

Research Article

Therapeutic Effect of Bicyclol Coupled with Aspirin Nanopreparation on Liver Function Damage Caused by Epstein-Barr Virus Infection in Children

Wenxia Yi ¹, Chunyan Zhang,² Chunyan Xu,¹ Wenqiu Tian,¹ Fei Xie,¹ and Bin Yue¹

¹Department of Pediatrics, Cangzhou Central Hospital, Hebei Province, China

²Department of Neonatology, Cangzhou Central Hospital, Hebei Province, China

Correspondence should be addressed to Wenxia Yi; jmxx1259@tom.com

Received 30 December 2021; Revised 25 January 2022; Accepted 31 January 2022; Published 18 March 2022

Academic Editor: Palanivel Velmurugan

Copyright © 2022 Wenxia Yi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aim of study effective treatment is to reach the appropriate part of the body and then maintain the required drug concentration at sufficient intervals in order to achieve the clinical therapeutic effect. *Problem of the Study.* Biological barrier hinders the flow of most drugs from the blood to the focus. *Methods of the Study.* A novel nanopreparation for inhibition of Epstein-Barr virus (EBV) was developed based on poly (lactic-co-glycolic acid) (PLGA) nanodrug carriers loaded with bicyclic alcohol (BA) and aspirin (Asp) (PLGA-BA/Asp). Among them, the addition of PLGA can effectively improve the low solubility of BA and poor oral availability. Results have revealed that PLGA-BA/Asp is a multifunctional therapeutic drug that integrates antiviral, treatment of liver damage, and regulation of the immune system. This discovery provides a new treatment method for the treatment of liver injury in children with EBV infection.

1. Introduction

Epstein-Barr virus (EBV) is a common lymphotropic virus in the γ subfamily of sporrash virus, which is mainly transmitted by blood transmission and saliva transmission. EBV usually enters the body, although part of it is cleared by the immune system, but some of it still enters the body's lymphocytes, disrupts the body's immune system, and multiplies wantonly in the body [1–3]. Under normal circumstances, EBV will only lead to elevated body temperature and partial inflammation, and in a few cases will lead to tissue cell proliferation, resulting in tissue and organ damage. Meanwhile, due to the incomplete development of children's immune system, children are more likely to suffer from tissue and organ damage after being infected with EBV [4]. Its clinical manifestations are basically divided into infectious mononucleosis (IM), chronic active EBV infection (CAEBV), EBV-associated haemophilic lymphohistiocytosis (EBV-HLH), and related tumor diseases [5, 6]. Among

them, IM is a benign disease, which is usually relieved or cured in about a week, such as fever, tonsillitis, enlarged lymph nodes, nonspecific congestive rash, and hepatosplenomegaly [7, 8]. CAEBV is a kind of systemic lymphoid tissue hyperplasia, which usually lasts for more than half a year. It is characterized by fever, hepatosplenomegaly, and lymphomegaly with normal immune function [9–11]. EBV-HLH is mainly caused by EBV infection of T-cells or NK cells and is characterized by excessive inflammatory diseases such as hepatosplenomegaly, decrease of blood cell count, abnormality of central nervous system, hypertriglyceridemia, and hypofibrinogenemia, which can seriously endanger patients' lives [12–14]. In addition, EBV can transform and proliferate B lymphocytes and form malignant tumors.

In almost all childhood diseases caused by EBV infection, patients have symptoms of liver injury [15]. Although EBV cannot directly infect hepatocytes, CD8⁺ T-cells infected by EBV can be captured by the liver, resulting in

TABLE 1: General patient data analysis.

		PLGA-BA/Asp group (n = 81)	Control group (n = 76)
Sex	Male	42	37
	Female	39	39
Age (years)		6.51 ± 2.63	6.74 ± 2.11
Weight (kg)		25.36 ± 2.37	25.53 ± 1.96

liver injury [16–18]. This phenomenon suggests that researchers should pay attention to the treatment of liver injury in children caused by EBV infection.

Bicyclol alcohol (BA) is a new enzyme-lowering drug for the treatment of liver injury, which can effectively prevent liver fibrosis, promote liver cell regeneration, reduce the activities of blood transaminase and adenosine triphosphatase (ATP), improve the function and structure of liver cells, and inhibit the formation of mitochondrial peroxy liposomes and triglyceride accumulation in liver cells [19]. It is effective in the treatment of chronic hepatitis and liver cirrhosis. At the same time, it can help youngsters with the symptoms of liver damage caused by EBV infection, although it is unclear if it has inhibitory properties. It has an effect on the EBV virus. In addition, BA has low solubility and poor oral availability as a common tablet, so it is very important to find a dosage form that can improve its solubility. Aspirin (Asp), also known as acetylsalicylic acid, is a derivative of salicylic acid, and is widely used in the treatment of cardiovascular diseases [20, 21]. In addition, its pharmacological effects are mainly antipyretic, analgesic, anti-inflammatory, antithrombus, and reducing the risk of tumorigenesis [22]. Li et al. found that Asp can effectively improve the liver fibrosis induced by thioacetamide in rats, which proves that Asp may have a therapeutic effect on liver injury to some extent [23]. The combination of BA and Asp is expected to enhance its therapeutic effect on liver injury caused by EBV infection in children and improve its fever and inflammation to some extent.

Nanopreparation is a new type of pharmaceutical technology, which can be used to treat diseases by constructing nanocarriers to carry active drugs. According to the different choices of nanocarriers, it can usually improve the biosafety, biodegradability, and sustained release and targeting of active drugs, and it is the most widely studied new drug delivery system. Polylactic acid glycolic acid copolymer (PLGA) is a new material made by random polymerization of lactic acid and glycolic acid. As a degradable functional polymer organic compound, it has good biocompatibility, nontoxicity, and biodegradability. And the degradation rate is controllable and the performance of encapsulation and film formation is good, so it is widely used in pharmaceuticals, medical engineering materials, and modern industrial fields. It has been made into artificial catheters, drug slow-release carriers, and tissue engineering scaffold materials. At the same time, PLGA as a drug carrier can effectively improve the low solubility of BA and poor oral availability [24–26]. The contribution of study prepared PLGA-BA/Asp nanopreparation with PLGA as a nanocarrier, BA and

Asp, at the same time, and studied its therapeutic effect on liver injury caused by EBV in children.

The effective treatment is to reach the appropriate part of the body and then maintain the required drug concentration at sufficient intervals in order to achieve the clinical therapeutic effect.

2. Materials and Methods

2.1. Reagent. PLGA, BA, Asp, dichloromethane, pyridine, 4-dimethylaminopyridine, petroleum ether, ethyl acetate, dimethyl sulfoxide (DMSO), tetramethyl thiazolyl blue (MTT), and acetonitrile were purchased from Shanghai Tongwei Biotechnology Co., Ltd. (Shanghai, China). Poloxamer is provided by Wuhan Weiselman Biological Engineering Co., Ltd. (Hubei, China). DMEM medium and fetal bovine serum were purchased from Sichuan Weikeqi Biotechnology Co., Ltd. (Sichuan, China). Ganciclovir antiviral injection is provided by Shandong Phoenix Pharmaceutical Co., Ltd. (Shandong, China). Placental polypeptide injection was purchased from Guizhou Taibang Biological Products Co., Ltd. (Guizhou, China). Compound glycyrrhizin capsules were purchased from Beijing Cain Technology Co., Ltd. (Beijing, China).

2.2. Instrument. The 5.0L automatic rotary evaporator Nmur2100 was purchased from Tokyo Physicochemical equipment Co., Ltd. (Tokyo, Japan). ACQUITY UPLC HSS high strength silica gel chromatographic column is provided by Waterworld (Massachusetts, USA). C1650R-230 V micro-high-speed freezing centrifuge was purchased from Beijing Leiputer Scientific instrument Co., Ltd. (Beijing, China). Beijing Sihuan freeze dryer LGJ-30G was purchased from Sihuan Fricoyi Technology Development Co., Ltd. (Beijing, China). Hitachi high resolution cold field emission scanning electron microscope SU9000 is provided by Hitachi Company (Tokyo, Japan). Zetasizer WT potential measuring instrument is purchased from Malvern Analytical instrument Co., Ltd. (Malvern, UK). Rywald gasket carbon dioxide incubator D180Murp was purchased from Shenzhen Ryward Life Technology Co., Ltd. (Guangzhou, China). Human normal liver cells (HL-7702) are provided by Tongpai Biotechnology Co., Ltd. (Shanghai, China).

2.3. Preparation of PLGA-BA/Asp Nanoparticles

2.3.1. Preparation of BA/Asp Conjugate. BA and Asp were dissolved in 30 mL dichloromethane at a mass ratio of 2:1. After completely dissolved, 50 mg pyridine and 50 mg 4-dimethylaminopyridine were added as catalysts for overnight reaction at 25°C. After filtration, the filtrate was eluted with petroleum ether and ethyl acetate successively in the silica gel column. After the organic solvent was removed by rotary evaporator, the BA/Asp conjugate was obtained by freeze drying.

2.3.2. Preparation and Characterization of PLGA-BA/Asp Nanoparticles. Exactly 12 mg of BA/Asp conjugate and 500 mg of PLGA were dissolved in 20 mL acetonitrile solution as organic phase solution. Poloxamer 407 with 100 mg

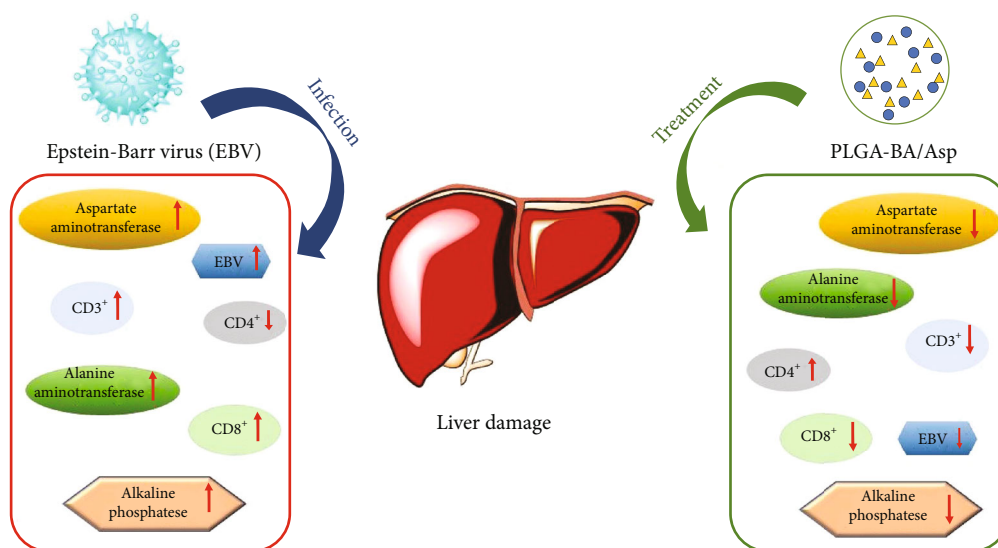


FIGURE 1: The mechanism of poly(lactic acid glycolic acid) copolymer modified bicyclic alcohol coupled aspirin nanoformulation (PLGA-BA/Asp) on EBV infection causing liver damage in children.

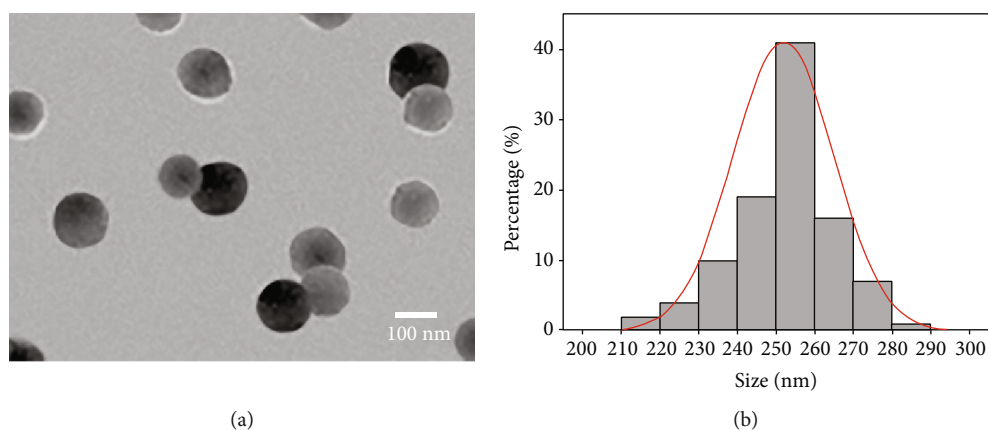


FIGURE 2: Characterization of poly(lactic-co-glycolic acid) modified bicyclic alcohol coupled aspirin nanoformulation (PLGA-BA/Asp). (a) Electron microscope characterization diagram. (b) Graph of particle size distribution.

was dissolved in 20 mL double distilled water as aqueous phase solution. The organic phase solution of 5 mL was dropped into the aqueous phase solution at the rate of 1.0 mL/min and stirred continuously for 16 h to obtain water-in-oil emulsion. PLGA-BA/Asp suspension was obtained by rotating evaporation at 40°C for 1.0 h. The supernatant was discarded by high-speed centrifugation at 15,000 rpm for 40 min, and the precipitate was washed. 200 mg sucrose was added and dissolved in 10 mL double distilled water. After completely dissolved, freeze-dried, white PLGA-BA/Asp freeze-dried nanoparticles were obtained. It was characterized by Hitachi high resolution cold field emission scanning electron microscope (SU9000) and Zetasizer WT potential meter.

2.4. Toxicity Determination of PLGA-BA/Asp Nanoparticles. HL-7702 cells were used to study the cytotoxicity of PLGA-BA/Asp nanoparticles in vitro. HL-7702 cells were

cultured in DMEM medium containing 10% fetal bovine serum in a 37°C incubator containing 5.0% CO₂. The prepared PLGA-BA/Asp nanoparticles and BA were dissolved in DMSO and diluted to 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 µg/mL, respectively, which were divided into the PLGA-BA/Asp group and BA group (the control group has the same amount of DMSO solution). HL-7702 cells were treated and sucked out after 24 h and then added with 5.0 mg/mL MTT reagent. After coculture for 4.0 h, DMSO solution was added and the cell viability was detected at 490 nm.

2.5. Study on the Application of PLGA-BA/Asp Nanoparticles in the Treatment of Liver Injury Caused by EBV Infection in Children

2.5.1. General Data Analysis. A total of 157 children with liver function injury caused by EBV infection were treated in Cangzhou Central Hospital from June 2018 to October

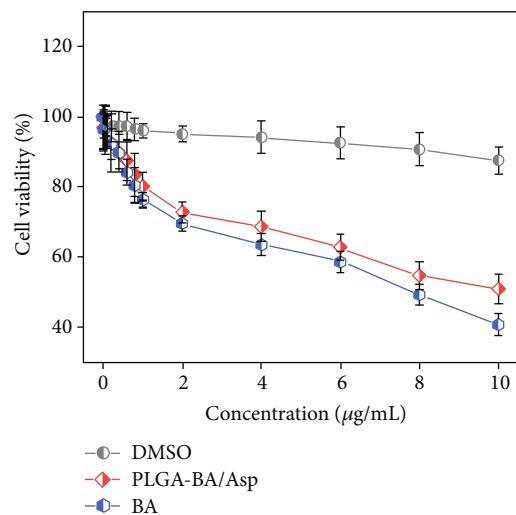


FIGURE 3: Cytotoxicity of poly(lactic-co-glycolic acid) modified bicyclic alcohol coupled aspirin nanoformulation (PLGA-BA/Asp).

2020. According to the wishes of the patients, they were divided into two groups: the PLGA-BA/Asp group ($n = 81$) and control group ($n = 76$). The specific data were recorded in Table 1. All patients and their guardians have signed informed consent.

2.5.2. Inclusion and Exclusion Criteria. The inclusion criteria are as follows. All the selected children have been confirmed by laboratory examination that their liver injury is caused by EBV infection. All the selected children had no congenital and hereditary diseases. All the selected children were not complicated with cardio-cerebrovascular diseases, and other organ diseases such as the liver, lung, and kidney.

The following are the exclusion criteria: Children who got antiviral, enzyme-lowering, and immunotherapy within one month. Hematology and medical history were used to investigate children infected with type A or hepatitis B virus, CMV, and hepatitis virus. Children with drug-induced hepatitis, autoimmune hepatitis, and alcoholic hepatitis.

2.5.3. Treatment Method. All children were given intravenous ganciclovir antiviral injection and placental polypeptide for basic immune treatment. The children in the PLGA-BA/Asp group were given 1.5 mg/kg of PLGA-BA/Asp nanopreparation daily in addition to the basic treatment, which was taken three times in the morning, middle, and evening, and its therapeutic mechanism was such in Figure 1. The children in the control group were treated with compound glycyrrhizin capsule, and the dosage was taken according to the instructions. The treatment cycle of both groups was 8 weeks.

2.5.4. Observation Index. The fasting venous blood of the two groups was taken before and after treatment, and the copy number of EBV-DNA was determined by the laboratory department of our hospital. At the same time, the levels of serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were

detected by automatic biochemical analyzer. The ratios of $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ in the peripheral blood were calculated by flow cytometry. In addition, the incidence of adverse reactions such as liver pain, vomiting, rash, and fatigue were compared between the two groups.

2.6. Statistical Analysis. All the data involved in this study were statistically analyzed by the SPSS 22.0 statistical analysis software. The measurement data in this study are all expressed by t -test in the form of average \pm standard deviation ($\bar{x} \pm SD$). The counting data were tested by χ^2 test. Among them, $P < 0.05$ indicates that it is statistically significant.

3. Results and Discussion

3.1. Characterization of PLGA-BA/Asp Nanoparticles. The prepared PLGA-BA/Asp nanoparticles were observed and characterized by Hitachi high resolution cold field emission scanning electron microscope (SU9000) (Figure 2). Its surface is a sphere of uniform size, the surface is smooth and evenly distributed. It was found that the average particle size was 253.16 ± 25.47 nm, zeta and the average potential was -27.56 ± 3.69 mV.

3.2. Cytotoxicity of PLGA-BA/Asp Nanoparticles. HL-7702 cells were used to evaluate the cytotoxicity of PLGA-BA/Asp nanoparticles in vitro (Figure 3) by MTT method. With DMSO as the control group, the toxic effect of its concentration on cells changed little, and the cell proliferation rate was more than 80%. With the increase of the concentration of PLGA-BA/Asp group and BA group, the greater the toxic effect on HL-7702 cells, the lower the ability of cell proliferation, and compared with the BA group, the effect of PLGA-BA/Asp group on cell proliferation was smaller. It is suggested that PLGA-BA/Asp nanoparticles can reduce the cytotoxicity induced by BA.

3.3. Effect of PLGA-BA/Asp Nanoparticles on EBV Load Before and After Treatment. EBV-DNA copy number was used to evaluate the change of EBV load before and after treatment (Figure 4). The results showed that the EBV load decreased significantly in both groups after treatment, especially in the PLGA-BA/Asp group. This shows that compared with the control group, PLGA-BA/Asp nanoparticles have a stronger effect on EBV and can treat children with EBV infection more effectively.

3.4. Effect of PLGA-BA/Asp Nanoparticles on Liver Function Index. ALT, ALP, and AST, as common indicators of liver function, will significantly increase after EBV infection. This study used this as an evaluation index to evaluate the therapeutic effect of two groups of drugs on liver function damage caused by EBV infection (Figure 5). The results showed that the liver function indexes of the two groups decreased significantly after treatment ($P < 0.05$). In the control group, ALT, ALP, and AST decreased to 79.87 ± 17.54 U/L, 86.17 ± 22.33 U/L, and 79.11 ± 19.32 U/L, respectively. However, the values of ALT, ALP, and AST in PLGA-BA/Asp group decreased to 51.62 ± 17.54 U/L, 62.53 ± 20.51 U/L, and

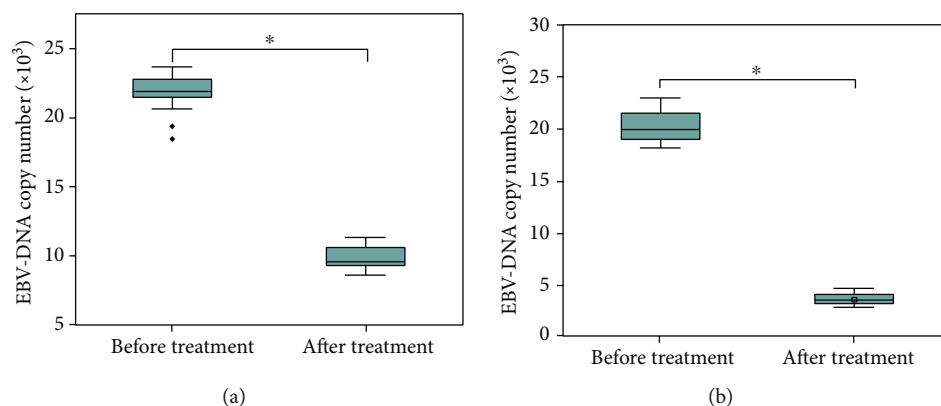


FIGURE 4: The effect of poly(lactic acid glycolic acid copolymer modified bicyclic alcohol coupled aspirin nanoformulation (PLGA-BA/Asp) on EBV load before and after treatment. (a) Control group. (b) PLGA-BA/Asp group.

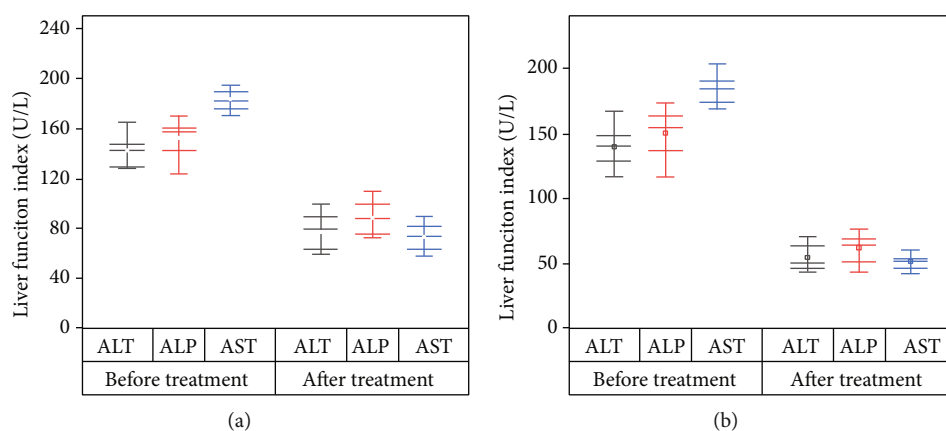


FIGURE 5: The effect of poly(lactic-co-glycolic acid) modified bicyclic alcohol coupled aspirin nanoformulation (PLGA-BA/Asp) on liver function indexes. (a) Control group. (b) PLGA-BA/Asp group.

50.67 ± 18.64 U/L. The results showed that the decrease of liver function index of PLGA-BA/Asp nanoparticles was more obvious than that of the control group, and it was more effective in the treatment of liver function damage caused by EBV infection.

3.5. Effect of PLGA-BA/Asp Nanoparticles on T-Cell Subsets Before and After Treatment. EBV infection can lead to abnormal immune function and T-cell imbalance in children. In this study, the changes of T-cell subsets before and after treatment were used to further evaluate its therapeutic effect on liver function damage caused by EBV infection (Figure 6). The results showed that PLGA-BA/Asp nanoparticles could reduce the increase of CD3⁺ and CD8⁺ levels caused by EBV infection and increase the levels of CD4⁺ and CD4⁺/CD8⁺ at the same time. In addition, the effect of PLGA-BA/Asp nanoparticles on T-cell subsets was more obvious than that of the control group.

3.6. Analysis of Adverse Reaction Rate. As vomiting, fatigue, dull pain in the liver, rash, and other adverse reactions are usually caused during treatment, the adverse reactions of the two groups were recorded in Figure 7. The total incidence of adverse reactions in the control group was

19.73%, including 2 patients with rash, 1 patient with dull pain in the liver area, 3 patients with fatigue, 2 patients with vomiting, and 7 children with other adverse reactions. The total incidence of adverse reactions in the PLGA-BA/Asp group was 9.88%, including 0 patients with rash, 1 patient with dull pain in the liver area, 3 patients with fatigue, 1 patient with vomiting, and 3 children with other adverse reactions. This shows that the incidence of adverse reactions of PLGA-BA/Asp nanoparticles is lower.

3.7. Discussion. As a kind of lymphotropic virus, the infection rate of EBV is extremely high. About 95% of human beings have been infected with EBV. The main symptoms are fever and some inflammatory reactions, and the infection mainly occurs in childhood [27–29]. Because EBV does not have the ability to damage hepatocytes, it mainly causes liver injury and cholestasis through T lymphocyte infiltration. At the same time, EBV infection can also lead to intracellular lipid peroxidation, further causing hepatocyte damage [30]. If it cannot be treated in time, severe cases can develop into hepatitis and life-threatening.

In this study, PLGA-BA/Asp nanopreparation was prepared by using PLGA as a nanocarrier, BA and Asp, at the same time. The results of in vitro toxicity test showed that

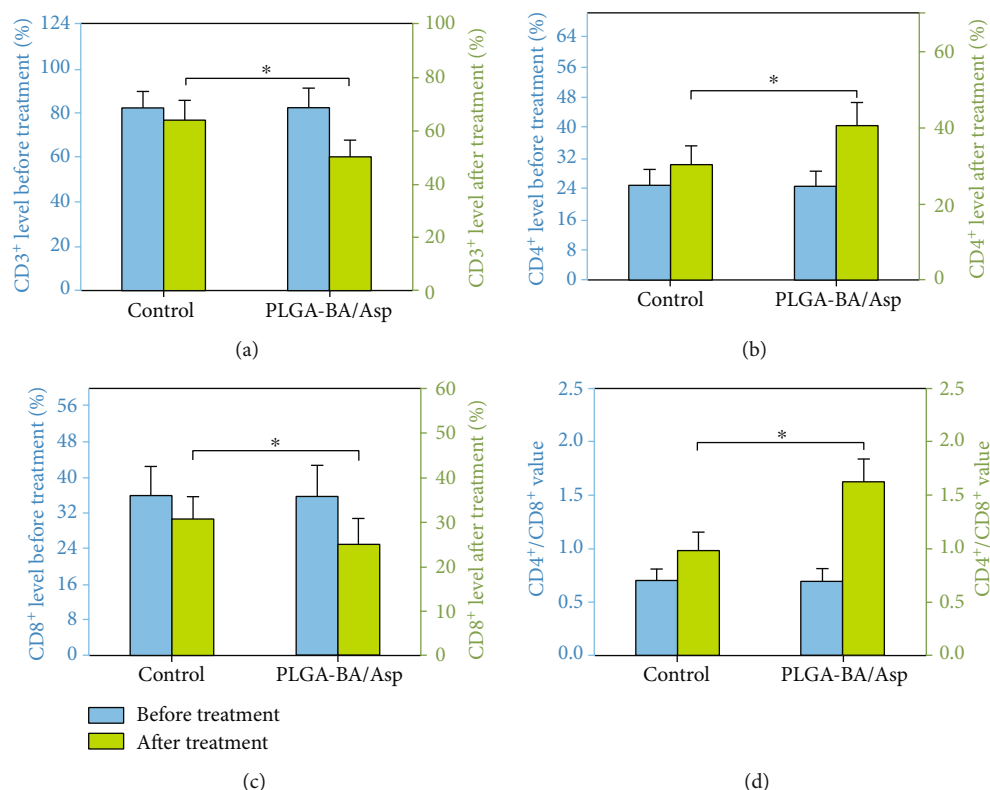


FIGURE 6: The effect of poly(lactic acid glycolic acid) copolymer modified bicyclic alcohol coupled aspirin nanoformulation (PLGA-BA/Asp) on T-cell subsets before and after treatment. (a) Changes in CD3⁺ levels before and after treatment. (b) Changes in CD4⁺ levels before and after treatment. (c) Changes in CD8⁺ levels before and after treatment. (d) Changes in CD4⁺/CD8⁺ levels before and after treatment.

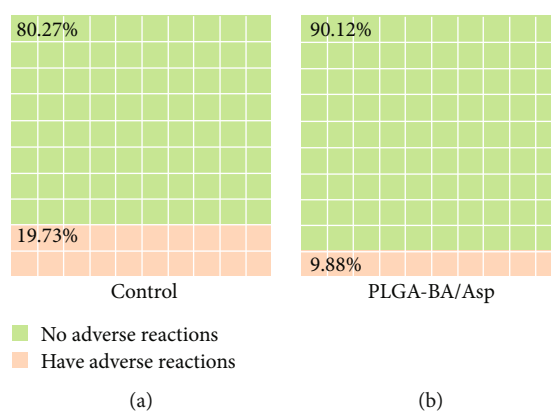


FIGURE 7: Analysis of the incidence of adverse reactions. (a) Control group. (b) PLGA-BA/Asp group.

PLGA-BA/Asp nanopreparation had lower cytotoxicity than BA which has the effect of treating liver injury. At the same time, compared with compound glycyrrhizin capsules in the control group, PLGA-BA/Asp nanoparticles could significantly reduce the number of EBV-DNA copies in the treatment of children with liver injury caused by EBV infection. Because the basic antiviral drug ganciclovir was used at the same time, it was not possible to determine whether the antiviral effect of PLGA-BA/Asp nanoparticles came from PLGA-BA/Asp nanoparticles, but both groups were treated with antiviral drugs at the same time. The decrease of EBV-DNA viral load in the PLGA-BA/Asp group was

greater than that in the control group, indicating that its antiviral effect was stronger than that in the control group. The results of liver function index further proved that PLGA-BA/Asp nanoparticles can alleviate liver injury while anti-virus.

EBV patients usually have obvious abnormal immune function, which is easy to cause Th1/Th2 cell imbalance in patients. Based on this, this study compared the level of T-cell subsets between the two groups before and after treatment. It was found that the two groups of drugs can regulate the imbalance of Th1/Th2 and enhance the ability of immune regulation, and the regulatory effect of PLGA-BA/

Asp nanoparticles is better. In addition, according to the statistics of the occurrence of adverse reactions in the two groups, it was found that the total incidence of adverse reactions in the PLGA-BA/Asp group was lower, and there was no occurrence of adverse reactions such as rash. This finding proves that PLGA-BA/Asp nanoparticles are safer and more suitable for the treatment of liver injury caused by EBV infection.

4. Conclusion

In this research, PLGA was employed as a nanocarrier, and BA and Asp were used concurrently to synthesize PLGA-BA/Asp nanopreparation. The treatment outcomes of children with EBV-induced liver injury revealed that, as compared to the control group, it had superior biological safety, immunomodulatory capacity, and liver injury treatment ability. This discovery has a high therapeutic practical value, since it provides a novel therapy for EBV-related liver damage in children.

Data Availability

The data underlying the results presented in the study are available within the manuscript.

Ethical Approval

Research experiments conducted in this article with animals were approved by the Medical Ethics Committee of Cangzhou Central Hospital following all guidelines, regulations, legal, and ethical standards as required for animals.

Conflicts of Interest

There are no conflicts to declare.

Acknowledgments

Many thanks are due to our colleagues and laboratory staff at Cangzhou Central Hospital for providing the equipment.

References

- [1] J. R. Kerr, "Epstein-Barr virus (EBV) reactivation and therapeutic inhibitors," *Journal of Clinical Pathology*, vol. 72, no. 10, pp. 651–658, 2019.
- [2] S. G. Tangye, "Genetic susceptibility to EBV infection: insights from inborn errors of immunity," *Human Genetics*, vol. 139, no. 6–7, pp. 885–901, 2020.
- [3] C. Venturini, C. J. Houldcroft, A. Lazareva et al., "Epstein-Barr virus (EBV) deletions as biomarkers of response to treatment of chronic active EBV," *British Journal of Haematology*, vol. 195, no. 2, pp. 249–255, 2021.
- [4] N. M. Ferressini Gerpe, A. G. Vistarop, A. Moyano, E. De Matteo, M. V. Preciado, and P. A. Chabay, "Distinctive EBV infection characteristics in children from a developing country," *International Journal of Infectious Diseases*, vol. 93, pp. 139–145, 2020.
- [5] J. I. Cohen, L. Dropulic, A. P. Hsu et al., "Association of GATA2 deficiency with severe primary Epstein-Barr virus (EBV) infection and EBV-associated cancers," *Clinical Infectious Diseases*, vol. 63, no. 1, pp. 41–47, 2016.
- [6] M. Imajoh, Y. Hashida, M. Murakami et al., "Characterization of Epstein-Barr virus (EBV) BZLF1 gene promoter variants and comparison of cellular gene expression profiles in Japanese patients with infectious mononucleosis, chronic active EBV infection, and EBV-associated hemophagocytic lymphohistiocytosis," *Journal of Medical Virology*, vol. 84, no. 6, pp. 940–946, 2012.
- [7] J. M. Grimm-Geris, S. K. Dunmire, L. M. Duval et al., "Screening for Epstein-Barr virus (EBV) infection status in university freshmen: acceptability of a gingival swab method," *Epidemiology and Infection*, vol. 147, pp. e140–e140, 2019.
- [8] K. Rostgaard, H. H. Balfour Jr., R. Jarrett et al., "Primary Epstein-Barr virus infection with and without infectious mononucleosis," *PLoS One*, vol. 14, no. 12, article e0226436, 2019.
- [9] N. Kobayashi, T. Mitsui, Y. Ogawa et al., "A rare case of chronic active Epstein-Barr virus (EBV) infection accompanied by the infiltration of EBV-infected CD8⁺ T cells into the muscle," *Journal of Pediatric Hematology/Oncology*, vol. 40, no. 3, pp. e171–e175, 2018.
- [10] Y. D. Wang, L. L. Wu, L. Y. Ma et al., "Chronic active EBV infection associated with NK cell lymphoma and hemophagocytic lymphohistiocytosis in a 27-year-old woman," *Medicine*, vol. 98, no. 2, p. e14032, 2019.
- [11] Y. Xing, H. M. Song, M. Wei, Y. Liu, Y. H. Zhang, and L. Gao, "Clinical significance of variations in levels of Epstein-Barr virus (EBV) antigen and adaptive immune response during chronic active EBV infection in children," *Journal of Immunotoxicology*, vol. 10, no. 4, pp. 387–392, 2013.
- [12] Y. L. Wang, J. H. Ai, Z. D. Xie et al., "IL-10-592 A/C polymorphisms is associated with EBV-HLH in Chinese children," *Hematology*, vol. 21, no. 2, pp. 95–98, 2016.
- [13] Y. Chikagawa, K. Hikishima, H. Mizumaki et al., "Resolution of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis associated with rapid immune reconstruction after a single course of CHOP therapy," *International Journal of Hematology*, vol. 112, no. 6, pp. 889–893, 2020.
- [14] S. Imashuku, "Treatment of Epstein-Barr virus-related hemophagocytic lymphohistiocytosis (EBV-HLH); update 2010," *Journal of Pediatric Hematology/Oncology*, vol. 33, no. 1, pp. 35–39, 2011.
- [15] J. H. Hu, H. Zhao, D. F. Lou et al., "Human cytomegalovirus and Epstein-Barr virus infections, risk factors, and their influence on the liver function of patients with acute-on-chronic liver failure," *BMC Infectious Diseases*, vol. 18, no. 1, pp. 1–8, 2018.
- [16] B. Chatterjee, Y. Deng, A. Holler et al., "CD8⁺ T cells retain protective functions despite sustained inhibitory receptor expression during Epstein-Barr virus infection in vivo," *PLoS Pathogens*, vol. 15, no. 5, article e1007748, 2019.
- [17] R. J. Abbott, A. Pachnio, I. Pedroza-Pacheco et al., "Asymptomatic primary infection with Epstein-Barr virus: observations on young adult cases," *Journal of Virology*, vol. 91, no. 21, article e00382-17, 2017.
- [18] A. J. J. Worth, C. J. Houldcroft, and C. Booth, "Severe Epstein-Barr virus infection in primary immunodeficiency and the normal host," *British Journal of Haematology*, vol. 175, no. 4, pp. 559–576, 2016.
- [19] N. Q. Wu, L. S. Wang, Z. Y. Han et al., "A multicenter and randomized controlled trial of bicyclol in the treatment of statin-

- induced liver injury,” *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, vol. 23, pp. 5760–5766, 2017.
- [20] A. Mogul, E. E. Leppien, E. Laughlin, and S. A. Spinler, “Aspirin for primary prevention of cardiovascular disease: a review of recent literature and updated guideline recommendations,” *Expert Opinion on Pharmacotherapy*, vol. 22, no. 1, pp. 83–91, 2021.
- [21] M. Paseban, R. M. Marjaneh, M. Banach, M. M. Riahi, S. Bo, and A. Sahebkar, “Modulation of microRNAs by aspirin in cardiovascular disease,” *Trends in Cardiovascular Medicine*, vol. 30, no. 5, pp. 249–254, 2020.
- [22] M. Dovizio, P. Ballerini, R. Fullone, S. Tacconelli, A. Contursi, and P. Patrignani, “Multifaceted functions of platelets in cancer: from tumorigenesis to liquid biopsy tool and drug delivery system,” *International Journal of Molecular Sciences*, vol. 21, no. 24, p. 9585, 2020.
- [23] C. J. Li, Z. H. Yang, X. L. Shi, and D. L. Liu, “Effects of aspirin and enoxaparin in a rat model of liver fibrosis,” *World Journal of Gastroenterology*, vol. 23, no. 35, pp. 6412–6419, 2017.
- [24] M. Mir, N. Ahmed, and A. Rehman, “Recent applications of PLGA based nanostructures in drug delivery,” *Colloids and Surfaces B: Biointerfaces*, vol. 159, pp. 217–231, 2017.
- [25] M. K. Anwer, M. Mohammad, M. Iqbal et al., “Sustained release and enhanced oral bioavailability of rivaroxaban by PLGA nanoparticles with no food effect,” *Journal of Thrombosis and Thrombolysis*, vol. 49, no. 3, pp. 404–412, 2020.
- [26] M. H. Park, H. S. Jun, J. W. Jeon et al., “Preparation and characterization of bee venom-loaded PLGA particles for sustained release,” *Pharmaceutical Development and Technology*, vol. 23, no. 9, pp. 857–864, 2018.
- [27] J. S. Pagano, C. B. Whitehurst, and G. Andrei, “Antiviral drugs for EBV,” *Cancers*, vol. 10, no. 6, article 197, 2018.
- [28] S. K. Dunmire, P. S. Verghese, and H. H. Balfour, “Primary Epstein-Barr virus infection,” *Journal of Clinical Virology*, vol. 102, pp. 84–92, 2018.
- [29] T. Yasuda, T. Wirtz, B. C. Zhang et al., “Studying Epstein-Barr virus pathologies and immune surveillance by reconstructing EBV infection in mice,” *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 78, pp. 259–263, 2013.
- [30] N. A. Smith, C. B. Coleman, B. E. Gewurz, and R. Rochford, “CD21 (complement receptor 2) is the receptor for Epstein-Barr virus entry into T cells,” *Journal of Virology*, vol. 94, no. 11, article e00428-20, 2020.