



## A prospective study of isolated human hepatocyte function following liver resection for colorectal liver metastases: The effects of prior exposure to chemotherapy

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**Background/Aims:** Chemotherapy is increasingly used prior to resection of colorectal liver metastases, yet few studies have addressed its effect on the viability and function of hepatocytes. This study evaluated the effect of pre-operative chemotherapy on human hepatocytes.

**Methods:** Studies were carried out on isolated hepatocytes from 47 patients undergoing hepatectomy for colorectal metastases. The function of drug metabolising enzymes in hepatocytes was assessed *in vitro*, as well as hepatocyte integrity and culture longevity. These results were compared between patients undergoing no pre-operative chemotherapy ( $N = 8$ ), 5 fluorouracil (5FU) post-bowel resection ( $N = 20$ ), and neo-adjuvant 5FU alone ( $N = 7$ ) or in a combination with oxaliplatin ( $N = 12$ ).

**Results:** Average cell viability at isolation determined by trypan blue dye exclusion was 71% with no significant difference between the no chemotherapy or pre-resection chemotherapy groups. There was no significant difference in LDH leakage and cellular ATP content over a 96-h time course between the patient treatment groups. The function of cytochromes P450 (CYP1A2, CYP2A6, CYP2C9, CYP2D6, CYP2E1, CYP3A4) and phase II enzymes (UDP-glucuronosyltransferase and sulphotransferase) was not adversely affected by pre-operative chemotherapy.

**Conclusions:** Pre-resection chemotherapy does not impair the function or culture integrity of hepatocytes isolated at the time of liver resection.

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**Keywords:** Neo-adjuvant chemotherapy; Liver cell culture; Isolated human hepatocytes; Oxaliplatin; Hepatectomy

### 1. Introduction

Colorectal cancer (CRC) is the fourth most common malignancy worldwide. Approximately 150 000 new cases are diagnosed in Europe each year and 60 000 will

die from the disease [1–3]. Metastases develop in approximately 60% of patients of which half are confined to the liver [4]. Surgery remains the only curative treatment, although the majority of patients at presentation have tumours that are deemed unresectable either due to their size, number or location [5–7]. Recent efforts have been directed at increasing resectability rates using new surgical approaches, pre-operative portal vein embolisation and neo-adjuvant chemotherapy using oxaliplatin and irinotecan [8–12]. Resecting a portion of a liver that has been exposed to systemic

Received 5 December 2005; received in revised form 23 January 2006; accepted 6 February 2006

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chemotherapy is therefore becoming more prevalent. The success of liver resection depends on the ability of the liver to maintain function and regenerate functional mass [13,14]. It is therefore essential that the functional capacity of the hepatocytes within the remnant liver is not compromised by pre-operative chemotherapy.

The liver plays a key role in the metabolism of many xenobiotics [15] including some drugs used to treat CRC metastases such as 5 fluorouracil (5FU). This agent has not been reported to cause liver damage when given as an oral preparation, and only rare reports of hepatotoxicity have been observed when it is given intravenously [16]. Oxaliplatin is an increasingly used drug that has been reported to cause a mild derangement of liver function in contrast to cisplatin that causes significant dose-related hepatotoxicity [17,18]. Transient elevations of serum transaminases and bilirubin can occur in up to 25% of patients treated with irinotecan [19]. Hepatic arterial infusion of floxuridine, however, has been shown to cause an acute hepatitis and dose-related hepatocyte necrosis, steatosis and micronodular cirrhosis that can be fatal [20–22].

Although the liver in patients who have received pre-operative chemotherapy may appear abnormal at the time of surgery [8,23,24], there is no objective evidence that hepatocyte function is altered. A few studies have analysed the effect of pre-operative systemic chemotherapy on the outcome of liver resection, one of which related to the use of oxaliplatin-based treatment [23,24]. No studies have assessed the effect of pre-operative administration of chemotherapy on the viability and function of hepatocytes isolated at the time of liver resection. These provide a versatile and sensitive model system that allows specific biochemical functions to be evaluated. In this study, cell viability was assessed by several methods including *in vitro* culture survival, whilst liver-specific function was assessed by the activity of the cytochromes P450 (CYP).

## 2. Materials and methods

### 2.1. Patient selection and demographics

Liver samples were obtained from 47 patients undergoing liver resection for colorectal metastases during a 4-year period. Informed consent was obtained pre-operatively from each patient involved in the study that was approved by the Ethics Committee of the Royal Free Hospital. Data on pre-operative co-morbidities, medication and serum liver function tests were collected. These measurements included liver enzymes aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),  $\gamma$ -glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) as markers of hepatic damage; the synthetic functions accorded to the liver for circulating plasma albumin, and the clotting index that provided a global view of liver physiology to rank alongside the hepatocyte functions (see below). The hepatectomy procedure was standardised throughout the time period of the study.

### 2.2. Tissue collection

Immediately following resection of the liver the specimen was transferred to the operating theatre back bench. Under sterile conditions a wedge of normal liver including the capsular surface and away from the tumour was resected from the specimen. A major vessel in the specimen was isolated and perfused with cold preservation solution. The specimen was then prepared for transport as described previously [25]. Hepatocyte isolation was carried out in the cell isolation laboratories of Pharmagene Laboratories within 4–6 h – a period documented in previous studies to be compatible with the isolation of large numbers of intact and viable human hepatocytes [25].

### 2.3. Hepatocyte isolation

The liver specimen was photographed, weighed and prepared for hepatocyte isolation, using a modified 2-step perfusion technique [26] with non-filtered collagenase type IV, *Clostridium histolyticum* at 0.05% w/v (Sigma–Aldrich Ltd.; Poole, Dorset, UK) for up to 45 min depending on the size and texture of the liver. The cells were dispersed by gentle shaking and subsequently filtered through a 150  $\mu$ m nylon bolting cloth (Clarcor UK (Lockertex), Warrington, Cheshire, UK), and washed three times in ice-cold William's Medium E (WME; Sigma–Aldrich Ltd.) with centrifugation (23g for 6 min at 4 °C). The cell number and initial viability were determined by trypan blue dye exclusion [27] and the total number of viable cells was then calculated per gram of liver tissue. Cell plating and experimentation using monolayer cultures were performed if the hepatocyte viability was greater than 60%.

### 2.4. Hepatocyte metabolism measurement

Hepatocytes were resuspended in WME at  $1 \times 10^6$  per mL and investigated immediately for their ability to metabolise seven different probe substrates to establish the activity of six different CYP isoenzymes and two phase II enzymes (UDP-glucuronosyltransferase (UGT) and sulphotransferase (SULT)). Table 1 shows the substrates, the key metabolising enzymes and the metabolites produced. Probe substrates and metabolites were assayed using high performance liquid chromatography (Agilent 1100 series HPLC; Agilent Technologies UK Ltd., West Lothian, UK) with either UV or fluorescence detection. Samples were injected using a Presearch HTC PAL autosampler (Presearch Ltd., Hitchin, Hertfordshire, UK). The initial rates of disappearance of the parent compound and/or production of the specific metabolite were calculated and expressed as pmol/min/ $10^6$  cells.

### 2.5. Hepatocyte integrity and longevity measurements

From each liver specimen further aliquots of hepatocytes were resuspended in WME supplemented with L-glutamine (2 mM), penicillin (100 U/mL), streptomycin (100  $\mu$ g/mL), fungizone (2.5  $\mu$ g/mL), insulin (0.01  $\mu$ g/mL), hydrocortisone (0.005  $\mu$ g/mL) and 10% foetal bovine serum (all Sigma–Aldrich Ltd.). Cells were plated at a density of  $2 \times 10^6$  per well onto Type I collagen-coated 6-well plates and incubated for 4–8 h to allow cell attachment. The cells were then washed gently with Phosphate Buffered Saline Dulbeccos (PBS; Sigma–Aldrich Ltd.) and 1 mL serum-free WME added to each well. Culture assays were performed in triplicate at 0, 4, 24, 48 and 96 h after attachment.

Hepatocyte LDH leakage was measured in the supernatant from each well relative to that in the remaining cells (after lysis with 1 mL Triton X-100 (2% v/v)) using a commercial kit (LDH Cytotoxicity Detection kit, Roche Diagnostics Ltd., Lewes, East Sussex, UK). Cellular ATP content was measured in cell lysates using the commercial reagents for bioluminescence assay (ATP Bioluminescence Assay kit HSII, Roche Diagnostics Ltd., Lewes, East Sussex, UK).

The hepatocyte isolation and function studies were performed in a blinded fashion without clinical details regarding the liver specimens. Once the functional information was obtained, the code was broken and the hepatocyte samples were divided into one of four groups depending on the pre-operative chemotherapy regime of the patients

**Table 1**

**Individual substrates used, metabolites produced and type of reaction performed for the in vitro evaluation of the function of hepatic CYP isoenzymes and phase II enzymes**

CYP	Substrate	Metabolite	Reaction
1A2	7-Ethoxycoumarin	7-Hydroxycoumarin	O-Deethylation (ECOD)
2A6	Coumarin	7-Hydroxycoumarin	7-Hydroxylation
2C9	Diclofenac	4-Hydroxydiclofenac	4-Hydroxylation
2D6	Bufurolol	1-Hydroxybufurolol	1-Hydroxylation
2E1	Chlorzoxazone	6-Hydroxychlorzoxazone	6-Hydroxylation
3A4	Testosterone	6 $\beta$ -Hydroxytestosterone	6 $\beta$ -Hydroxylation
UGT <sup>a</sup>	7-Hydroxycoumarin	7-Hydroxycoumarin glucuronide	
SULT <sup>a</sup>	7-Hydroxycoumarin	7-Hydroxycoumarin sulphate	

<sup>a</sup> Phase II enzymes: UGT, UDP-glucuronosyltransferase; SULT, sulphotransferase.

in the study: group 1 (NA Ox), patients receiving neo-adjuvant oxaliplatin and 5FU/FA; group 2 (NA 5FU), patients receiving neo-adjuvant 5FU/FA only; group 3 (AD 5FU), patients receiving adjuvant 5FU/FA after their initial colorectal resection, but no pre-hepatectomy chemotherapy; group 4 (None), patients receiving no chemotherapy at any stage.

### 2.6. Statistical analysis

All data were entered directly into a specifically designed Microsoft<sup>®</sup> Access<sup>®</sup> database and analysed using a combination of statistical software packages (Microsoft<sup>®</sup> Excel<sup>®</sup>, GraphPad Prism 4<sup>®</sup> and SPSS for Windows<sup>®</sup>). Categorical variables were compared between groups using the  $\chi^2$  test and quantitative variables were compared with the Kruskal–Wallis test for multiple groupings.

## 3. Results

### 3.1. Demographic and pre-operative data

The demographics for the 47 patients providing liver tissue are shown in Table 2. Patient samples were well matched for age, sex and number of metastatic tumour deposits within the liver. The colorectal primary

**Table 2**

**Demographic patient data and co-morbidity**

Variable	Chemotherapy group			
	NA Ox	NA 5FU	AD 5FU	None
Number of patients	12	7	20	8
Age (years)*	57 (42–75)	59 (38–74)	61 (33–76)	51 (39–61)
Male/female	5/7	5/2	11/9	4/4
Co-morbidity				
Cardiovascular	4	2	9	
Respiratory	2		1	
Renal			1	1
Diabetes	1		1	
Coagulopathy	1	1	1	
Alcohol >20u/week	1	1	1	1
Smokers	1	3	3	2

*Key.* Co-morbidities: Cardiovascular: hypertension, ischaemic heart disease and atrial fibrillation; Respiratory: asthma and chronic obstructive pulmonary disease (COPD); Renal: chronic renal impairment; Coagulation abnormalities: thrombophilic disorders, previous venous thrombosis and current anticoagulant therapy.

\* Median (range).

tumours were mainly located in the sigmoid colon or rectum with only 18% originating from other parts of the colon. The number of patients with synchronous metastases was significantly greater in the neo-adjuvant as opposed to the no neo-adjuvant groups (12/19, 63% vs. 4/28, 14%;  $p = 0.001$ ). There were also a higher number of metastases in the neo-adjuvant groups although not significantly so (median 2, range 1–6 vs. median 1, range 1–5;  $p = 0.4$ ). Patients did not routinely undergo pre-operative quantitative measurements of hepatic reserve.

### 3.2. Chemotherapy durations and regimens

All values marked \* are median.

#### 3.2.1. Neo-adjuvant oxaliplatin + 5FU/FA group

Of the 12 patients within this group, 10 (83%) had also received adjuvant chemotherapy with 5FU/FA after their initial colorectal resection (6\* cycles, range 3–16) with one patient also receiving adjuvant irinotecan. These patients underwent 5\* cycles of neo-adjuvant chemotherapy (range 2–12) that was stopped 55\* days prior to surgery (range 15–120).

#### 3.2.2. Neo-adjuvant 5FU/FA group

Six (86%) of these patients had undergone adjuvant chemotherapy following resection of the primary cancer with 5FU/FA for 9\* cycles (range 6–30). After diagnosis of the metastases, neo-adjuvant 5FU/FA therapy was given for 6\* cycles (range 6–12), and was stopped 55\* days prior to surgery (range 25–90).

#### 3.2.3. Adjuvant 5FU/FA group

These patients underwent 12\* cycles of chemotherapy (range 1–30) following their initial colorectal resection.

### 3.3. Serum tests of liver function

The serum tests of liver function on the day prior to surgery are shown in Table 3. There were no statistically significant differences in the values between the four groups.

**Table 3**  
**Comparison of pre-operative serum tests of liver function with chemotherapy status**

Variable	Chemotherapy group				<i>p</i> value Kruskal–Wallis test 6–8
	NA Ox median (range)	NA 5FU median (range)	AD 5FU median (range)	None median (range)	
Range of <i>N</i> <sup>a</sup>	8–12	4–7	14–20	6–8	
Albumin	42 (39–50)	42 (35–46)	43 (37–48)	42.5 (40–48)	0.9
Bilirubin	7.0 (5–19)	12 (6–32)	7.0 (5–22)	6.5 (3–75)	0.4
AST	29 (17–74)	30 (19–149)	26.5 (14–54)	20.5 (15–216)	0.26
ALT	29.5 (16–89)	27 (14–152)	22.5 (12–67)	20 (11–276)	0.3
ALP	<b>151</b> (65–294)	99 (82–111)	92 (45–201)	102 (54–1412)	0.3
GGT	<b>62</b> (18–310)	<b>66.5</b> (9–275)	29.5 (17–112)	30.5 (15–919)	0.4
LDH	<b>476.5</b> (273–865)	<b>483</b> (282–963)	386.5 (274–995)	384.5 (311–934)	0.6
INR	0.95 (0.9–1.1)	1.0 (0.9–1.2)	1.0 (0.8–1.2)	1.0 (0.9–1.0)	0.3

Key. Albumin (normal range 35–50 g/L), bilirubin (5–17 μmol/L), AST, aspartate transaminase (5–40 U/L); ALT, alanine aminotransferase (5–40 U/L); ALP, alkaline phosphatase (42–128 U/L); GGT, γ-glutamyl transpeptidase (8–35 U/L); LDH, lactate dehydrogenase (230–460 U/L); INR, international normalised ratio.

Median values represented in bold were higher than the upper limit of normal.

<sup>a</sup> Not all results were available for each subject.

### 3.4. Hepatectomy

The extent of the hepatectomy was similar across all groups. Prior to surgery, 4 out of the 47 patients (8.5%) underwent portal vein embolisation (PVE) (all in the NA Ox group). Anaesthesia was induced with propofol or etomidate and maintained using isoflurane or desflurane. Hilar inflow occlusion was used in seven patients (equally distributed across the groups) with ischaemic times varying from 15 to 50 min. Median blood transfusion was 3 units (range 0–22). Pre-operative chemotherapy did not influence transfusion requirements.

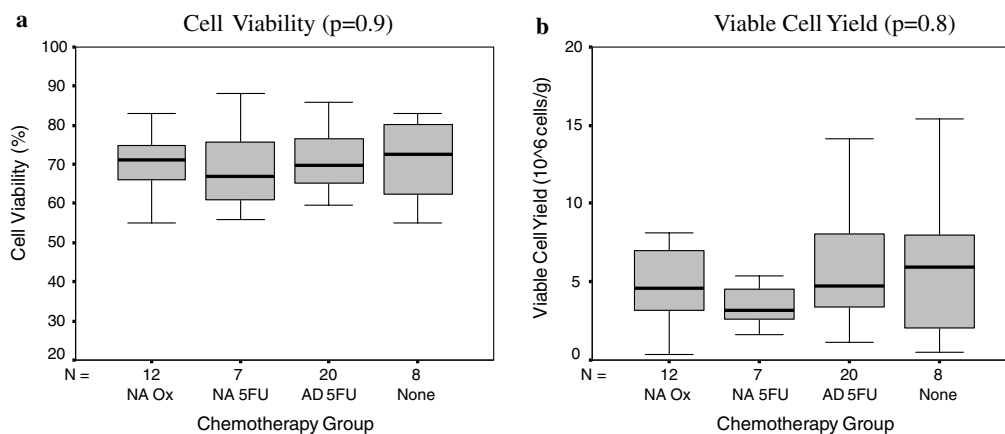
### 3.5. Hepatocyte isolation data

Median cell viability as determined by trypan blue dye exclusion was 71% with no significant differences

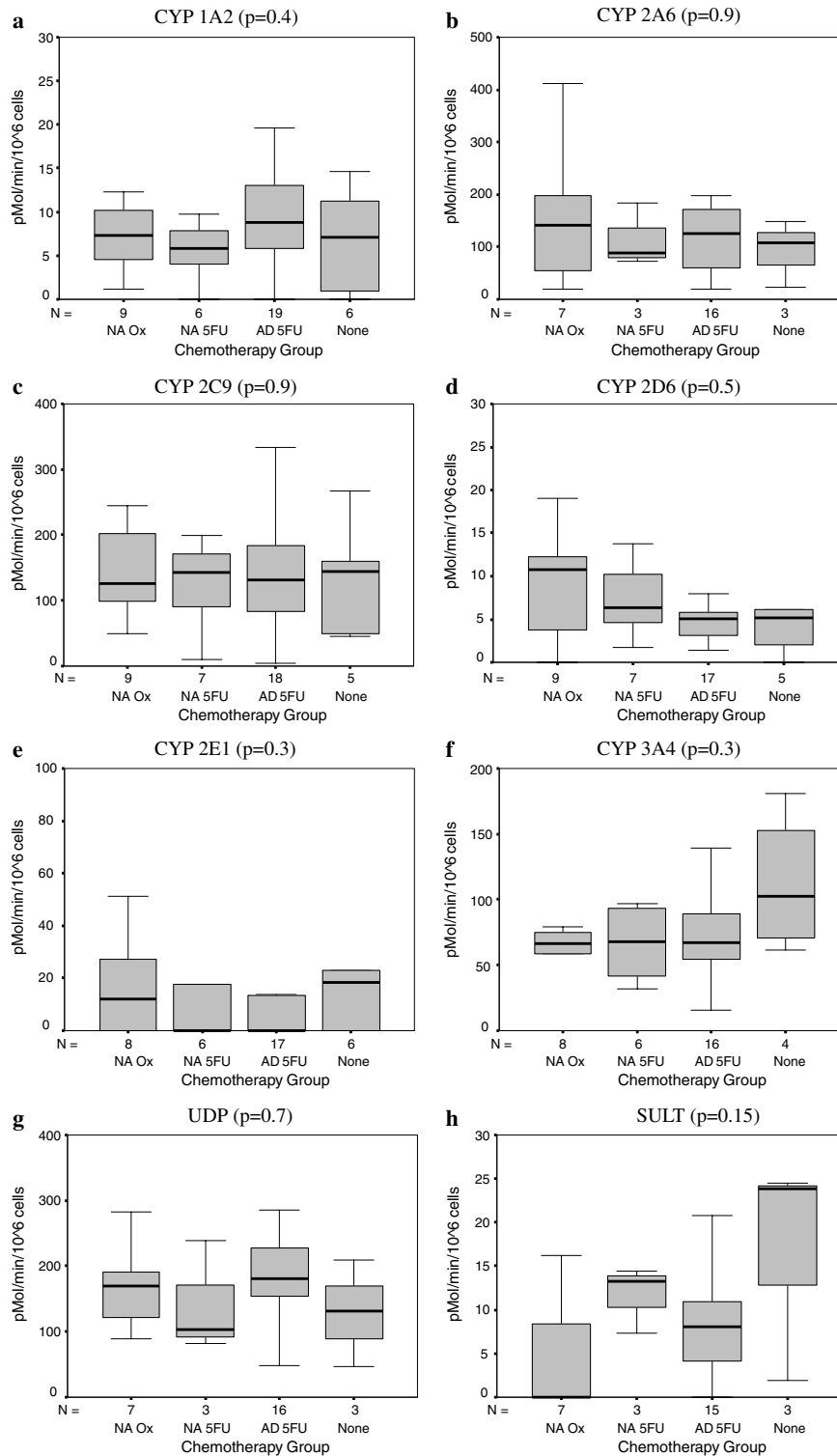
between the groups (Fig. 1a). There was a median of  $4.6 \times 10^6$  viable cells isolated per gram of liver tissue and this again showed no significant differences between groups (Fig. 1b).

### 3.6. Metabolism data

The CYP activity data are shown in Fig. 2(a)–(h). Not all liver specimens could be tested with each of the seven substrates; therefore numbers for individual metabolic activities differ. The rates of metabolism presented for each individual probe substrate fell within the ranges previously documented for over 150 hepatocyte preparations (Pharmagene Laboratories Ltd., unpublished data). There were no statistically significant differences in the rates of metabolism of any of the substrates tested between the four groups.



**Fig. 1.** Comparison of isolated cell viability and viable cell yield with pre-operative chemotherapy status, using box-and-whisker plots. (a) The initial hepatocyte viability determined by trypan blue dye exclusion, and (b) the yield of viable cells (number of viable hepatocytes isolated per gram of parenchymal tissue). Key. NA Ox, neo-adjuvant oxaliplatin; NA 5FU, neo-adjuvant 5 FU; AD 5FU, adjuvant 5 FU; None, no chemotherapy. The x-axis shows the *N* values for cell preparations studied in each of the four chemotherapy groups. The box length of the box-and-whisker plots represents the interquartile range that contains the median value shown as a horizontal line. The whiskers represent the 95% confidence intervals. No measured value was <0. *p* values are shown for comparisons between the four chemotherapy groups and determined using the Kruskal–Wallis test.



**Fig. 2.** Comparison of a range of CYP function with pre-operative chemotherapy status measured using rate of production of metabolites from appropriate probe substrates, using box-and-whiskers plots. (a) CYP 1A2 activity (25  $\mu$ M 7-ethoxycoumarin); (b) CYP 2A6 activity (25  $\mu$ M coumarin); (c) CYP 2C9 activity (50  $\mu$ M diclofenac); (d) CYP 2D6 activity (20  $\mu$ M bufurolol); (e) CYP 2E1 activity (200  $\mu$ M chlorzoxazone); (f) CYP 3A4 activity (100  $\mu$ M testosterone); (g) UDP-glucuronosyltransferase activity (25  $\mu$ M 7-hydroxycoumarin); (h) sulphotransferase activity (25  $\mu$ M 7-hydroxycoumarin). Key. NA Ox, neo-adjuvant oxaliplatin; NA 5FU, neo-adjuvant 5 FU; AD 5FU, adjuvant 5 FU; None, no chemotherapy. Data are expressed as pmol/min/10<sup>6</sup> cells. The x-axis shows the N values for cell preparations studied in each of the four chemotherapy groups. The box length of the box-and-whisker plots represents the interquartile range that contains the median value shown as a horizontal line. The whiskers represent the 95% confidence intervals. No measured value was <0 pmol/min/10<sup>6</sup> cells. p values are shown for comparisons between the four chemotherapy groups and determined using the Kruskal-Wallis test.

### 3.7. Hepatocyte integrity and longevity data

Samples from 18 patients were analysed for LDH leakage and ATP content, and were again grouped according to their pre-operative chemotherapy status (five NA Ox, two NA 5FU, eight AD 5FU and three with no chemotherapy). The results of hepatocyte ATP content of one specimen from the AD 5FU group were uninterpretable due to a technical problem and were therefore not included in the analysis.

For each specimen, data on LDH leakage against time following isolation were plotted, and linear regression analysis was applied to derive a slope, representing the rate of LDH release. These slopes were then compared between groups using the Kruskal–Wallis test. The leakage of LDH from the cells over time in an uninterrupted static primary culture is likely to increase due to gradual cell death. This was the case in all chemotherapy groups and comparison of the slopes demonstrated no significant differences ( $p = 0.4$ , data not shown). Cellular ATP content decreased with time in each of the four chemotherapy groups, and there was again no significant difference between the groups when the slopes were compared ( $p = 0.9$ , data not shown).

## 4. Discussion

Isolated human hepatocytes maintain most hepatic biochemical functions including the ability to metabolise drugs and other biologically active substances [28]. Cytochromes P450 play an important role, mediating phase I oxidative metabolism of a wide range of drugs and chemicals and therefore provide a good functional measure of the effects of systemic chemotherapy. The hepatocytes in this study were obtained from patient groups that were demographically well matched, although the patients in the neo-adjuvant groups had more advanced cancers, with a higher number of metastases and synchronous tumours. No other factors were identified that may have affected hepatocyte function. The operative technique and anaesthetic were standardised. The inhalational anaesthetics used (isoflurane and desflurane) can cause hepatotoxicity in patients pre-sensitised to halogenated anaesthetics, but the risk is appreciably smaller than with halothane [29]. Liver function prior to surgery was assessed using standard liver function tests. The levels of GGT and LDH were above normal in the two neo-adjuvant chemotherapy groups, and the ALP level was elevated in the NA Ox group. This would suggest mild hepatocyte injury and some impairment of biliary excretion. Measurement of the serum levels of plasma proteins such as albumin, and indirect measures of clotting factors such as INR are more representative of liver synthetic function but were similar between the groups.

Albumin is an indicator of chronic rather than acute liver damage but was unaffected by chemotherapy [30]. More sophisticated tests of liver metabolic capacity, e.g., galactose elimination, maximal rate urea synthesis, isotope measurement of protein synthesis or indocyanine green clearance [31,32] could have provided better quantisation of liver function but were not routinely applied in patients undergoing hepatic resection.

The assessment of the blood clotting parameter (INR) was included as a surrogate, although it was appreciated that this is only an indirect method of assessing liver function. Whether the elevations of the three liver function tests were due to the effect of the chemotherapy cannot be proven from this study. Oxaliplatin is not specifically metabolised in the liver but has been shown to cause limited effects on liver function tests, unlike other platinum agents which can cause significant dose-related hepatotoxicity [17,18]. Only rare cases of possible hepatotoxicity have been reported with intravenous 5FU [16]. In contrast hepatic arterial floxuridine commonly produces deranged liver function [33]. However it should be noted that the time period between chemotherapy being completed and hepatectomy was 2 months, which may have allowed a recovery period from hepatotoxicity.

In this study the viability of hepatocytes in culture was assessed by trypan blue dye exclusion, LDH leakage and cellular ATP content. Trypan blue is a simple test of cell integrity and demonstrated an average viability of greater than 70% that was not significantly different between the chemotherapy groups and similar to that previously reported for human hepatocytes [34]. Primary isolated hepatocytes in simple monolayer culture gradually de-differentiate and die over a period of days [35]. It is expected that LDH leakage from the cells will increase with time, and that cellular ATP concentration will decrease. The differential rates of LDH leakage and ATP content provide an accurate indication of the effect of chemotherapy on the viability and integrity of the cells [37]. There were no significant differences in LDH leakage or cellular ATP content between the four different groups. This suggests that pre-operative chemotherapy did not affect hepatocyte cellular integrity or viability.

The use of specific probe substrates is the method of choice for phenotyping CYP function in hepatocytes, as the rates of substrate decline and/or metabolite production highlight the activity of each specific CYP isoenzyme. A single isoform, however, is not programmed to catalyse a specific type of reaction, and multiple functions may reside in a single isoenzyme [36]. The rates of probe substrate metabolism and metabolite production in the isolated hepatocytes were not significantly different between the groups. Inter-patient differences in drug metabolism are largely the consequence of the variability of CYP expression. CYP genes are regulated by genetic as well as non-genetic factors (such

as physiological or pathological states) which could mask subtle differences between groups [37]. There was no evidence that pre-operative chemotherapy altered hepatocyte drug metabolism. These data therefore suggest that the isolation and storage of hepatocytes from these patients can be safely undertaken for hepatocyte functional and experimental transplantation studies, and supports the value of their use in specific studies as suggested by other authors [34,38].

This study has shown no objective evidence to support the subjective view that pre-operative chemotherapy produces significant hepatocyte damage. Damage to other liver cell types (e.g., stellate or endothelial cells) may have occurred, although investigation of this was outside the scope of the study. The period between chemotherapy and surgery may have allowed functional hepatocyte recovery and further studies are needed to address other possible mechanisms for this observation such as alterations to the liver histology or parenchymal microcirculation and damage to other cell types.

#### Acknowledgements

The isolation of hepatocytes and subsequent experimentation was carried out and funded by Pharmagene Laboratories Ltd., Hertfordshire, UK. Data from this study were presented at the 6th World Congress of the International Hepato-Pancreatico-Biliary Association, June 2004.

#### References

- [1] Great Britain Office for National Statistics. Cancer statistics registrations: registrations of cancer diagnosed in 1992. England and Wales Stationery Office; 1998.
- [2] Slevin ML, Papamichael D, Rougier P, Schmoll HJ. Is there a standard adjuvant treatment for colon cancer?. *Eur J Cancer* 1998;34:1652–1663.
- [3] Levitan N, Hughes KS. Management of non-resectable liver metastases from colorectal cancer. *Oncology (Huntingt)* 1990;4:77–84.
- [4] Fusai G, Davidson BR. Management of colorectal liver metastases. *Colorectal Dis* 2003;5:2–23.
- [5] Fong Y, Cohen AM, Fortner JG, Enker WE, Turnbull AD, Coit DG, et al. Liver resection for colorectal metastases. *J Clin Oncol* 1997;15:938–946.
- [6] Scheele J. Hepatectomy for liver metastases. *Br J Surg* 1993;80:274–276.
- [7] Scheele J, Stang R, Altendorf-Hofmann A, Paul M. Resection of colorectal liver metastases. *World J Surg* 1995;19:59–71.
- [8] Bismuth H, Adam R, Levi F, Farabos C, Waechter F, Castaing D, et al. Resection of nonresectable liver metastases from colorectal cancer after neoadjuvant chemotherapy. *Ann Surg* 1996;224:509–520.
- [9] Bismuth H, Adam R. Reduction of nonresectable liver metastasis from colorectal cancer after oxaliplatin chemotherapy. *Semin Oncol* 1998;25:40–46.
- [10] Adam R, Avisar E, Ariche A, Giacchetti S, Azoulay D, Castaing D, et al. Five-year survival following hepatic resection after neoadjuvant therapy for nonresectable colorectal. *Ann Surg Oncol* 2001;8:347–353.
- [11] Giacchetti S, Itzhaki M, Gruia G, Adam R, Zidani R, Kunstlinger F, et al. Long-term survival of patients with unresectable colorectal cancer liver metastases following infusional chemotherapy with 5-fluorouracil, leucovorin, oxaliplatin and surgery. *Ann Oncol* 1999;10:663–669.
- [12] Fusai G, Davidson BR. Strategies to increase the resectability of liver metastases from colorectal cancer. *Dig Surg* 2003;20:481–496.
- [13] Diehl AM, Rai R. Review: regulation of liver regeneration by pro-inflammatory cytokines. *J Gastroenterol Hepatol* 1996;11:466–470.
- [14] Ozawa K. Hepatic function and liver resection. *J Gastroenterol Hepatol* 1990;5:296–309.
- [15] Oinonen T, Lindros KO. Zonation of hepatic cytochrome P-450 expression and regulation. *Biochem J* 1998;329:17–35.
- [16] King PD, Perry MC. Hepatotoxicity of chemotherapy. *Oncologist* 2001;6:162–176.
- [17] Pollera CF, Ameglio F, Nardi M, Vitelli G, Marolla P. Cisplatin-induced hepatic toxicity. *J Clin Oncol* 1987;5:318–319.
- [18] Armand JP, Boige V, Raymond E, Fizazi K, Faivre S, Ducreux M. Oxaliplatin in colorectal cancer: an overview. *Semin Oncol* 2000;27:96–104.
- [19] Burris III HA, Fields SM. Topoisomerase I inhibitors. An overview of the camptothecin analogs. *Hematol Oncol Clin North Am* 1994;8:333–355.
- [20] Doria Jr MI, Shepard KV, Levin B, Riddell RH. Liver pathology following hepatic arterial infusion chemotherapy. Hepatic toxicity with FUDR. *Cancer* 1986;58:855–861.
- [21] Shepard KV, Levin B, Faintuch J, Doria MI, DuBrow RA, Riddell RH. Hepatitis in patients receiving intraarterial chemotherapy for metastatic colorectal carcinoma. *Am J Clin Oncol* 1987;10:36–40.
- [22] Pettavel J, Gardiol D, Bergier N, Schnyder P. Fatal liver cirrhosis associated with long-term arterial infusion of floxuridine. *Lancet* 1986;2:1162–1163.
- [23] Parc Y, Dugue L, Farges O, Hiramatsu K, Sauvanet A, Belghiti J. Preoperative systemic 5-fluorouracil does not increase the risk of liver resection. *Hepatogastroenterology* 2000;47:1703–1705.
- [24] Tanaka K, Adam R, Shimada H, Azoulay D, Levi F, Bismuth H. Role of neoadjuvant chemotherapy in the treatment of multiple colorectal metastases to the liver. *Br J Surg* 2003;90:963–969.
- [25] Caruana M, Battle T, Fuller B, Davidson B. Isolation of human hepatocytes after hepatic warm and cold ischemia: a practical approach using University of Wisconsin solution. *Cryobiology* 1999;38:165–168.
- [26] Berry MN, Edwards AM, Barritt GJ, Grivell MB. Isolated hepatocytes: preparation, properties and applications. Amsterdam, Oxford: Elsevier Publication; 1991.
- [27] Phillips HJ, Terryberry JE. Counting actively metabolizing tissue cultured cells. *Exp Cell Res* 1957;13:341–347.
- [28] Gomez-Lechon MJ, Donato MT, Castell JV, Jover R. Human hepatocytes as a tool for studying toxicity and drug metabolism. *Curr Drug Metab* 2003;4:292–312.
- [29] Lee NC. Inhalation anaesthetics and liver damage. *S Afr Med J* 1993;Suppl.:1–4.
- [30] Blumgart LH, Fong Y. Surgery of the liver and biliary tract. 3rd ed. London: W.B. Saunders; 2000.
- [31] Burra P, Masier A. Dynamic tests to study liver function. *Eur Rev Med Pharmacol Sci* 2004;8:19–21.
- [32] Gao L, Ramzan I, Baker AB. Potential use of pharmacological markers to quantitatively assess liver function during liver transplantation surgery. *Anaesth Intensive Care* 2000;28:375–385.
- [33] Hohn D, Melnick J, Stagg R, Altman D, Friedman M, Ignoffo R, et al. Biliary sclerosis in patients receiving hepatic arterial infusions of floxuridine. *J Clin Oncol* 1985;3:98–102.

- [34] Mitry RR, Hughes RD, Dhawan A. Progress in human hepatocytes: isolation, culture and cryopreservation. *Semin Cell Dev Biol* 2002;13:463–467.
- [35] Battle T, Stacey G. Cell culture models for hepatotoxicology. *Cell Biol Toxicol* 2001;17:287–299.
- [36] Guengerich FP. Reactions and significance of cytochrome P-450 enzymes. *J Biol Chem* 1991;266:10019–10022.
- [37] Donato MT, Castell JV. Strategies and molecular probes to investigate the role of cytochrome P450 in drug metabolism: focus on in vitro studies. *Clin Pharmacokinet* 2003;42: 153–178.
- [38] Mitry RR, Hughes RD, Bansal S, Lehec SC, Wendon JA, Dhawan A. Effects of serum from patients with acute liver failure due to paracetamol overdose on human hepatocytes in vitro. *Transplant Proc* 2005;37:2391–2394.