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The role of calcineurin inhibitors in the progression of hepatocellular carcinoma after liver transplantation

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Abstract

Introduction. *Orthotopic liver transplantation is the most radical method of treatment of hepatocellular carcinoma. The high recurrence rate limits the use of transplantation in patients with hepatocellular cancer. Immunosuppressive therapy may affect the frequency of oncoprogression after liver transplantation*

Aim. *To evaluate the role of immunosuppressive therapy in the postoperative progression of hepatocellular cancer in patients after liver transplantation*

Material and methods. *The recurrence rate of hepatocellular cancer and tumor free survival in 104 patients after liver transplantation were analyzed. To evaluate the effect of the immunosuppression main component concentration on the postoperative progression of hepatocellular carcinoma, we studied the mean baseline concentration (C_0) for the entire follow-up period for patients with a tumor-free period and the mean baseline concentration for patients with hepatocellular carcinoma progression, in whom only the duration of the tumor-free*

period was studied. According to the degree of tumor lesion, patients were distributed in accordance with the Milan criteria (based on the results of a pathologic and morphological examination of the recipient's explanted liver.

Results. *The values of the baseline blood level of tacrolimus>6.0 ng/ml and cyclosporine A>100 ng/ml is associated with a high rate of progression of hepatocellular cancer. Reducing the load of calcineurin inhibitors can reduce the incidence of cancer progression by at least 2 times. The values of 1-, 3- and 5-year relapse-free survival in patients with advanced cancer and low figures of the baseline blood level of calcineurin inhibitor are 82%, 70% and 70%, respectively.*

Conclusion. *Minimization of immunosuppression is of crucial importance in the prevention of posttransplant progression of hepatocellular cancer, especially among patients with its common form.*

Keywords: liver transplantation, hepatocellular carcinoma, immunosuppressive therapy, tacrolimus, cyclosporine A

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CyA - cyclosporine A

CNI − calcineurin inhibitor

HCC - hepatocellular carcinoma

IT - immunosuppressive therapy

mTOR − mammalian target of rapamycin

OS - overall survival

RFS - recurrence-free survival

Tac - tacrolimus

Introduction

To date, liver transplantation is the most radical treatment for hepatocellular carcinoma (HCC). The acute shortage of donor organs and the high rate of postoperative progression are the main reasons that hinder the use of this technique in patients with HCC. A key role in the post-transplant progression of HCC belongs to the tumor burden exceeding the Milan criteria and vascular invasion [1–4]. The significance of other prognostic factors in HCC recurrence (α-fetoprotein concentration, histological form of the tumor and its differentiation, etiology of the underlying disease, etc.) is still a matter of debate [2]. Immunosuppressive therapy (IT) is an integral part of the entire post-transplantation period and its role in the phenomenon of postoperative progression of HCC is not clearly defined. The number of studies on the significance of IT in the post-transplant progression of HCC is significantly inferior to the number of studies that investigate the role of tumor burden or other factors. At the same time, in the studies of M. Rodríguez-Perálvarez and M. Vivareli, the influence of IT on the duration of the relapse-free period after liver transplantation is noted [5–9]. As far as we know, there are no such publications in our country science. In this work, we analyzed the effect of IT on the progression of HCC after liver transplantation.

Aim of the study was to evaluate the role of IT in the postoperative progression of hepatocellular cancer in patients after liver transplantation.

Material and methods

The results of relapse-free (RFS) and overall survival (OS) in 104 patients who underwent liver transplantation for HCC in the period 2000–

2020 were analyzed. In the course of the analysis, the impact of calcineurin immunosuppressant on the post-transplant progression of HCC was assessed. Basic immunosuppression was represented by calcineurin inhibitors (CNIs): cyclosporine A (CyA) or tacrolimus (Tac).

In the postoperative period, the frequency of measuring the blood levels of CNIs was twice a week during the first month (8 times a month), 1 time per month during the first half year, 1 time per 3 months from the 6th to the 12th months after orthotopic liver transplantation. After the first year, the blood levels of CNIs were studied once every 6 months, as a rule. However, if the clinical situation (i.e. acute cellular rejection, drug toxicity) required a more frequent measuring the blood concentrations of CNIs and the IT correction, the measurements were performed as often as required.

To determine the effect of the blood concentration of the immunosuppression main component on the postoperative progression of HCC, we studied the mean baseline concentrations (C_0) for the entire follow-up period for patients with a relapse-free course and the mean baseline concentrations for patients with HCC progression, in whom only the duration of the relapse-free period was evaluated. The method for calculating the mean concentration was performed for each patient, followed by the study of the mean concentration in the study group.

According to the degree of tumor involvement, patients were distributed in accordance with the Milan criteria (according to the results of a pathological and morphological study of recipient's removed liver). Of 104 transplanted patients, only 43 (41.3%) had tumor lesions that met the Milan criteria ($T_{1–2}$ according to TNM classification) (Table 1).

Table 1. Characteristics of clinical parameters

Group	T_{1-2} (n, %)	T_{3-4} (n, %)	p-value
Floor			
Men/Women	33/10 (76.7/23.3)	44/17 (72/28)	0.59
Age(in years)	55±7.6	51±12	0.07
m-TOR inhibitors	31 (72%)	42 (68.8%)	0.7
CNI (Tac/CyA)	32/11 (74.4/25.6)	45/15 (75.4/24.6)	0.9
The number of IT components in the immediate postoperative period			
1	0	3 (4.9)	0.5
2	13 (30.2)	18 (29.5)	
3	18 (41.8)	28 (45.9)	
4	12 (28)	12 (19.7)	

Statistical analysis was performed using standard methods of descriptive statistics, x^2 test, Student's t-test. The Kolmogorov–Smirnov method was used to determine the normality of distribution, and the Kaplan–Meier method was used to determine survival rates. The ROC analysis and ROC curves were used to determine the cut-off criterion.

Results

Post-transplant progression of HCC was observed in 36 patients (34.6%). The relapse rate in the Milan criteria group (T_{1-2}) was 11.6% (5 of 43), and in the group of patients where the extent of the tumor corresponded to T_{3-4} (50.8%; 31 of 61). Differences between the groups were statistically significant (p=0.0005).

Tacrolimus was used as the base immunosuppressant in 78 cases (75%), CyA was used in 26 (25%) cases. The analysis of RFS and OS did not reveal statistically significant differences with regard to the CNI type.

The rates of the 1-, 3-, 5-year RFS for the Tac and CyA groups were 82%, 71%, 69% and 79%, 67%, 63%, respectively (p=0.3; Fig. 1).

The 1-, 3-, 5-year OS for the Tac and CyA groups were 93%, 78%, 71% and 89%, 82%, 78%, respectively (p=0.9; Fig. 2).

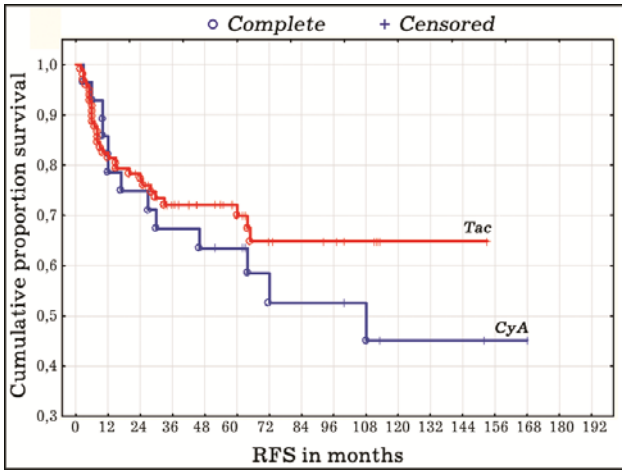

Fig. 1. Recurrence-free survival after liver transplantation against the background of the use of calcineurin receptor inhibitors

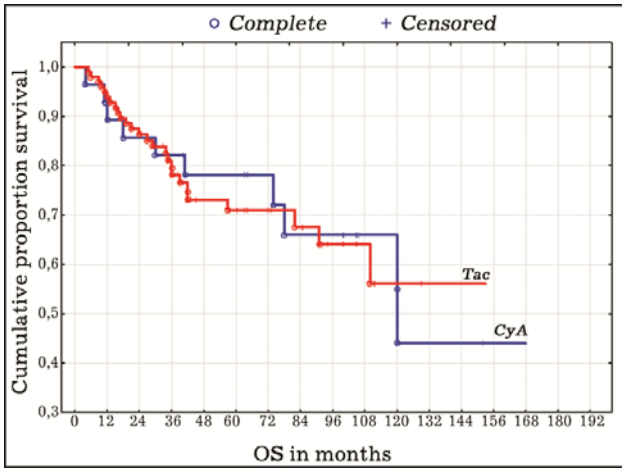

Fig. 2. Overall survival after liver transplantation

Cross-tab analysis did not reveal significant differences in the rate of postoperative progression depending on the type of CNI. Thus, in the group of patients treated with CyA, the relapse rate was 46.1% (12 out of 26) in the Tac group, it was 30.7% (24 of 78), p=0.15. It is important to note that among patients in the T_{1-2} group, Tac was used in 74.4% of patients; and in the T_{3-4} group it was used in 75.4% of patients.

Mean blood concentrations for Tac and CyA in the general population had a normal distribution (two-sided asymptomatic

significance over 0.05) and amounted to 6.2±1.7 ng/mL and 139±53 ng/mL, respectively. In the group of patients who received Tac and did not have a relapse of HCC, the blood level of Tac was 5.9±1.5 ng/mL, in the group of patients with progression of HCC − 7±1.9 ng/mL (p=0.01). The concentrations were 109±41 ng/mL in the group of patients who received CyA and did not have a relapse of HCC, and 168±48 ng/mL in the group of patients with HCC progression (p=0.004).

When distributing patients treated with Tac with regard to with the volume of tumor lesions, we found that in the group of patients within the Milan criteria, there were no significant differences in the blood level of Tac. Thus, the concentration was 6.0±1.6 ng/mL in patients who did not have the HCC recurrence, and 4.7±0.8 ng/ml in the group of patients with recurrence (p=0.2).

Significant differences were achieved in the group of patients who had a T_{3-4} volume of tumor lesions at baseline. Thus, in patients with a recurrence-free course, the Tac concentration was 5.7±1.5 ng/mL, while in the group of patients with proven HCC progression, this valuer was 7.3±1.9 ng/mL (p=0.002).

When studying the effect of blood CyA concentration on postoperative progression, we found that in the group of patients within in the Milan criteria with a relapse-free course, the CyA concentration was 146±47 ng/mL. In the only patient who had HCC progression in this group, the mean CyA concentration was 180 ng/mL. Significant statistical differences were achieved in the group of patients with a baseline tumor volume of $T_{3–4.}$ Thus, in patients with a relapse-free course, the concentration was 89±32 ng/mL, while in the group of patients with confirmed HCC progression, this indicator was 154±48 ng/mL (p=0.02).

Based on the above, it can be assumed that minimizing the immunosuppressive therapy to a blood level of no more than 6.0 ng/mL for Tac and no more than 100 ng/mL for CyA can reduce the incidence of post-transplant progression of HCC. To verify the correctness of this hypothesis, we combined all patients with a baseline concentration of CyA lower than 100 ng/mL and that of Tac lower than 6.0 ng/ml into one group. The second group of patients consisted of cases where the mean concentration of CyA or Tac exceeded 100 ng/mL and 6.0 ng/mL, respectively. So, the incidence of HCC progression was 22.2% in the first group, and 44% in the second group that was 2 times higher (p=0.02; Table 2). Similar calculations were made separately for patients who did not meet the Milan criteria. HCC progression rates in the groups were 28.5% and 69.7%, respectively (p=0.001; Table 3).

Table 2. The impact of the blood level of calcineurin inhibitor on the rate of hepatocellular carcinoma progression after liver transplantation

Blood level of calcineurin inhibitor	PCC relapse (n)	Relapse-free course (n)	Total (n)
Tac>6.0 ng/mL; CyA>100 ng/mL	26	33	59
Tac≤6.0 ng/mL; CyA≤100 ng/mL	10	35	45
Total	36	68	104

Table 3. The impact of the blood level of calcineurin inhibitor on the rate of hepatocellular carcinoma progression after liver transplantation in patients outside the Milan criteria ($T_{3–4}$)

Blood level of calcineurin inhibitor	HCC relapse (n)	Relapse-free course (n)	Total (n)
Tac>6.0 ng/mL; CyA>100 ng/mL	23	10	33
Tac≤6.0 ng/mL; CyA≤100 ng/mL	8	20	28
Total	31	30	61

The hypothesis about the effect of minimizing immunosuppression on postoperative HCC progression was subjected to ROC analysis, which showed that the sensitivity and specificity of HCC relapse occurrence at Tac concentrations in blood above 6.0 ng/mL would be 73% and 60%, respectively. Those parameters for CyA concentrations above 110 ng/mL would be 88% and 80%, respectively (Fig. 3, 4).

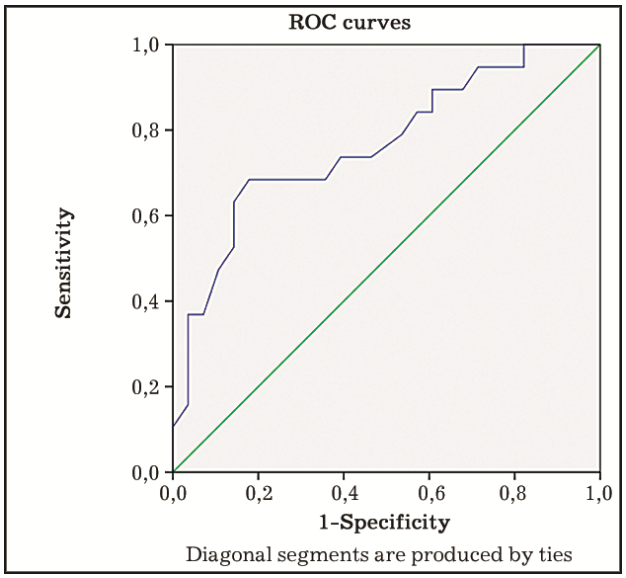

Fig. 3. ROC curve of hepatocellular carcinoma progression to blood level of tacrolimus for patients outside the Milan criteria. Area under the curve (AUC) 0.76; p=0.02

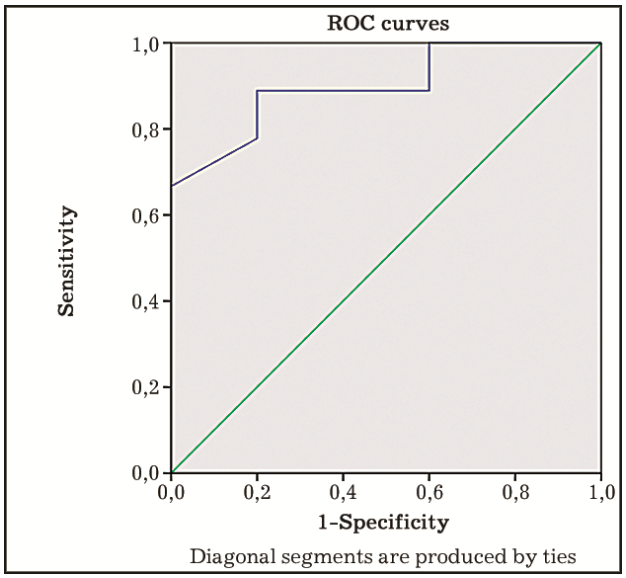

Fig. 4. ROC curve of the hepatocellular carcinoma progression to the cyclosporine A blood level for patients outside the Milan criteria. Area (AUC) under the curve 0.9; p=0.016

Finally, we tested our hypothesis by evaluating RFS values by distributing the patients into four groups based on tumor lesion ($T_{1–2}$ and $T_{3–4}$) and mean CNI concentrations (Fig. 5; Table 4). These results supported the assumption that minimizing IT increases the duration of the relapse-free period, especially in patients with advanced HCC (T_{3-4}). Differences in the groups were statistically significant (p=0.00003) mainly for the 4th group of patients. The statistical significance between groups 3 and 4 was p=0.034.

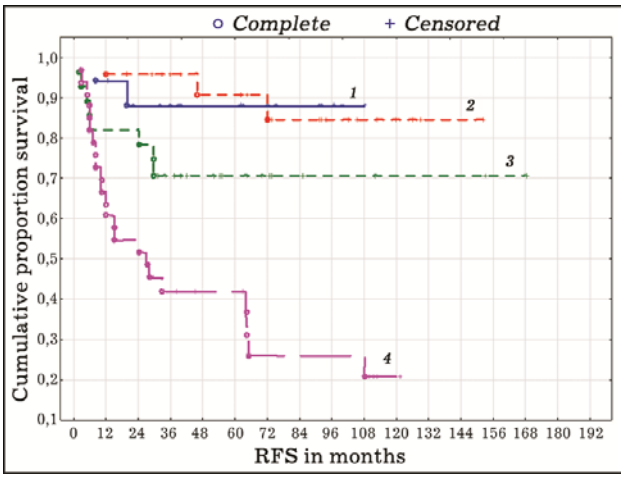

Fig. 5. Recurrence-free survival after liver transplantation in patients with hepatocellular carcinoma and blood level of calcineurin inhibitors: 1 - Milan criteria, Tac concentration ≤ 6.0 ng/mL; CyA ≤ 100 ng/mL; 2 – Milan criteria, Tac > 6.0 ng/mL; CyA > 100 ng/mL; 3 – outside the Milan criteria, Tac ≤ 6.0 ng/mL; CyA ≤ 100 ng/mL; 4 – outside the Milan criteria, Tac > 6.0 ng/mL; CyA > 100 ng/mL

Table 4. The impact of the blood level of calcineurin inhibitor on the rate of hepatocellular carcinoma progression after liver transplantation in patients

Patient groups	RFS			Median RFS, months
	1-year RFS, %	3-year RFS, %	5-year RFS, %	
Milan criteria, Tac concentration ≤ 6.0 ng/mL; CyA ≤ 100 ng/mL	96	96	90	62
Milan criteria, Tac>6.0 ng/ml; CyA > 100 ng/mL	93	88	88	91
Outside Milan criteria, Tac ≤ 6.0 ng/mL; CyA ≤ 100 ng/mL	82	70	70	40
Outside Milan criteria, Tac>6.0 ng /mL; CyA>100 ng/mL	60	42	42	27

Discussion

Immunosuppressive therapy is an integral and constant component of the post-transplant period. Common components of IT in liver

transplant recipients, CyA and Tac, are the most effective agents in the prevention of acute cellular rejection. On the other hand, we must not forget that the main role of antitumor protection belongs to body's natural immunity; and its artificial (and, alas, still inevitable) suppression can provoke the progression of oncological process [10]. The role of CNIs as a mediator of oncological disease progression is covered in detail in the studies of M. Rodríguez-Perálvarez, M. Vivarelli [5–9]. In particular, M. Vivarelli et al. note that the excess of CyA above 170 ng/mL and Tac above 8.6 ng/mL after liver transplantation is an independent risk factor (including according to the results of multivariate analysis) of HCC progression [5-6]. M. Rodríguez-Perálvarez et al. noted that exceeding the baseline tacrolimus concentration of 10 ng/mL during the first post-transplant month is an independent risk factor for HCC progression [8]. It is important to note that the positive effect of minimizing IT on the duration of OS has also been observed in patients with confirmed HCC progression [11]. J. Lerut has noted that both the type of immunosuppressive drug or IT regimen, and more importantly, the overall immunosuppressive burden matter in HCC relapse [10].

The results of our study suggest that the role of IT in the progression of HCC persists throughout the postoperative period. And the greater the initial tumor load is, the lower IT exposure should be. Worthwhile to note that we did not find a difference in the development of HCC relapse between the Tac and CyA administration. From the results of our study, it follows that for patients undergoing liver transplantation for HCC, the preferred baseline concentrations for Tac and CyA are the values not exceeding 6.0 ng/mL and 100 ng/mL, respectively. Compliance with this requirement reduces the risk of postoperative progression of HCC by twice, at least, which turned out to be especially relevant in patients who had an initial tumor load that

exceeded the Milan criteria. The results obtained, which made it possible to achieve 70% of the 5-year RFS in a group of patients with unfavourable prognosis in terms of oncprogression risk, currently form the basis of the IT protocol for patients with HCC operated on at our Center.

The choice of the optimal immunosuppressive therapy regimen requires careful and regular monitoring of laboratory parameters. The ideal scheme of immunosuppressive therapy should be represented by the minimum effective dose of an immunosuppressive agent or their combination and meet safety requirements, which would mean maintaining a balance between the prevention of acute cellular rejection and an increased immunosuppressive load that provokes the progression of the oncological process. Minimizing the exposure of CNIs is of paramount importance for patients with tumor burden exceeding T_2. In this regard, the most promising scheme of immunosuppressive therapy in patients with hepatocellular carcinoma seems to be a combination of low doses of CNIs and m-TOR receptor inhibitors [7, 10, 12].

Conclusion

Exposure to calcineurin inhibitors with blood concentrations above 6.0 ng/mL for tacrolimus and 100 ng/mL for cyclosporin A is associated with a two-fold increase in the incidence of hepatocellular carcinoma progression after liver transplantation in patients with initially its locally advanced type (the tumor size > T2).

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