

Original Article

Evaluation of the proliferative potential of a preparation from the bursa of broiler chicken

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ABSTRACT

This article evaluates the proliferative potential of a preparation derived from the bursa of broiler chickens. In the experimental groups, a decrease in the incorporation of the selective DNA precursor was observed compared to the control group: by 68.0% in the first group, 48.6% in the second group, and 45.6% in the third group. Conversely, there were opposite changes in the rates of incorporation of the selective RNA precursor and DNA. In the first group, there was a 59.6% increase in the incorporation of the selective RNA precursor, whereas in the second and third groups, there were decreases of 51.3% and 22.1%, respectively. The incorporation of the selective DNA precursor decreased by 30.2% in the first group, but increased by 58.1% and 27.1% in the second and third groups. The study also revealed a statistically significant increase in the amount of DNA in the spleen of mice. In the first group, there was a 119.4% increase in the incorporation of the selective DNA precursor, 149.0% in the second group, and 72.3% in the third group compared to the control group. Despite a 13.2% decrease in DNA in the first group, this suggests active DNA synthesis processes under the influence of bursal peptides, indicating cell transition into the S-phase of the cell cycle. Thus, the administered peptide drug highlights immunomodulatory properties, potentially enhancing the proliferation and differentiation of T- and B-lymphocytes.

Keywords: Bursanatal, Bursal peptide, Immunogenesis, Immune response, Immune modulation

Introduction

The significance of the complex effect of bursa peptides on immunogenesis is an important field of research. Numerous domestic and foreign studies show that bursa peptides can have a significant effect on cell proliferation, especially in the liver, which plays a key role in metabolism and immune response.

Many foreign authors [1-19] have investigated the immunoadjuvant activity of four bursa peptides (BP) from the Bursa Fabricius: BP-I primarily induced cell-mediated immune responses, BP-II significantly induced humoral immune responses, BP-III had no significant effect on the activity of humoral and cell-mediated immune responses compared with the control group, BP-IV enhanced both humoral and cell-mediated immunity and provided protection against H9N2 AIV virus infection in the lungs of mice. Thus, the four peptides exhibited different levels of humoral and cell-mediated immune responses, indicating that they acted as multifunctional peptides capable of implementing various immune functions.

In other studies, BP11 has been shown to promote the formation of pre-B-cell-lymphoid-embryonic-cells (pre-B-CFUs) and regulate B-cell differentiation, including increasing the proportion of immature and mature B cells in the spinal cord

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when cultured with IL-7. BP11 also exerted immunomodulatory effects on antigen-specific immune responses in BALB/c mice immunised with an inactivated AIV, H9N2 subtype vaccine, including enhancement of AIV-specific antibody and cytokine production. Specifically, BP11 stimulated antibody production and potentiated dose-dependent Th1 and Th2 immune responses in chickens.

In another study [1, 15, 16, 20-27], it is shown that incubation of lymphocytes from cancer patients with a peptide is accompanied by an increase in the expression of differentiation markers on the lymphocyte membrane. The investigated peptide has a broad spectrum of activity and stimulates the expression of membrane molecules of T, B lymphocytes, and NK cells: CD2, CD3, CD4, CD16, CD25, CD21, CD22, and CD23. Under the influence of the peptide, the expression of surface IgM on the membranes of lymphocytes of cancer patients increases significantly.

A separate investigation [28-37] revealed that the compound being examined exhibits a significant immunostimulatory effect, primarily influencing the B-cell component of the immune system. This compound targets lymphocytes at various developmental stages, ranging from precursor cells to fully developed antibody-secreting cells.

Materials and Methods

The study utilized original raw materials, semi-finished products, and the final product as test subjects. The preparation of the product involved several steps. First, the bursa was mechanically excised from the raw material. Next, the isolated bursa underwent a 3-hour wash in a cold (between +2°C and +4°C) 0.05% chlorhexidine solution. Afterward, the bursae were rinsed three times for 15 minutes each with a physiological saline solution (0.9% NaCl) at a temperature range of +20°C to +40°C to remove any residual chlorhexidine. Subsequently, a surgical scalpel was used to mechanically mince the bursae into pieces measuring 3-4 mm within a laminar flow hood, maintaining the temperature between +20°C and +40°C. Finally, the minced bursae were immersed in a physiological saline solution (0.9% NaCl) that had been cooled to a temperature range of +20°C to +40°C.

Subsequently, the bursae immersed in the physiological solution were transferred to a refrigeration chamber maintained at a temperature range of +20 to +40°C for an incubation period of 180 minutes. After this incubation, the bursae underwent heating to +55°C for 30 minutes. They were then returned to the refrigeration chamber at +20 to +40°C for an additional 240 minutes. Following this, the solution containing the

homogenized bursae was subjected to centrifugation at 800g for 30 minutes at 0°C. The resulting sediment was discarded, and the supernatant was carefully extracted using an automatic pipette and transferred into sterile flasks. These flasks were subsequently placed in a deep-freeze chamber at -85°C for 24 hours. After this freezing period, the flasks were taken out and allowed to thaw at room temperature (between +22°C and +24°C) until thoroughly defrosted. The thawed solution was then centrifuged at 13,000g for 60 minutes at 0°C, with the sediment being discarded, and the supernatant was again separated using an automatic pipette and stored in sterile flasks. The sterile solution in the flasks was frozen at -85°C for 24 hours. After this time, the flasks were removed from the deep freeze and allowed to thaw at room temperature (between +22°C and +24°C) until completely liquefied. The resulting mixture was then centrifuged at 20,000g for 60 minutes at 0°C, with the pellet being discarded. The supernatant was carefully extracted using an automatic pipette and transferred to sterile flasks. Next, the solution was filtered through a negative-pressure filtration unit with 0.22 µm pore size filters. The sterile filtrate was collected and stored in sterile test tubes. Finally, the finished product was placed in a freezer set at -20°C for long-term storage.

The total yield of the final product (sterile solution), accounting for all losses during the process, was 1.5 g of bursa per 1 ml of the final solution. As an additional preparation method, some samples underwent lyophilization. The powder obtained from this process was then reconstituted with physiological solution until fully dissolved.

The drug's proliferative potential was assessed using standardized testing methods, specifically *in vivo* with liquid scintillation radiometry. The measurement results were analyzed and presented using statistical techniques in the RStudio software environment, utilizing appropriate statistical packages for data visualization.

Results and Discussion

When analyzing liver samples, statistically significant results were obtained for certain indicators. The amount of RNA (mg/g of dry weight) increased by 11.7 % and 21.7 % in the first and third groups, respectively. The incorporation of the selective DNA precursor (Bq/mg of DNA) showed a 7.0% increase in the first group, while a decrease of 18.8 % and 8.7 % was observed in the second and third groups, respectively, compared to the control (**Table 1**). No other indicators showed significant changes during the study of the liver.

Table 1. Levels of nucleic acids and indicators of liver proliferative activity caused by a preparation based on the bursa of Fabricius of broiler chickens

Liver	Control	Experimental Group 1	Experimental Group 2	Experimental Group 3
DNA quantity (mg/g of dry weight)	7.55± 0.32	8.41± 1.15	8.00± 0.76	8.39± 0.84

RNA quantity (mg/g of dry weight)*	21.03± 3.20	23.49*± 1.40	21.59± 2.17	25.6*± 2.07
Incorporation of the selective DNA precursor (Bq/g of dry weight)	541.47± 156.64	577.04± 149.58	446.46± 186.94	480.35± 61.10
Incorporation of the selective RNA precursor (Bq/g of dry weight)	1197.79± 264.95	1039.24± 174.89	1172.71± 292.74	1156.87± 125.73
Incorporation of the selective DNA precursor (Bq/mg of DNA)*	4.42± 0.39	4.73*± 0.53	3.59*± 0.67	4.04*± 0.54
Incorporation of the selective RNA precursor (Bq/mg of RNA)	3.66± 0.81	3.08± 0.31	3.59± 0.33	3.2 0± 0.62

* - reliability ($p \leq 0.05$) in relation to the value for the control group

Based on the data obtained, a significant increase in RNA amount was found in the liver tissue of the two experimental groups compared to the control group. This rise in RNA per gram of dry weight (mg/g) could be due to transcription activation in the cells of the sample being studied, leading to RNA accumulation, without a corresponding increase in liver tissue mass. This could be compared to a simplified representation of the G1 phase of the cell cycle (**Figure 1**), potentially indicating cellular synchronization influenced by the drug. Further evidence to support this hypothesis should be sought by examining additional parameters related to metabolic plasticity.

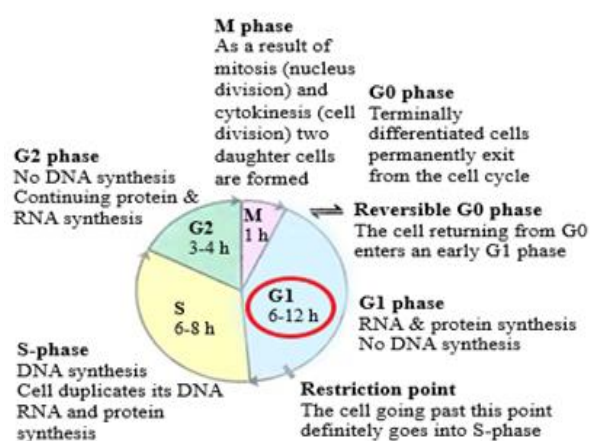


Figure 1. Cell cycle phases in the context of the liver samples study

However, subsequent data assessing the incorporation of selective nucleic acid precursors per tissue weight and mg of DNA did not definitively confirm the earlier findings. The lack of statistically significant differences in the incorporation of radionuclides, except for a 7% increase in DNA incorporation in the first group and a decrease by 8.1% and 9.1% in the second and third groups, respectively, along with unchanged other parameters, does not provide enough evidence to consider these changes as physiologically significant. This suggests a neutral effect of the chicken bursa preparation on the mouse liver.

In this study, a particular scientific interest was the potential for activating the proliferation of perisinusoidal liver cells, which are considered regional progenitors of liver tissue, and Ito cells, which facilitate the restoration of the parenchyma during liver regeneration through the production of growth factors, such as hepatocyte growth factor, mesenchymal morphogenic protein, epimorphin, and pleiotrophin.

The observation of minimal deviations in the synthetic activity of the liver in response to the administration of the peptide extract derived from the bursa necessitates a more nuanced interpretation. This can be attributed to specific functions inherent to liver tissue, which plays a crucial role in the detoxification process of xenobiotics. Central to this process is the cytochrome P-450 enzyme system, which facilitates microsomal oxidation. Following this phase of metabolism, the resultant compounds are subsequently conjugated with organic acids, enhancing their solubility and facilitating excretion.

Cells derived from the ectodermal germ layer exhibit varying degrees of inherent properties. However, the function of the bursa of Fabricius as the avian primary lymphoid organ, responsible for B-lymphocyte development, suggests a potential lack of analogous effects on tissues originating from other eukaryotic germ layers. This implies a degree of growth selectivity limited to the ectodermal lineage. While investigating the regulation of hematopoiesis, the paracrine effects of various factors on the production of key regulators cannot be disregarded. This is particularly pertinent to erythropoietin and neurotrophins, as well as the expression of vascular cell adhesion molecule-1 (VCAM-1) by Ito cells. These molecules play a crucial role in both erythropoiesis and granulopoiesis and are essential for maintaining the adhesion of hematopoietic progenitors to bone marrow stromal cells.

Furthermore, hepatic cells express stromal cell-derived factor-1 (SDF-1), a chemokine known to attract hematopoietic stem cells (HSCs). This chemotactic effect is mediated by the interaction of SDF-1 with its receptor, CXCR4, a crucial morphogen in hematopoietic development. Therefore, despite the absence of demonstrable, bursa-dependent alterations in hepatic synthetic activity, we hypothesize that the liver has an indirect influence on hematopoietic plasticity.

Analysis of bone marrow samples (**Table 2**) revealed statistically significant differences between the experimental and control groups. Specifically, experimental groups demonstrated a reduction in selective precursor DNA incorporation (Bq/g of dry weight) compared to the control group. Reductions of 68.0%, 48.6%, and 45.6% reductions were observed in groups one, two, and three, respectively. Furthermore, the data indicated multidirectional alterations in the incorporation of selective precursor RNA (Bq/g of dry weight) and DNA (Bk/mg DNA). The first experimental group exhibited a 59.6% increase in selective precursor RNA inclusion. In contrast, the second and third groups showed decreases of 51.3% and 22.1%, respectively, compared to the control group. Selective precursor DNA inclusion (Bk/mg DNA) decreased by 30.2% in the first

group, while the second and third groups displayed increases of 58.1% and 27.1%, respectively, relative to the control group. A significant elevation in selective RNA precursor inclusion (Bk/mg RNA) was observed across all experimental groups

compared to the control group, with increases of 231.7%, 32.4%, and 71.1% in the first, second, and third groups, respectively.

Table 2. Levels of nucleic acids and indicators of bone marrow proliferative activity when influenced by "BursaNatal"

Bone Marrow	Control Group	Experimental Group 1	Experimental Group 2	Experimental Group 3
DNA quantity (mg/g dry weight)	44.92± 1.29	35.20± 0.96	33.64± 0.86	36.60± 0.98
RNA quantity (mg/g dry weight)	19.30± 0.84	15.77± 0.63	16.33± 0.69	16.66± 0.71
Incorporation of the selective DNA precursor (Bq/g of dry weight)*	5806,13± 721.86	1859.61*± 323.69	2982.30*± 422.37	3157.43*± 472.89
Incorporation of the selective RNA precursor (Bq/g of dry weight)*	2738,08± 285.03	4370.34*± 336.62	1334.75*± 53.10	2131.97*± 122.25
Incorporation of the selective DNA precursor (Bq/mg of DNA)*	1.29± 0.16	0.90*± 0.15	2.04*± 0.29	1.64*± 0.24
Incorporation of the selective RNA precursor (Bq/mg of RNA)*	1.42± 0.15	4.71*± 0.36	1.88*± 0.07	2.43*± 0.14

* - reliability ($p \leq 0.05$) in relation to the value for the control group

Of particular interest in the context of the data obtained in the study of liver samples is **Figure 3**, which displays a statistically significant increase in the degree of incorporation of the selective DNA precursor per milligram of nucleic acid by 58.1% and 27.1% in the second and third groups, respectively, compared to the control group. Despite a slight decrease in the studied indicator by 30.2% in the first group, it does not change the overall focus of the discussion.

In the analysis of liver samples obtained during our study, we observed significant variations in the incorporation of the selective DNA precursor among different groups. This figure demonstrates a statistically significant increase in the level of incorporation per milligram of nucleic acid, with a 58.1% increase in the second group and a 27.1% increase in the third group compared to the control group. These results indicate a strong response to the experimental conditions applied to these groups, potentially suggesting increased metabolic or genetic activity related to the selective DNA precursor in liver cells.

The likely cause of induction is the direct stimulating effect of bursal peptides, as well as an indirect effect through the activation of morphogenesis factors produced by the liver for hematopoietic cells involved in erythro- and myelopoiesis processes.

This observation suggests that bone marrow progenitor cells may be in G-1 phase with a transition to the S phase of the cell cycle (**Figure 2**).

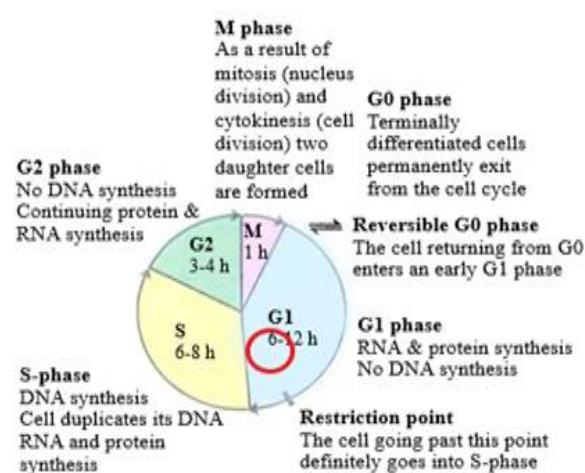


Figure 2. Cell cycle phases in the context of the study of bone marrow samples

The increase in the inclusion of the selective RNA precursor per mg shown in **Figure 3** (by 231.7% in the first group and 32.4% and 71.1% in the second and third groups, respectively) can be explained by the increase in transcription as a stage of cell preparation for further division, which supports the hypothesis. In other words, in bone marrow tissue, under the influence of the studied drug, there is an increase in synthetic activity either indirectly, due to liver growth factors, or directly, by stimulating the proliferation of colony-forming and blast cell elements, including precursors of B-lymphopoiesis.

The most intriguing question pertains to the tropism of the preparation being studied towards specific types of tissue and cells: is the increase in synthetic activity observed a result of the selectivity of the influence of bursal peptides on bone marrow lymphoid sprout cells through specific interactions, or is it simply a general stimulating effect? To address this question, we must

examine the findings of a study on the primary lymphoid organ of mammals - the spleen.

The study of spleen samples yielded statistically significant results (**Table 3**) for the following parameters: DNA amount (mg/g dry weight) showed a 13.2% decrease in the first group and a 6.7% increase in the second group, relative to the control group. The experimental groups exhibited an increase in the inclusion of the selective DNA precursor per gram of dry weight (Bk/g dry weight) by 122.3 %, 140.79 % and 97.18 % in the first, second, and third groups, respectively, compared to the control.

A similar pattern was observed for the degree of incorporation of the selective RNA precursor per gram of dry matter (Bk/g dry weight) with increases of +47.97 %, +53.5 % and +38.8 % in the corresponding groups. In terms of the degree of incorporation of the selective precursor DNA (Bk/mg DNA), there was an increase of 119.4 %, 149.0 % and 72.3 % in the first, second, and third groups, respectively, compared to the control. In relation to the degree of incorporation of the selective precursor RNA (Bk/mg RNA), there was an increase of +40.4% and +53.7 % in the first and second groups, respectively.

Table 3. The levels of nucleic acids and indicators of proliferative activity in the spleen when influenced by BursaNatal"

Spleen	Control	Experimental group 1	Experimental group 2	Experimental group 3
DNA quantity (mg/g dry weight) *	14.42± 2.30	12.52*± 1.38	15.34*± 1.63	14.04± 1.79
RNA quantity (mg/g dry weight)	42.11± 5.79	41.44± 4.68	48.69± 5.58	47.17M± 4.63
Incorporation of selective DNA precursor (Bq/g of dry weight)*	1478,70± 346.93	3287.28*± 1082.63	3560.68*± 1180.08	2915.83*± 1032.66
Incorporation of a selective RNA precursor (Bq/g of dry weight)*	3727,24± 917.59	5515.02*± 1519.39	5721.48*± 1538.90	5175.3 0*± 1280.78
Incorporation of selective DNA precursor (Bq/mg of DNA)*	2.53± 1.28	5.55*± 0.85	6.30*± 1.95	4.36*± 1.22
Incorporation of a selective RNA precursor (Bq/mg of RNA)*	2.03± 0.46	2.85*± 0.49	3.12*± 0.52	2.32± 0.56

* - reliability ($p \leq 0.05$) in relation to the value of the control group

The results of the study on spleen samples from mice exposed to bursal peptide extracts are of great scientific interest and continue the discussion about the selectivity of the drug being studied.

There is a statistically significant increase in the amount of DNA in mouse spleen tissue (by 6.38% in the second group compared to the control) and incorporation of the selective DNA precursor per mg (by 119.4% in the first group and 149.0 % and 72.3% in the second and third groups, respectively, compared to the control). When considering these indicators together, despite a slight decrease in the amount of DNA in the first group (by 13.2% compared to the control), there is a reason to believe that intensive DNA synthesis processes are occurring in the cells of the experimental samples due to the direct stimulating effect of bursal peptides. In other words, the spleen cells appear to have entered the S-phase of the cell cycle (**Figure 3**).

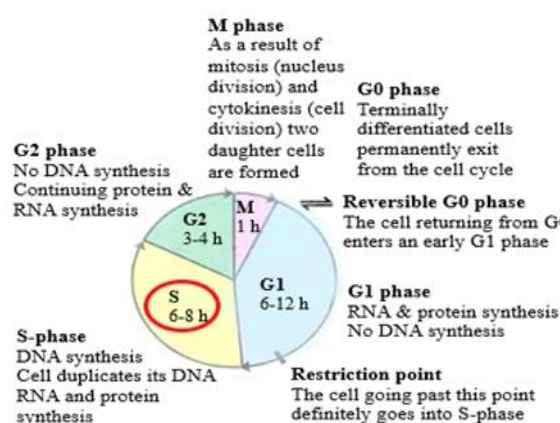


Figure 3. Phases of the cell cycle in the context of the study of spleen samples

The incorporation of selective RNA precursor per gram of dry weight (mg/g) increased by 47.96 % in the first group and by 53.5% and 38.85 % in the second and third groups, respectively. The amount of nucleic acid increased by 40.4 % and 53.7 % in the first and second groups, respectively, as determined in **Figures 2 and 3**. These results indicate that there are actively occurring transcription processes in cells, which aligns with our current understanding.

The significant increase observed in the most important indices of plastic metabolism in the tissues of the mouse spleen samples cannot be solely explained by substrate regulation. The spleen, as a peripheral organ of the immune system, is responsible for the secondary antigen-dependent differentiation of T and B lymphocytes. The white pulp, which contains periaarterial sheaths with T lymphocytes and lymphoid nodules (follicles) with T and B lymphocytes, along with the red pulp, which consists of splenic cords containing components of the blood, macrophages, and plasma cells in a reticular stroma, form the morphological basis of the organism's immunogenesis. This structure shows similarities with the Bursa Fabricius in chickens. Given this background, it is likely that a receptor-ligand mechanism is present for the influence of the investigated drug on the immune system [38-40].

The most important confirmation of the presence of a specific component of the receptor-ligand interaction is the similarity in the levels of change in proliferative activity of organs under the influence of the drug "BursaNatal" in all three experimental groups. If we consider the drug concentration in the second and third groups was 50% and 10% of the first experimental group, then a concentration-dependent "stepwise" change should be observed with substrate regulation alone. Although in some cases

a stepwise effect occurs (for example, when studying liver RNA synthesis per mg NC), in the overwhelming majority of cases, no concentration dependence is observed. Furthermore, for a number of indicators, particularly in the spleen and bone marrow, the level of proliferative activity in the second and third experimental groups is not lower than in the first group, and sometimes even higher [41-43].

This does not allow us to disregard the significant contribution of specific regulatory interaction of the "ligand-receptor" type to the effects of the drug "BursaNatal".

Conclusion

Based on the research data obtained, it can be assumed that the peptide preparation administered to laboratory animals exhibits tropism for the organs of immunogenesis, primarily the spleen, likely due to specific receptor-ligand regulation. This demonstrates pronounced immunomodulatory properties presumably consisting of enhancing the proliferation and differentiation of T- and B-lymphocytes.

It should be noted that the peptide preparation has a more pronounced effect on the organs of primary and secondary immunogenesis, specifically the spleen and bone marrow. However, the data obtained from the effect of the peptide preparation on liver samples does not demonstrate a physiologically significant stimulating effect. A possible reason for this is the targeted tropic effect on the cellular link of immunity, explained by the origin of the studied preparation/ The bursa of Fabricius in chickens is an important specialized organ of the immune system responsible for the formation of mature immunocompetent B-lymphocytes. This indicates the ability of the studied preparation to primarily affect immune tissues, and possibly influence immunogenesis and its various stages.

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Ethics statement: The study was conducted according to the guidelines of the Declaration of Helsinki.

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