F. Amiodarone-induced enhancement of doxorubicin and 4′-deoxydoxorubicin cytotoxicity to rat colon cancer cells *in vitro* and *in vivo*. *Cancer Res* 1986; **46**:825–830.
35 Pérez-Tomàs R. Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. *Curr Med Chem* 2006; **13**:1859–1876.
36 Guiu B, Collin C, Cercueil JP, Loffroy R, Guiu S, Ferrant E, *et al.* Pilot study of transarterial chemoembolization with pirarubicin and amiodarone for unresectable hepatocellular carcinoma. *Am J Clin Oncol* 2009; **32**:238–244.
37 Mankhetkorn S, Dubru F, Hesschenbrouck J, Fiallo M, Garnier-Suillerot A. Relation among the resistance factor, kinetics of uptake, and kinetics of the P-glycoprotein efflux of doxorubicin, daunorubicin, 8-(S)-fluoroidarubicin, and idarubicin in multidrug-resistant K562 cells. *Mol Pharm* 1996; **49**:532–539.
38 Goebel M. Oral idarubicin: an anthracycline derivative with unique properties. *Ann Hematol* 1993; **66**:33–43.
39 Berman E, McBride M. Comparative cellular pharmacology of daunorubicin and idarubicin in human multidrug-resistant leukemia cells. *Blood* 1992; **79**:3267–3273.
40 Vander Borght S, Komuta M, Libbrecht L, Katoonizadeh A, Aerts R, Dymarkowski S, *et al.* Expression of multidrug resistance-associated protein 1 in hepatocellular carcinoma is associated with a more aggressive tumour phenotype and may reflect a progenitor cell origin. *Liver Int* 2008; **28**:1370–1380.