American Journal of Biomedical and Life Sciences
2019; 7(4): 73-78
http://www.sciencepublishinggroup.com/j/ajbls
doi: 10.11648/j.ajbls.20190704.11
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

Study of the Exogenous Peptide Effect on the TGF-β1 Expression-A Risk Factor for the Hepatocellular Carcinoma Recurrence

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To cite this article:
Vadym Shypulin, Oksana Stoliarova, Volodymyr Konovalenko, Oleksiy Tatskyy, Gennadiy Didenko, Sergii Konovalenko. Study of the Exogenous Peptide Effect on the TGF-β1 Expression-A Risk Factor for the Hepatocellular Carcinoma Recurrence. *American Journal of Biomedical and Life Sciences.* Vol. 7, No. 4, 2019, pp. 73-78. doi: 10.11648/j.ajbls.20190704.11

Received: June 20, 2019; **Accepted**: July 22, 2019; **Published**: August 6, 2019

Abstract: An article deals with the influence of the transforming growth factor beta-1 (TGF-β1) on the carcinogenesis of hepatic cells, as well as attention is given to the role of this factor in the hepatocellular carcinoma (HCC) progression. We monitored two groups of patients: in the treatment group, patients were administered cancer vaccine therapy and anti-relapse immune corrector Arecur®-a complex of exogenous peptides with anti-inflammatory, anti-infective and regenerating properties; in the reference group, patients were not administered immune corrector. The study showed that the cancer vaccine has a positive effect on the activity of T-cell immunity and interleukin-2 expression level. The result of the inclusion of anti-relapse immune corrector Arecur® in the patients' management regimen was the reduction of TGF-β1 expression. The lower recurrence rate of HCC in the treatment group suggests that Arecur® may prevent possible recurrences and metastasis of HCC by limiting TGF-β1 expression. Future researches of anti-relapse immune correction regimens for patients with hepatic tumours may improve the therapeutic strategy and prevention of the disease progression in the long term. In addition, immune correction using exogenous peptides is likely capable of preventing the malignancy in patients with chronic hepatitis and liver cirrhosis.

Keywords: Hepatitis, TGF-β1, Hepatocellular Carcinoma, Anti-relapse Immune Correction, Exogenous Peptides

1. Introduction

One of the major complications in patients with hepatic fibrosis is the development of hepatocellular carcinoma (HCC). Understanding of the molecular mechanisms leading to HCC is essential in the development of novel pharmacological agents that serve either to prevent or mitigate the effects of this malignant neoplasm. Transforming growth factor-beta (TGF-β) and its isoforms trigger signal cascade, which is closely related to hepatic fibrosis, hepatic cirrhosis and subsequent progression to HCC. Because of its role in these stages of disease progression, TGF-β, apparently, plays a unique role in the molecular pathogenesis of HCC. Recent studies have shown that the inhibition of TGF-β signals leads to multiple synergistic effects, which can probably improve the clinical outcome in HCC [1, 2].

Hepatocellular carcinoma occurs in patients as a consequence of the long-existing liver diseases, including viral hepatitis, alcohol abuse or metabolic disorder. In such liver diseases, TGF-β plays an important role in the

formation of growth-supporting microenvironment to tumour cells and stimulation of the epithelial-mesenchymal transition (EMT) [3, 4].

It should also be noted that HCC is the most common histological subtype of liver cancer. It accounts for 70-85% of all cases of primary liver cancer in the world. Mortality from HCC is high because of early metastasis [5, 6]. Cell adhesion, progressively increasing migration and invasiveness are key factors in early tumour metastasis [7, 8]. Consequently, understanding the cellular adhesion, migration, and invasion is useful for molecular diagnostics and prevention of HCC metastasis.

The majority of tumours acquire metastatic phenotype during progression, developing the ability to penetrate into the surrounding tissues to migrate and grow in remote locations through an EMT-dependent process. The accumulated data show that TGF-β1 has tumour effects at an advanced stage, in particular, to stimulate EMT and dissemination of cancer [9-11] It is also necessary to add that transcription factor glioma-associated oncogene 1 (GLI 1) modulates EMT through direct up-regulation of SNAI1 and serves as a downstream effector of the TGF-β1 pathway. Overexpression of GLI 1 increased proliferation, viability, migration, invasion, and colony formation by HCC cells. Increased GLI 1 expression in HCC tissues was significantly correlated with more rapid tumor recurrence after surgical resection of the primary tumor [12].

The role of viruses and toxic substances in the malignancy of normal cells is known. This process is governed by the trigger peptides [13-16]. For example, under the conditions of chronic hepatocellular damage, the significance of a specific protein, glypican-3, was determined. In case of the alcoholic liver disease, toxic and viral hepatitis, the role of this peptide on cell membranes is recognized as determining in the development of the disease. And the higher the glypican activity, the greater the probability of hepatic cell neoplastic transformation [17-19]. The expression of glypican-3 was upregulated in HCC tumor tissues compared with normal and benign liver diseases and contributed to promoting the growth of HCC by stimulating Wnt signalling. [20].

In preclinical and clinical trials, success has been demonstrated in using a combination of cancer vaccines (CV) with chemotherapy to achieve a synergistic effect, even if the dose and regimen for administering the appropriate agents needed to be optimized. It was demonstrated that some drugs, for example, doxorubicin and cyclophosphamide induce immunological destruction of tumour cells, docetaxel increases the expression of tumour-associated antigens, HLA-peptide complexes, and in such a manner sensitizing tumour for vaccine induced T-cell killing [21]. The antitumour activity of the vaccines created on the basis of chicken embryo proteins (CEP) of 7 days of gestation and B. subtilis B-7025 peptides with molecular mass of 18.5 and 70 kDa as adjuvants was evaluated [22-24].

Currently, more and more special biological products, contributing to the liver function normalization, are becoming widespread [25, 26]. Some of them are used for prevention and treatment of liver cancer, including hepatocellular carcinoma [27-29]. As already mentioned above, the trigger factor TGF-β1 is played a pivotal role in the development, progression, as well as recurrence of hepatocellular carcinoma. Therefore, it is of interest to study the question of how vaccine therapy with combination of anti-relapse immune correction affects the expression of this factor in patients with HCC, and therefore the possible risk of disease recurrence.

2. Objective

To explore the possibility of the vaccine therapy influence combining with the complex of exogenous peptides Arecur® on the risk level of possible hepatocellular carcinoma recurrence.

3. Materials and Methods

In the laboratory of oncoimmunology and design of cancer vaccines of the Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology during the period of 2013/2018, after special treatment of hepatocellular carcinoma-surgical removal of the tumour and chemotherapy-96 patients were administered vaccine therapy. A complex of exogenous peptides anti-relapse immune corrector Arecur® was administered to 64 patients as a means for antitumour immune surveillance enhancement.

Arecur® is a preparation that contains the substance camelyn consisting of biologically active substances, including exogenous peptides, derived from haemocytes and secretory cells of the Apis mellifera bee. The substance camelyn, which is a part of various drugs, has demonstrated its immunoprotective properties, including the ability to enhance antitumour immunity and prevent the cancer recurrence [30]. The substance camelyn inter alia contains defensin-1 and royal jelly-1 protein (RJP-1). The exogenous peptides defensin-1 and RJP-1 are similar in structure and function to human anti-infective proteins. These substances help the immune cells to detect viruses and bacteria, as well as are able to activate macrophages that clean the liver from failed cells and toxins. Therefore, it is logical to assume that getting into the liver, exogenous peptides activate metabolic processes in it and trigger internal defence mechanisms against infections and toxins. It is quite reasonable that the reduction of infectious load and a decrease in inflammation lead to the anti-inflammatory cytokine synthesis and reduced activity of factors provoking the growth of fibrotic fibers in the liver.

In the treatment group, the patients were administered cancer vaccine therapy along with Arecur® according to such dosage regimen as: 1 capsule 2 times a day for sixty days. The patients of reference group received only vaccine therapy without immune corrector. Before treatment initiation, patients were screened for the immunological indicators CD3, CD4, CD8, the assessment of the IL-2

production and TGF-β1 expression. Monitoring of these indicators in both groups was provided on the 30th and 60th days after the initiation of treatment with Arecur®, and also one month after the termination of treatment course.

The statistical significance of indicator changes over time was estimated by using the Wilcoxon's test. Survival analysis was conducted according to Kaplan-Meier.

4. Results and Discussion

Before using the vaccine and initiation of treatment with Arecur®, the immunological parameters CD3, CD4, CD8, the IL-2 production and TGF-β1 expression were determined in patients. At the time of the first screening, the quantitative indicators of T-cells were recorded at the lower limit of normal on average in the patients of treatment group and were as follows: CD3-692.7±126.4 cells/μL, CD4-594.4±61.1 cells/μL, CD8-236.3±46.9 cells/μL. After thirty days from the introduction of the cancer vaccine and against the background of the preparation Arecur® administration, there were a statistically significant ($p<0.05$) increase in the concentration of CD3-CD8 T-cell subpopulations, which is also manifested on the 60th day and, subsequently, a month after the completion of the first treatment course using Arecur® (Table 1). The interleukin-2 expression in the patients of treatment group at the study initiation visit was on average 2.0±0.18 ng/mL, gradually increased from 30th to 60th day of follow-up, exceeded the normal level, but then again demonstrated the tendency to decline. In the patients of reference group at the 1st visit, the quantitative indicators of T-cells were as follows: CD3-599±62 cells/μL, CD4-602±22 cells/μL, CD8-266±55 cells/μL. After thirty days from the introduction of the cancer vaccine, an increase in the T-cell concentration was recorded as well, which also continued after 60 days and then after another 30 days.

The interleukin-2 concentration in the patients of comparison group prior to the vaccine introduction was 3.16±0.68 ng/mL, and a month after the vaccine administration, it increased to 7.18±1.06 ng/mL. On the 60th day of follow-up, the interleukin level in patients of this group decreased and almost returned to the baseline values after a month (see Table). That is, it was observed how the cancer xenogeneic vaccine stimulates cellular immunity and induces the interleukin-2 activity-this effect persisted for at least 3 months.

Table 1. *Quantitative indicators of CD3-CD8 subpopulations of T-lymphocytes and the interleukin-2 expression level in the patients of treatment and reference groups.*

	Indicators		Before treatment	30th day	60th day	30 days after treatment
Treatment group	CD3 abs. cells/μL	M±SD	692.7±126.4	808.1±146.6	867.3±160.9	785.1±145.9
		Δ (95%CI)	-	+115.4 (67-163)*	+174.6 (124-225)*	+92.4 (44-140)*
	CD4 abs. cells/μL	M±SD	594.4±61.1	662.8±68.0	706.1±72.4	678.6±69.0
		Δ (95%CI)	-	+68.4 (45-91)*	+111.7 (88-135)*	+84.1 (61-107)*
	CD8 abs. cells/μL	M±SD	236.3±46.9	277.9±55.1	345.2±68.5	336.0±68.4
		Δ (95%CI)	-	+41.6 (24-59)*	+108 (88-129)*	+99.7 (79-120)*
	IL-2 pg/mL	M±SD	2.0±0.18	7.9±2.17	7.11±1.86	4.14±1.06
		Δ (95%CI)	-	+5.9 (5.3-6.4)*	+5.1 (4.6-5.5)*	+2.1 (1.9-2.4)*
	TGF-β1 ng/mL	M±SD	212.5±23.5	298.0±33.2	191.0±16.5	112.6±14.8
		Δ (95%CI)	-	+85.6 (75-96)*	-21.4 (14.3-28.5)*	-99.8 (93-106)*
Reference group	CD3 abs. cells/μL	M±SD	595.5±63.5	704.6±73.9	800.7±83.7	661.8±69.6
		Δ (95%CI)	-	+109.1 (74-143)*	+205.2 (168-242)*	+66.3 (32-99)*
	CD4 abs. cells/μL	M±SD	601.2±39.0	649.2±41.3	747.2±58.2	691.3±53.7
		Δ (95%CI)	-	+48 (27-68)*	+146.0 (121-170)*	+90.1 (66-113)*
	CD8 abs. cells/μL	M±SD	271±54.1	298.8±60.5	291.2±59.4	304.9±59.7
		Δ (95%CI)	-	+27.8 (-1- +56)	+20.2 (-8 - +48)	+33.9 (5-62)*
	IL-2 pg/mL	M±SD	3.14±0.68	7.17±0.94	5.93±0.79	3.42±0.79
		Δ (95%CI)	-	+4.0 (3.6-4.4)*	+2.8 (2.4-3.2)*	+0.28 (-0.9 - +0.6)
	TGF-β1 ng/mL	M±SD	262.0±18	309.6±17.2	246.5±18.5	248.8±14.8
		Δ (95%CI)	-	+47.6 (39-57)*	-15.5 (6.2-24.8)*	-13.2 (4.0-22.4)*

M±SD-mean and standard deviation; Δ (95%CI)-different in comparison with initial level (95% Confidential Interval); * - different is statistically significant ($p<0.05$, Wilcoxon test).

The transforming growth factor beta-1 (TGF-β1) expression level was determined in both groups of patients. It is important to note that both in the treatment group and in the reference group before vaccination, the TGF-β1 concentration was approximately at the same level and increased markedly on the 30th day of follow-up: from 212.5±23.5 to 298.0±33.2 and from 262.0±18 to 309.6±17.2 ng/mL, respectively. But most importantly, in the treatment group, by the 60th day of follow-up, expression of the factor again decreased by -21.4 (95% CI: 14.3-28.5) ng/mL compared with the baseline level ($p<0.05$) and the reducing trend still retained in a month and made up -99.8 (95% CI: 93-106) ng/mL ($p<0.05$). In the comparison group, the TGF-β1 level on the 60th day of follow-up also significantly decreased -15.5 (95% CI: 6.2-24.8) ng/mL ($p<0.05$) in comparison with the baseline level; however, after another 30 days, the average figures ceased going downward and even slightly increased (Figure 1).

During the year, out of 64 patients who were administered the vaccine along with Arecur®, three patients had local recurrences of hepatocellular carcinoma and one patient had a single metastasis to the lumbosacral region of the spine.

Thus, the disease progression was observed in the treatment group in 6.3% of patients. In the comparison group, the HCC recurrences were recorded in six patients and the distant metastases were diagnosed in six patients; i.e., the disease progression occurred in twelve (37.5%) patients, which indicates a statistically significant recurrence risk reduction by 89.9% during the first year of follow-up against the background of Arecur® administration-OR=0.111 (0.02–0.43), p=0.0002.

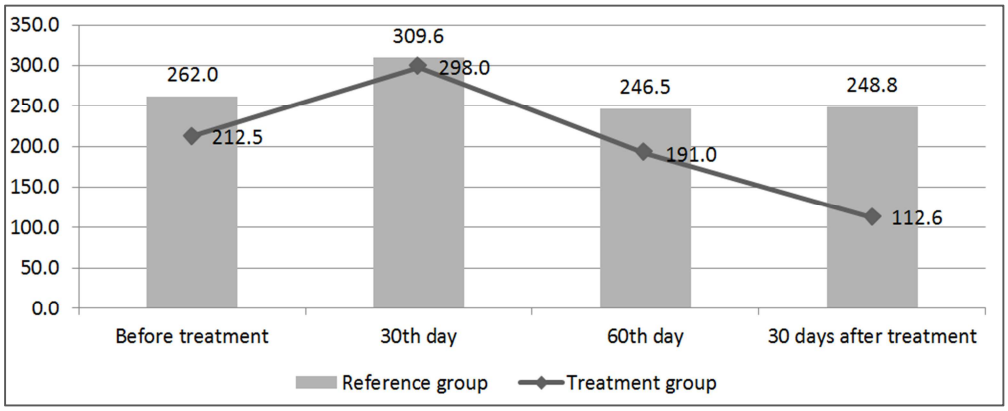

Figure 1. *The TGF-β1 expression level (ng/mL) in patients of the treatment and reference groups over time.*

As a trigger factor for launching the connective tissue formation in the liver, TGF-β1 is at the same time recognized as a key influence agent on the progress of hepatocellular carcinoma. [31-33]. Moreover, a retrospective analysis of clinical studies has shown that HCC patients with higher TGF-β1 expression presented a shorter overall survival than those with lower TGF-β1 expression (HR = 1.42, $p < 0.05$). [34] Because of the critical role of TGF-β1 in the process of metastasis, in recent years, research continues on possible paths of influence on biochemical cascades involving this regulatory protein. [35, 36]. It is assumed that exogenous peptides of natural origin can activate new interactions in the hepatic cells, violating the cascades of reactions involving TGF-β1 and glypican-3 and thereby improving the disease prognosis. [37]. These assumptions need future studies and researches will continue.

The accumulated 5-year treatment experience of hepatocellular carcinoma using anti-relapse immune corrector Arecur® in the comprehensive treatment allowed the conduction of a comparative analysis of the survival rates among the patient populations comparable with the disease severity with and without Arecur® administration. Stratification of patients was carried out with a baseline evaluation of the general condition according to the ECOG scale (0-1) and class A-B Child-Pugh scores. The comparative analysis included 88 patients with the experience of immune corrector Arecur® and 96 patients in the comparison group. The results of the survival analysis are shown in Figure 2.

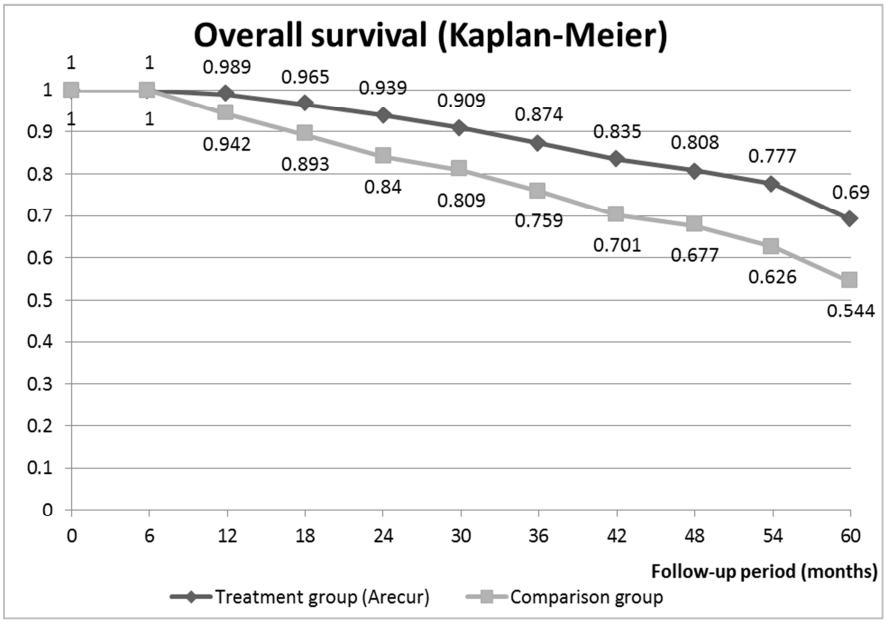

Figure 2. *Kaplan-Meier survival analysis over a 5-year period.*

The overall survival over a 5-year period was 69% in the treatment group (using Arecur® as recommended) and 54.4% in the reference group. Differences between groups are statistically significant and indicate a decrease in the relative risk of mortality in the treatment group-hazard ratio (HR) with 95% confidence interval: 0.49 (0.27-0.86), p=0.013. The median survival in the treatment group was 67 months, and in the control group – 61 months.

5. Conclusions

1. The obtained data indicate the positive influence of immune corrector Arecur® on the recurrence-free course of HCC in the studied group of patients. In addition, the study of the effects of the alimentary administration of exogenous peptides requires further research, which can improve the treatment strategy and hepatocellular carcinoma prevention, including in patients with chronic hepatitis and liver cirrhosis.
2. In the group of patients who, besides vaccination, were administered Arecur®, a decrease in TGF-β1 expression was observed, which probably contributed to a risk reduction in the recurrence and metastasis of HCC. The TGF-β1 activity reducing also creates conditions for preventing the progression of hepatitis and cirrhosis.
3. The use of Arecur® increases the median of patient survival.
4. The cancer vaccine has a positive effect on the activity of T-cell immunity and the interleukin-2 expression level.

For Information

The authors report no conflicts of interest in this work.

Acknowledgements

The authors express thanks to the International Institute of Biotherapy for providing samples of the exogenous peptide complex Arecur® for inclusion in the patients' management regimen, as well as for support in conducting research.

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