World J Surg (2013) 37:192–201
DOI 10.1007/s00268-012-1781-z

World Journal of Surgery

Does Hepatic Ischemia–Reperfusion Injury Induced by Hepatic Pedicle Clamping Affect Survival after Partial Hepatectomy for Hepatocellular Carcinoma?

Feng Xia · Wan-Yee Lau · Yanmin Xu · Lin Wu · Cheng Qian · Ping Bie

Published online: 11 September 2012

Abstract

Background Liver resection offers a chance of cure for patients with hepatocellular carcinoma (HCC). Hepatic pedicle clamping (HPC) is commonly used to reduce blood loss during hepatectomy. Hepatic ischemia-reperfusion (I/R) injury has recently been reported to be a major factor in accelerated tumor growth. We therefore evaluated the effect of intermittent HPC on the prognosis of patients after liver resection.

Methods The clinicopathological features and serum/tissue samples of 386 HCC patients who underwent curative liver resection were prospectively collected. The patients were divided into the HPC group (over 30 min) and the non-HPC group. Disease-free survival and overall survival were analyzed using multivariate analyses, Kaplan–Meier curves, and log-rank tests. Matrix metalloproteinases and E-selectin were measured to study hepatic I/R injury.

Results The preoperative clinicopathological data were comparable between the HPC group ($n = 224$) and the non-HPC group ($n = 162$). During the study period, 257 of the 386 patients (66.6 %) developed tumor recurrence. The overall tumor recurrence and intrahepatic tumor recurrence rates were not significantly different between the two groups. There were no significant differences between the two groups with respect to the 1-, 3-, and 5-year disease-free and overall survival rates. Similarly, subgroup analyses also showed no marked difference in survival rates for patients with cirrhosis in the two groups. The levels of mRNA in liver tissues and serum concentrations of MMP-2, MMP-9, and E-selectin showed no significant differences between the pre- and post-occlusion periods.

Conclusions Intermittent HPC produced no adverse effect on disease-free and overall survival for patients who underwent liver resection for HCC.

F. Xia · W.-Y. Lau · L. Wu · P. Bie (✉)
Institute of Hepatobiliary Surgery and Southwest Cancer Center, Southwest Hospital, Third Military Medical University, Chongqing, China
e-mail: bieping@medmail.com.cn

F. Xia
e-mail: frankfxia@gmail.com

W.-Y. Lau
Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China

Y. Xu · C. Qian (✉)
Institute of Pathology and Southwest Cancer Center, Southwest Hospital, Third Military Medical University, Chongqing, China
e-mail: cqian3184@yahoo.com.cn

Introduction

Hepatocellular carcinoma (HCC) is the cause of the second most common cancer-related death worldwide. It is especially common in Southeast Asia, and its incidence is rising in Western countries [1, 2]. Surgical resection, local ablative therapies, and liver transplantation are potentially curative treatment modalities in selected patients. Liver resection has now been established as the first-line therapy for HCC. Unfortunately, the postoperative recurrence rate is high, with a 3-year recurrence rate of up to 60 % [3]. Intermittent hepatic pedicle clamping (HPC) is commonly used to reduce blood loss during hepatectomy. Recent animal studies showed that hepatic ischemia–reperfusion (I/R) injury due to hilar vascular clamping caused accelerated tumor growth, stimulated tumor cell adhesion, and promoted metastases [4, 5]. The underlying mechanism is that I/R injury causes cellular damage by inducing free-radical formation, upregulating

inflammatory cytokines, dysregulating mitochondrial calcium handling, and upregulating matrix metalloproteinases. These events have been shown to promote metastases. However, in humans, three recent retrospective studies showed no evidence that HPC adversely affected long-term overall and disease-free survival after hepatectomy for colorectal liver metastases [6–8]. At present, it is still unknown whether I/R injury in general, and HPC in particular, affect recurrence and prognosis after hepatic resection for HCC [9, 10], especially in patients with cirrhosis. The answers to these questions will determine whether HPC should be used.

In this study we evaluated the effect of intermittent Pringle's maneuver on the overall and disease-free survivals of HCC patients after curative resection, taking into account the diversity of patient subpopulations. Matrix metalloproteinases (MMPs) and E-selectin were measured to study hepatic I/R injury.

Patients and methods

Between January 2001 and December 2006, a total of 565 consecutive patients underwent hepatic resection for HCC in our institute. The study was approved by the local review board, and written informed consent was obtained from all patients before treatment. The clinicopathological features and serum/tissue samples of 386 patients were collected prospectively in a specially designed computerized database. Patients who satisfied the following criteria were entered into this study: (1) histologically proven hepatocellular carcinoma with curative resection. The curative nature of the resection was established by a clear tumor resection margin (>0 cm) on postoperative histology and by a negative chest radiograph, ultrasonography, triphasic abdominal contrast-enhanced spiral computed tomography (CT) scans, and abdominal magnetic resonance imaging (MRI) carried out within 2 months after surgery. (2) Either HPC was not used or the total HPC time was more than 30 min. (3) There was no evidence of residual lymph node metastases. Sixty-nine patients were excluded from this study for the following reasons: liver resection had been carried out for recurrent or spontaneously ruptured HCC, the patients dropped out for follow-up within 1 month of the operation, or HPC was done but the occlusion time was >30 min. The remaining 386 patients were divided into two groups: HPC ($n = 224$) and non-HPC ($n = 162$).

Preoperative management

All patients followed the same preoperative evaluation protocol, which included blood biochemistry, percutaneous ultrasonography, spiral CT and CT angiography of the abdomen, chest X-ray, and electrocardiogram (ECG). Liver function was assessed by the Child-Pugh grading, an oral glucose tolerance test (OGTT), and/or an indocyanine green clearance test (ICG-R15). Major liver resections were carried out when ICG-R15 was less than 14 %, as we have previously reported [11, 12].

Surgical management

Liver resection included nonanatomical resection, subsegmentectomy, segmentectomy, hemihepatectomy, and trisecmentectomy. A major hepatectomy is defined as resection of three or more of Couinaud's segments.

Surgery was performed as we have previously reported [11, 12]. The operation started with a right subcostal incision, or a midline incision with a right horizontal extension. A low central venous pressure was routinely used. If inflow occlusion was used, it was achieved by tightening a 4-mm tape around the portal triad. Intermittent HPC consisted of cycles of occlusion/unocclusion of 15/5 min. Liver transection was carried out using a Kelly clamp or a CUSA (Cavitron Ultrasonic Surgical Aspirator System 200; Valleylab Inc., Boulder, CO, USA) with a 23-kHz standard tip, 70 W, 4 mL/min flush, and sensitivity of 100 %. A TissueLink (Force FX-8C; Valleylab, Tyco Healthcare, Boulder, CO, USA) was used to coagulate the vessels and bile ducts, using an output power at 70 W. The radiofrequency (480 kHz) energy was focused at the tips of the instrument through a channel inside the device and conveyed to the liver tissue by a low flow of saline solution (one drop per second) to induce thermocoagulation. HPC was not routinely started at the beginning of liver transection but only when significant bleeding occurred in the operative field. Blood transfusion was given only when the hemoglobin dropped below 8.0 g/L.

Postoperative management

All patients received the same postoperative care by the same team of surgeons, as reported previously [11, 12]. After discharge from hospital and on follow-up, the patients were monitored by ultrasonography, contrast CT scan, or MRI every 2 months in the first year and every 3 months thereafter. Recurrence was defined as the appearance of a new lesion with radiologic features typical of HCC on two or more imaging modalities. Disease-free survival was defined as the interval between the operation and the date of diagnosis of the first recurrence. Overall recurrence was defined as the interval between the operation and the date of death or the last date of follow-up.

Serum MMP-2, MMP-9, and E-selectin levels

Human blood samples were collected at three time points: before ischemia and 24 and 48 h after initiation of reperfusion. The samples were centrifuged at 1,500×*g* for 15 min. Total serum MMP-2, MMP-9, and E-selectin protein concentrations were determined using highly specific enzyme-linked immunosorbent assays (ELISA) (R&B Systems). All samples were analyzed in triplicate. Standard curves were generated according to the manufacturer's instructions.

Quantitative real-time PCR analysis for mRNA expression

Liver tissue samples were collected before liver transection (the Control group) and at the end of the operation (the Liver I/R group). Total RNA was extracted from the liver specimens with the use of Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. First-strand cDNA synthesis and amplification were performed according to the protocol of the PrimeScript™ RT Reagent Kit (perfect real-time) (TaKaRa, Shiga, Japan). Quantitative determination of gene expression levels was done on a Bio-Rad CFX96 Real Time PCR System (Bio-Rad, Hercules, CA, USA), with the use of the SYBR® premix Ex Taq (TaKaRa).

Western blotting analysis

Liver tissue samples were collected before liver transection (the Control group) and at the end of the operation (the Liver I/R group). Total proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10 % gel. Rainbow molecular weight markers were used to visualize protein migration. When electrophoresis was over, the specimen was then transferred onto a 0.45-μm nitrocellulose membrane (Millipore, Billerica, MA, USA) in a buffer containing 25 mmol/L Tris-HCl (pH 8.3), 192 mmol/L glycine, and 20 % methanol and blocked with 5 % fat-free dry milk in PBS for 2–4 h. The membranes were incubated with primary antibodies anti-MMP-2 and anti-GAPDH, followed by the addition of a secondary antibody anti-rabbit IgG or anti-mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The bound antibodies were detected using a chemofluorescence detection kit (Amersham, Piscataway, NJ, USA) following the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the χ^2 test or the Fisher exact test to compare discrete variables, and Student's *t* test was used to compare continuous variables. The survival curves of the two groups, including the cumulative survival and the disease-free survival, were generated by the Kaplan–Meier method and compared using log-rank tests. Multivariate analysis for 17 variables in Table 1 using the Cox proportional hazards regression model was used to identify independent prognostic factors in predicting disease-free survival and overall cumulative survival. A p value <0.05 was considered to be significant. Statistical analyses were performed using the SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Perioperative features of HCC patients

The background characteristics in the HPC and non-HPC groups are given in Table 1. Most patients (75.9 % of 386) were male. The platelet count in the HPC group was significantly lower than in the non-HPC group ($p = 0.046$). The number of patients with multiple tumors was also significantly higher in the HPC group than the non-HPC group ($p = 0.034$). On the other hand, gender, ICG-R15, total bilirubin, AST, hyaluronic acid, tumor size, portal vein invasion, and other variables showed no significant difference between the two groups. Table 2 gives the results of surgery in the two groups. The postoperative AST on day 3 was significantly lower in the HPC group than in the non-HPC group ($p = 0.042$). However, there was no significant difference in total bilirubin on postoperative day 3 between the two groups. The type of resection, intraoperative blood loss, red cell transfusion, and morbidity were similar between the two groups. The median of the total occlusion time of intermittent HPC was 50 min (range = 30–98 min).

Analysis of prognosis of HCC patients after hepatectomy

At the time of censor of the study, 257 of 386 patients (66.6 %) had developed tumor recurrence. The mean time to recurrence was 26.1 months (median = 23; range = 3–111). Of the 257 patients with recurrence, 159 patients (61.9 %) developed hepatic recurrence only, 55 patients (21.4 %) extrahepatic recurrence only, and 43 patients (16.7 %) both intra- and extrahepatic recurrence. Overall, hepatic recurrence occurred in 202 patients (78.6 %). The overall recurrence rate and the liver-specific recurrence rate were not significantly different between the two groups (Table 3).

Multivariate analysis identified only one significant factor (cirrhosis) for overall survival rate and five significant factors (tumor size ≥5 cm, cirrhosis, Child–Pugh grade, tumor number, and portal vein invasion) for disease-free survival rate (Table 4). Cirrhosis was an independent factor for both overall and disease-free survival: the hazard

Table 1 Characteristics of patients who underwent hepatic resection with or without intermittent HPC

Clinical parameters	HPC	Non-HPC	p[b]
No. of patients	224	162	–
Male	173	119	0.403
Age (years)[a]	48 (21–78)	57 (18–79)	0.141
Platelet count (10^9/L)[a]	121 (31–368)	156 (30–317)	0.046
Serum albumin (g/L)[a]	38 (26–52)	36 (24–51)	0.383
Serum total bilirubin (μmol/L)[a]	14.6 (5.1–48.3)	16.4 (7.6–51.5)	0.283
AST (IU/L)[a]	56 (11–526)	59 (12–498)	0.528
Hemoglobin (g/L)[a]	12.5 (6.6–18.6)	11.5 (5.9–17.3)	0.272
AFP ≥400 (mg/L)	143	93	0.206
Hepatitis B virus infection	209 (93.3 %)	149 (92.0 %)	0.692
ICG retention at 15 min (%)[a]	9.7 (1.5–27.2)	9.9 (2.3–26.8)	0.489
Child–Pugh grade			0.915
A	141	101	
B	83	61	
Hyaluronic acid (μg/L)[a]	217 (41–872)	229 (35–799)	0.103
Prothrombin time (s)[a]	14 (10–17)	14 (10–19)	0.221
Cirrhosis			0.463
Yes	169	128	
No	55	34	
Tumor size[a]	6.4 (2.8–20.2)	5.9 (2.9–21.3)	0.096
Tumor number			0.034
Single	145	122	
Multiple	79	40	
Portal vein invasion			0.239
Absent	148	97	
Present	76	65	

AFP α-fetoprotein, *AST* aspartate aminotransferase, *ICG* indocyanine green

[a] Value expressed as median with range in parentheses

[b] Occlusion group compared with nonocclusion group

Table 2 Surgical outcomes

Variable	HPC	Non-HPC	p[c]
Type of resection			0.254
Minor	131	85	
Major[a]	93	77	
Hilar occlusion time (min)	50 (30–98)	0	
Intraoperative blood loss (mL)[b]	500 (50–3,600)	450 (50–3,800)	0.078
Intraoperative blood transfusion (mL)[b]	650 (0–3500)	600 (0–3,600)	0.128
No. of patients without transfusion (%)	168 (75.0)	132 (81.48)	0.139
Postoperative AST on day 3 (IU/L)	226 (48–896)	327 (68–1,237)	0.043
Postoperative total bilirubin on day 3 (μmol/L)	42 (19–276)	39 (18–212)	0.137
Infectious morbidity	16	11	1.000
Lung infection	5	3	
Abdominal collection	6	4	
Infection of incisional wound	5	3	
Sepsis	0	1	
Noninfectious morbidity	11	11	1.000
Pleural effusion	7	5	
Bile leak	3	4	
Liver failure	1	2	

[a] Major hepatectomy was defined as ≥3 segments

[b] Value expressed in median with range in parentheses

[c] Occlusion group compared with nonocclusion group

Table 3 Type of recurrence

Type of recurrence	HPC	Non-HPC	p[a]
Hepatic	88	71	0.402
Extrahepatic	29	26	0.461
Hepatic + extrahepatic	31	12	0.050
Overall hepatic recurrence	119	83	0.757
Overall extrahepatic	60	38	0.479
Total recurrence	148 (66.1 %)	109 (67.3 %)	0.828

[a] Occlusion group compared with nonocclusion group

Table 4 Multivariate analysis: predictors for disease-free survival and overall survival

Variables	Hazard ratio	95 % CI	p
Disease-free survival			
Tumor size ≥5 cm	1.35	1.01–1.76	0.04
Cirrhosis	0.64	0.44–0.96	0.03
Child–Pugh grade	1.43	1.08–1.78	0.01
Tumor number	1.32	1.03–1.77	0.04
Portal vein invasion	0.56	0.33–0.88	0.02
Overall survival			
Cirrhosis	1.54	1.13–2.09	0.009

ratios with 95 % confidence intervals and the p values were 1.54, 1.13–2.09, $p = 0.009$, and 0.64, 0.44–0.96, $p = 0.03$, respectively (Table 4).

The overall and disease-free survival curves of the HPC and non-HPC groups are shown in Fig. 1a, b, respectively. Overall survival was similar in the two groups: the 1-, 3-, and 5-year survival rates were 95.6, 72.3, and 45.1 % in the non-HPC group vs. 86.4, 70.9, and 43.9 % in the HPC group ($p = 0.146$). The disease-free survival rates at 1, 3, and 5 years were 77.9, 31.7, and 13.8 % in the non-HPC group vs. 62.4, 44.5, and 17.9 % in the HPC group ($p = 0.554$). Similarly, there were no significant differences in disease-free survival (cirrhotic and noncirrhotic, $p = 0.103$, 0.351) and overall survival (cirrhotic and noncirrhotic, $p = 0.275$, 0.691) in patients with and without cirrhosis between the two groups (Fig. 2a–d). However, the postoperative long-term survivals for all of the patients were significantly better in the noncirrhotic group than in the cirrhotic group in terms of the overall and disease-free survival rates ($p = 0.004$ and 0.016, respectively). The overall 1-, 3-, and 5-year survival rates were 91.9, 76.8, and 57.8 % in the noncirrhotic group and 89.8, 70.2, and 40.9 % in the cirrhotic group, respectively. The 1-, 3-, and 5-year disease-free survival rates were 79.2, 51.7, and 25.4 % in the noncirrhotic group and 73.6, 36.4, and 14.8 % in the cirrhotic group, respectively (Fig. 3a, b).

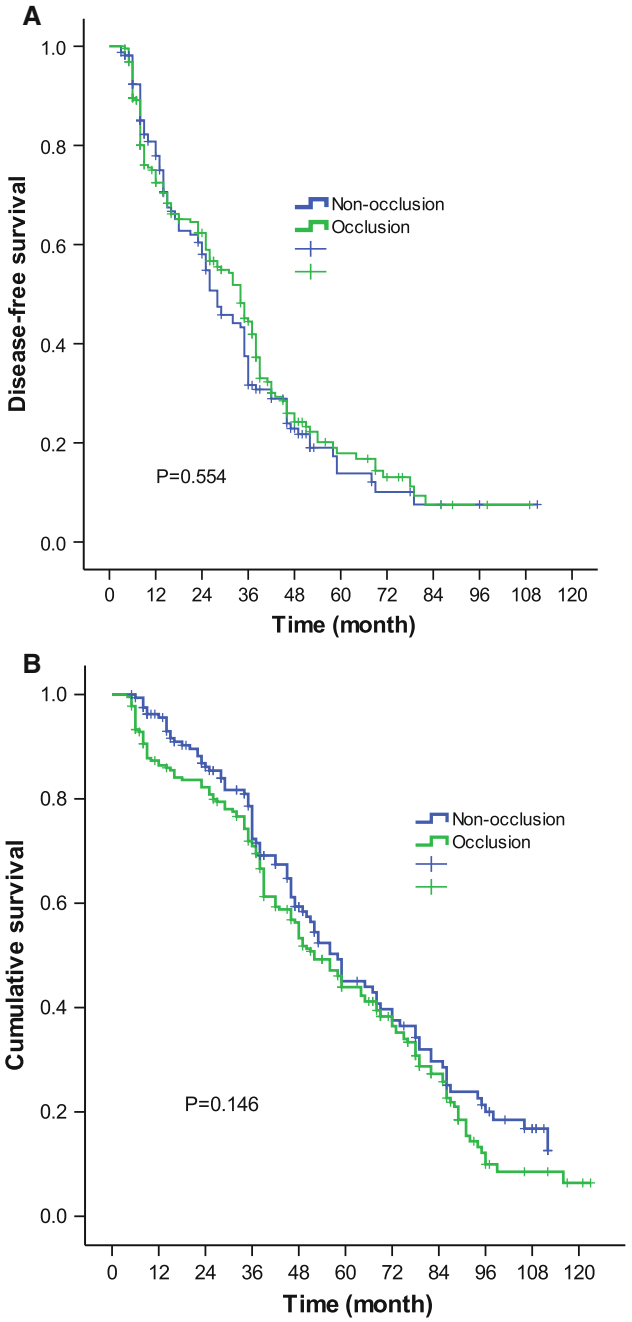


Fig. 1 Disease-free survival (**a**) and overall survival (**b**) for all patients (Kaplan–Meier, log-rank test)

Gene expression of MMP-2, MMP-9, and E-selectin

As shown in Fig. 4, the serum levels of MMP-2, MMP-9, and E-selectin were similar before surgery and 24 and 48 h after surgery. Hepatic expressions of MMP-2, MMP-9, and E-selectin were measured by real-time PCR in nontumorous liver tissues obtained from HCC patients before HPC and after HPC. There was no significant difference in the hepatic expressions of MMP-2, MMP-9, and E-selectin

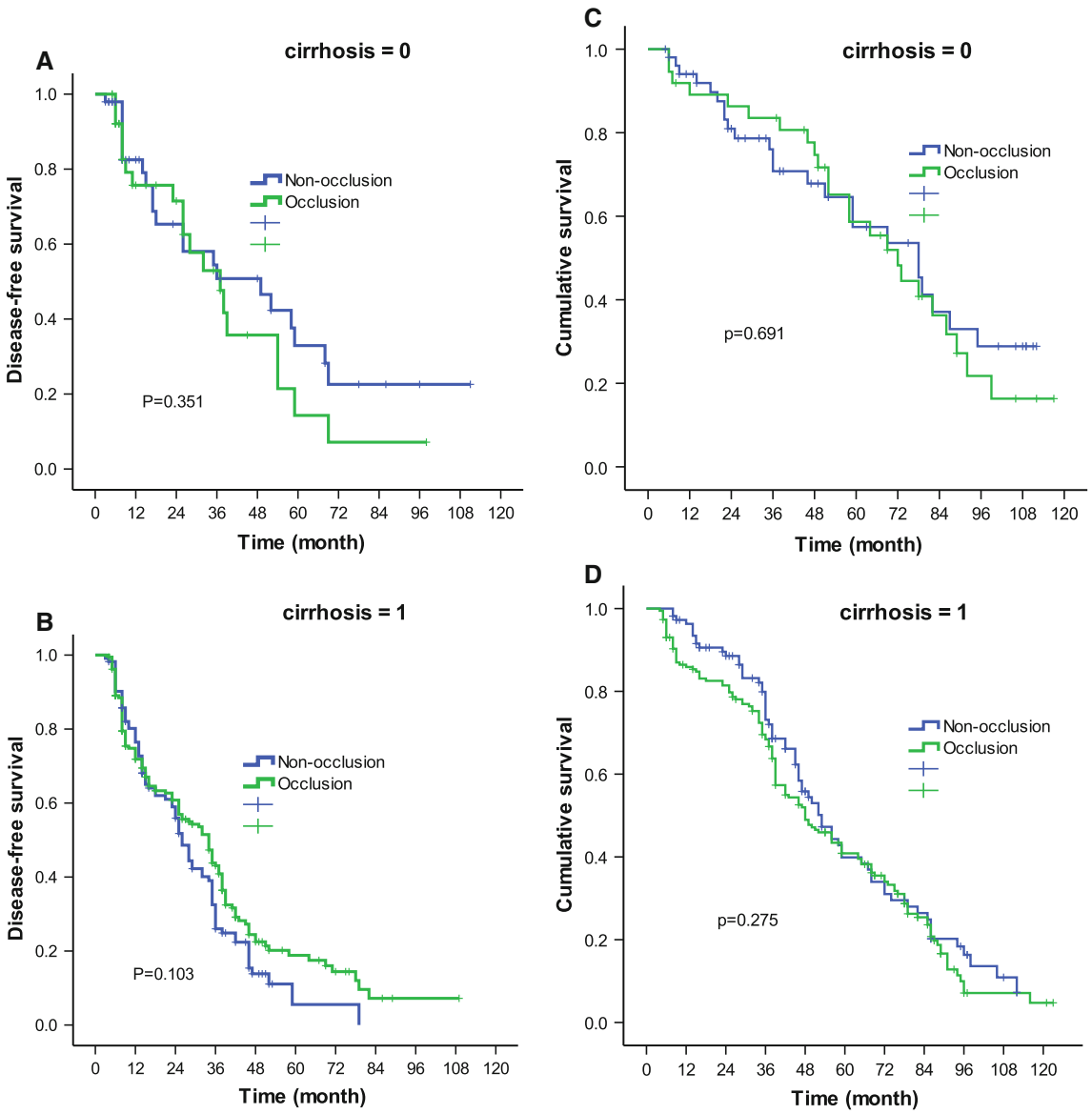


Fig. 2 Disease-free survival (**a**, **b**) and overall survival (**c**, **d**) for the patients divided into two subsets according to the presence of liver cirrhosis (Kaplan–Meier, log-rank test)

before and after occlusion (Fig. 5). The hepatic expression of MMP-2 was further analyzed at protein level by western blot analysis. The protein level of MMP-2 was similar in liver tissues before and after HPC (Fig. 6). Our data indicated that there was no upregulation of MMP-2, MMP-9, and E-selectin in patients who underwent hepatectomy with HPC.

Discussion

The present study showed that HPC did not affect overall and disease-free survival following curative liver resection for HCC. It also failed to show any significant rise in inflammatory mediators induced by hepatic ischemia–reperfusion injury caused by HPC using cycles of occlusion/unocclusion of 15/5 min.

Experimental and clinical studies have shown that even short clamping times produced some degree of ischemia–reperfusion injury, which resulted in hepatocellular damage and postoperative liver dysfunction. Hepatic I/R injury causes cellular damage by inducing free-radical formation, upregulating inflammatory cytokines, dysregulating mitochondrial calcium handling, and upregulating matrix metalloproteinases (MMPs) [13, 14]. Some experimental studies reported that ischemia–reperfusion injury stimulated

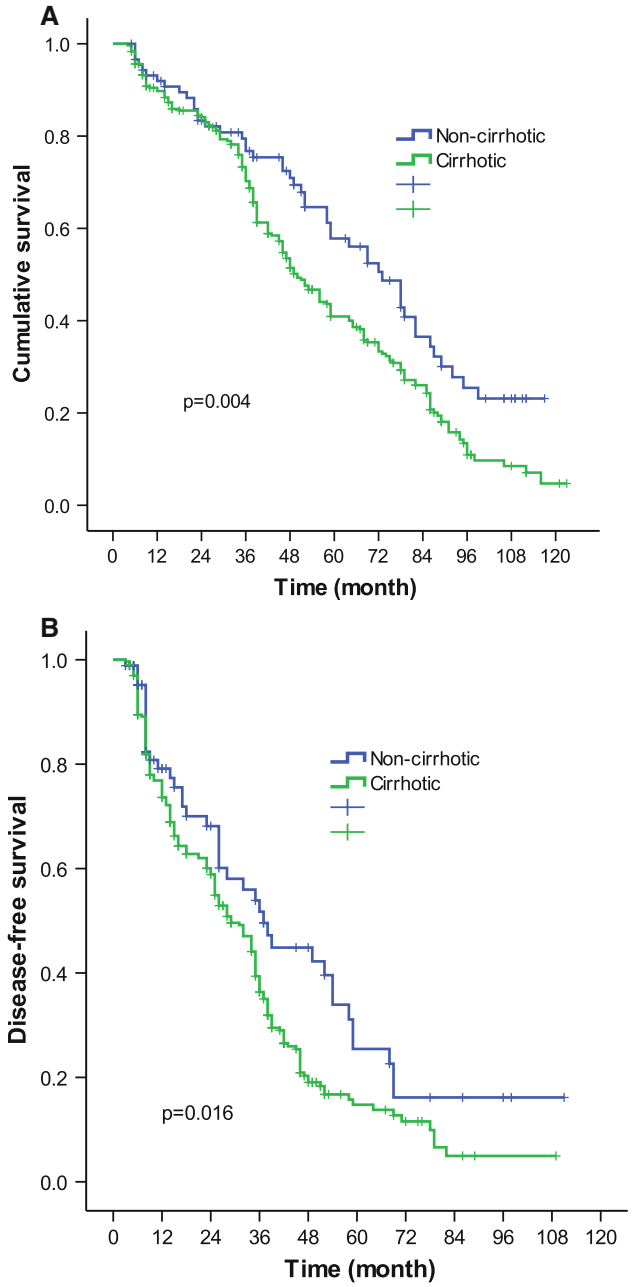


Fig. 3 Disease-free survival (**a**) and overall survival (**b**) for noncirrhotic and cirrhotic patients (Kaplan–Meier, log-rank test)

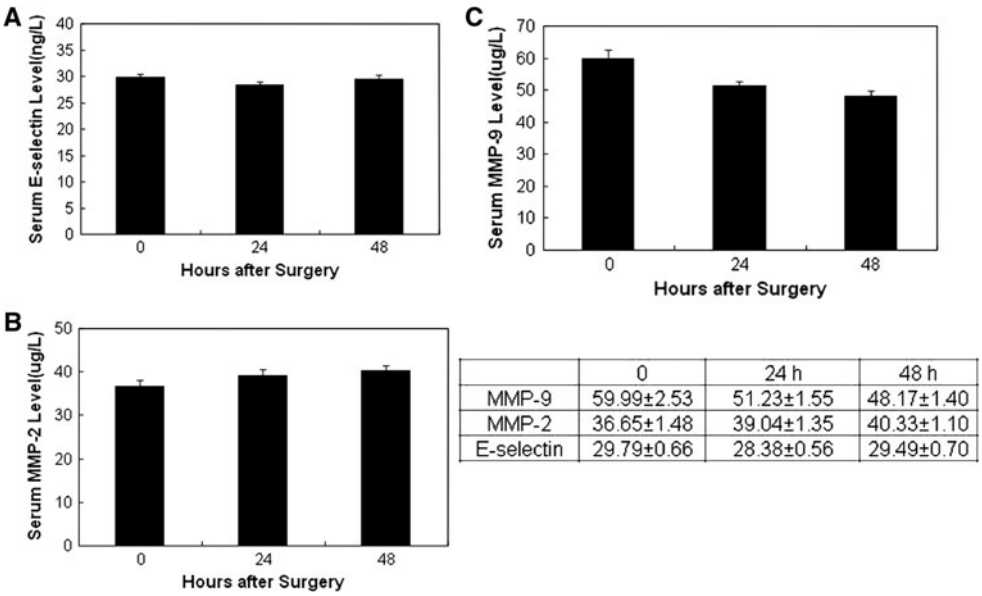
outgrowth of pre-established micrometastases and consequently adversely influenced prognosis in patients with colorectal cancer. Yoshida et al. [15] reported that sustained ischemia and reperfusion in the liver accelerated growth of established hepatic metastases, and minimization of I/R injury through intermittent ischemia attenuated metastasis of colorectal cancer to the liver following surgical stress.

However, recent clinical studies showed that HPC did not affect survival of patients with liver metastases. Wong et al. [6] reported that there was no difference in the median disease-free survival after hepatectomy for colorectal metastasis (CRLM) between patients who received intermittent HPC and those who did not (21.1 vs. 19.9 months; $p = 0.199$). Ferrero et al. [7] showed that survival of patients with liver metastases who underwent hepatectomy with ($n = 39$ patients, the HPC group) or without ($n = 41$ patients, the NHPC group) pedicle clamping was the same, i.e., HPC did not affect survival. Similarly, a recent bi-institutional study showed that HPC did not affect long-term survival and liver-specific disease-free survival following hepatectomy for CRLM analyzed according to the use, type, and duration of HPC [8].

The other concerns of HPC are its safety and effectiveness in the early results of liver resections [16]. Makuuchi et al. [17] reported that HPC can be used for the donor hepatectomy in living-donor liver transplantation without inducing any ischemic injury attributable to the technique itself. Furthermore, the incidence of biliary complications and the 1-year survival rates were not influenced by whether or not the graft was harvested using HPC.

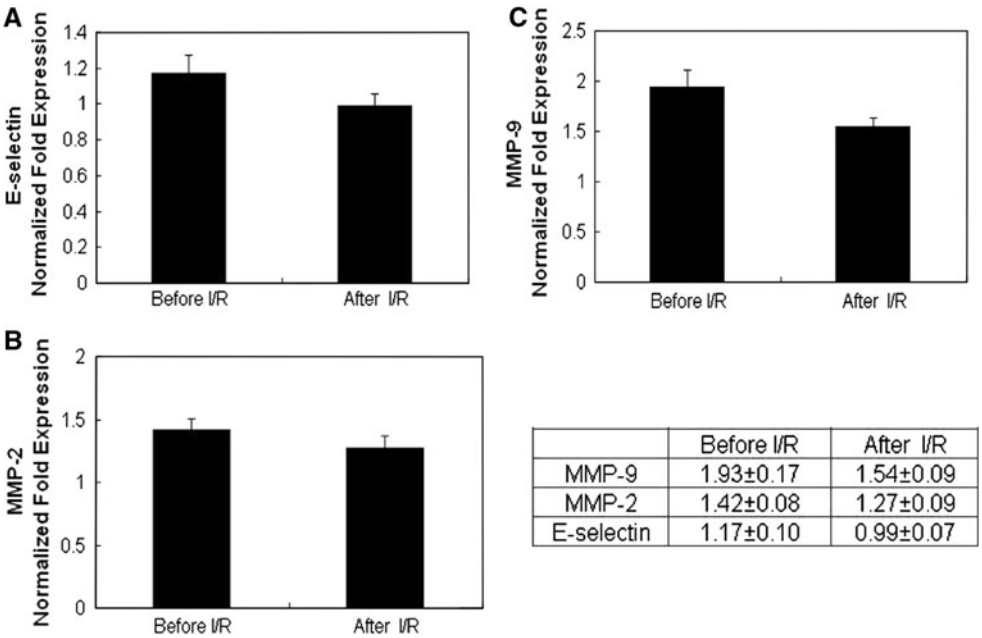
In our study, there was no difference in the postoperative total bilirubin on day 3, although postoperative AST was higher in the nonocclusion group, which was attributed to the more extensive coagulation of liver parenchyma by the TissueLink. There was also no significant difference in intraoperative blood loss and blood transfusion between the two groups. Thus, it cannot be argued that the possible negative effect was compensated by a decrease in intraoperative blood loss when HPC was used. It is well known that an increased intraoperative blood loss is associated with an increase in postoperative complications and in hepatic tumor recurrence [18, 19].

Many previous studies on hepatic I/R injury demonstrated that cytokines are released through induction of adhesion molecules, and chemokines promote neutrophil activation and accumulation and thereby contribute to progression of parenchymal injury by releasing reactive oxygen species (ROS) and proteases. In particular, hepatic expression of MMP2 and MMP9 has been linked to hepatic I/R injury through recruitment of neutrophils and T cells [9, 10]. MMPs promote tumor growth and metastases by a variety of mechanisms, including proteolysis of extracellular matrix that allows initial migration and seeding of tumor cells [20–23]. MMP2 and MMP9 are widely implicated in the metastasis of colorectal carcinoma. Nicoud et al. [23] reported that hepatic I/R-induced elevation of MMP9 contributed to the growth of metastatic colorectal carcinoma in mouse liver and that postresection MMP9 inhibition was clinically beneficial in preventing recurrence following hepatic surgery. Similarly, E-selectin is an adhesion molecule that is transiently expressed on the surface of activated endothelial cells. It participates in the rolling of leukocytes upon the endothelial cell surface and has been implicated in the initial events of neutrophil extravasation in inflammatory response. Ligands for E-selectin, sLe-X and sLe-a, have



	0	24 h	48 h
MMP-9	59.99±2.53	51.23±1.55	48.17±1.40
MMP-2	36.65±1.48	39.04±1.35	40.33±1.10
E-selectin	29.79±0.66	28.38±0.56	29.49±0.70

Fig. 4 Serum concentrations of E-selectin (**a**), MMP-2 (**b**), and MMP-9 (**c**) before and after surgery. Human blood samples were collected before surgery and 24 and 48 h after surgery. Serum concentrations of E-selectin, MMP-2, and MMP-9 were analyzed by enzyme-linked immunosorbent assay. The data are presented as mean ± SD ($n = 14$). No significant differences were observed at any time point



	Before I/R	After I/R
MMP-9	1.93±0.17	1.54±0.09
MMP-2	1.42±0.08	1.27±0.09
E-selectin	1.17±0.10	0.99±0.07

Fig. 5 Gene expression of E-selectin, MMP-2, and MMP-9 in nontumor livers before and after surgery. Nontumor liver samples were collected before and after hepatic pedicle clamping during operation. The mRNA levels of E-selectin (**a**), MMP-2 (**b**), and MMP-9 (**c**) in liver tissues were measured by real-time PCR. The data are presented as mean ± SD ($n = 15$)

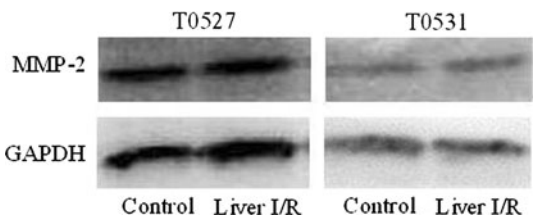


Fig. 6 Protein level of MMP-2 in nontumor liver tissue samples before and after surgery. Nontumor liver samples were collected before and after hepatic pedicle clamping during surgery. The protein level of MMP-2 in liver tissues was measured by western blot

been identified as markers of progression of cancer. The interaction between E-selectin and sLe-X or sLe-a affects tumor cell adhesion to sinusoidal endothelial cells. Doi et al. [24] indicated that hepatic I/R injury produced an increase in liver metastases of rat colon cancer with an increase in expression of E-selectin. Because a shorter duration of liver ischemia resulted in a smaller number of liver tumor nodules and a lower expression of E-selectin mRNA, shortening the ischemic period is worthwhile in liver resection carried out on patients with liver malignancy [24, 25]. However, we have not observed any significant difference in the serum

concentrations and the tissue mRNA levels of E-selectin, MMP-2, and MMP-9 between the pre- and postoperative periods using HPC in human HCC patients. This study showed that hepatic changes in cytokine expression in animals did not occur in human. These data suggested that the changes in the expression of cytokines such as E-selectin, MMP-2, and MMP-9 were mild. As a consequence, the changes were not enough to lead to an increase in liver metastases or tumor recurrence in HCC patients when intermittent HPC was used in our clinical setting.

To the best of our knowledge, there have not been any clinical or experimental studies on the influence of HPC on the long-term prognosis of patients with hepatocellular carcinoma after partial hepatectomy, although there have been such studies on colorectal metastases to the liver. HPC is commonly used for liver resection and its influence on the outcome of surgery is important. In this study, although the baseline data in the two groups were not entirely comparable, the fact that there were more cases with multiple tumors in the HPC group did not influence the outcomes. Furthermore, the use of matrix metalloproteinases and E-selectin supported the conclusion that intermittent HPC was safe and efficacious. Several experimental studies showed that the degree of hepatic injury after I/R was significantly greater in cirrhotic livers than in normal livers [26]. The results from randomized controlled trials and from a meta-analysis of hepatic inflow clamping showed that chronically diseased livers are more susceptible to I/R injury than normal livers [27, 28]. Our subgroup analysis showed that HPC had no significant negative impact on the long-term prognosis of cirrhotic patients. However, a properly conducted randomized controlled trial is necessary to clarify the effects of HPC on the long-term prognosis of HCC patients after curative resection.

In conclusion, HPC was safe and efficacious in partial hepatectomy for HCC. It had no significant negative impact on disease-free survival and overall survival, even in patients with cirrhosis. Cirrhosis, however, was an independent risk factor for both the disease-free survival and overall survival for HCC patients.

Acknowledgements This work was supported by the National S&T Major Project (No. 2008ZX10002-026).

Conflict of interest The authors have no potential and real conflicts of interest with this study to disclose.

References

1. Rahbari NN, Mehrabi A, Mollberg NM et al (2011) Hepatocellular carcinoma: current management and perspectives for the future. Ann Surg 253:453–469
2. Llovet JM, Burroughs A, Bruix J (2003) Hepatocellular carcinoma. Lancet 362:1907–1917
3. Forner A, Reig ME, de Lope CR et al (2010) Current strategy for staging and treatment: the BCLC update and future prospects. Semin Liver Dis 30:61–74
4. van der Bilt JD, Kranenburg O, Nijkamp MW et al (2005) Ischemia/reperfusion accelerates the outgrowth of hepatic micrometastases in a highly standardized murine model. Hepatology 42:165–175
5. van der Bilt JD, Kranenburg O, Borren A et al (2008) Ageing and hepatic steatosis exacerbate ischemia/reperfusion-accelerated outgrowth of colorectal micrometastases. Ann Surg Oncol 15:1392–1398
6. Wong KH, Hamady ZZ, Malik HZ et al (2008) Intermittent Pringle manoeuvre is not associated with adverse long-term prognosis after resection for colorectal liver metastases. Br J Surg 95:985–989
7. Ferrero A, Russolillo N, Viganò L et al (2010) Does Pringle maneuver affect survival in patients with colorectal liver metastases? World J Surg 34:2418–2425. doi:10.1007/s00268-010-0682-2
8. Giuliante F, Ardito F, Pulitanò C et al (2010) Does hepatic pedicle clamping affect disease-free survival following liver resection for colorectal metastases? Ann Surg 252:1020–1026
9. Bahde R, Spiegel HU (2010) Hepatic ischaemia-reperfusion injury from bench to bedside. Br J Surg 97:1461–1475
10. Klune JR, Tsung A (2010) Molecular biology of liver ischemia/reperfusion injury: established mechanisms and recent advancements. Surg Clin North Am 90:665–677
11. Xia F, Lau WY, Qian C et al (2011) Continuous occlusion of hepatic artery proper for prevention of blood loss in partial hepatectomy for ruptured hepatocellular carinoma: a case-matched comparative study. Ann Surg Oncol 18:1638–1643
12. Xia F, Lau WY, Qian C et al (2010) Surgical treatment of giant liver hemangiomas: enucleation with continuous occlusion of hepatic artery proper and intermittent pringle maneuver. World J Surg 34:2162–2167. doi:10.1007/s00268-010-0592-3
13. Lentsch AB, Kato A, Yoshidome H et al (2000) Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology 32:169–173
14. Selzner N, Rudiger H, Graf R et al (2003) Protective strategies against ischemic injury of the liver. Gastroenterology 125:917–936
15. Yoshida M, Horiuchi T, Uchinami M et al (2003) Intermittent hepatic ischemia-reperfusion minimizes liver metastasis in rats. J Surg Res 111:255–260
16. Casillas-Ramirez A, Mosbah IB, Ramalho F et al (2006) Past and future approaches to ischemia-reperfusion lesion associated with liver transplantation. Life Sci 79:1881–1894
17. Imamura H, Takayama T, Sugawara Y et al (2002) Pringle's manoeuvre in living donors. Lancet 360:2049–2050
18. Benoist S, Salabert AS, Penna C et al (2006) Portal triad clamping (TC) or hepatic vascular exclusion (VE) for major liver resection after prolonged neoadjuvant chemotherapy? A case-matched study in 60 patients. Surgery 140:396–403
19. Ercolani G, Ravaioli M, Grazi GL et al (2008) Use of vascular clamping in hepatic surgery: lessons learned from 1260 liver resections. Arch Surg 143:380–387 discussion 388
20. Chen CF, Leu FJ, Chen HI et al (2005) Oxygen radicals and matrix metalloproteinases mediate reperfusion liver injury. Transpl Proc 37:4547–4549
21. Cursio R, Mari B, Louis K et al (2002) Rat liver injury after normothermic ischemia is prevented by a phosphinic matrix metalloproteinase inhibitor. FASEB J 16:93–95
22. Khandoga A, Kessler JS, Hanschen M et al (2006) Matrix metalloproteinase-9 promotes neutrophil and T cell recruitment and migration in the postischemic liver. J Leukoc Biol 79:1295–1305
23. Nicoud IB, Jones CM, Pierce JM et al (2007) Warm hepatic ischemia-reperfusion promotes growth of colorectal carcinoma micrometastases in mouse liver via matrix metalloproteinase-9 induction. Cancer Res 67:2720–2728

24. Doi K, Horiuchi T, Uchinami M et al (2002) Hepatic ischemia-reperfusion promotes liver metastasis of colon cancer. J Surg Res 105:243–247
25. Bevilacqua MP, Stengelin S, Gimbrone MA et al (1989) Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 243:1160
26. Sugiyama Y, Ishizaki Y, Imamura H et al (2010) Effects of intermittent Pringle's manoeuvre on cirrhotic compared with normal liver. Br J Surg 97:1062–1069
27. Capussotti L, Nuzzo G, Polastri R et al (2003) Continuous versus intermittent portal triad clamping during hepatectomy in cirrhosis. Results of a prospective, randomized clinical trial. Hepatogastroenterology 50:1073–1077
28. Rahbari NN, Wente MN, Schemmer P et al (2008) Systematic review and meta-analysis of the effect of portal triad clamping on outcome after hepatic resection. Br J Surg 95:424–432