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Immunomodulatory properties of lysozyme dimer under conditions of stimulation or suppression of the immune system – preclinical trials

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Abstract: Lysozyme dimer is the active ingredient of Lydium-KLP (Nika Health Products), an immunomodulating drug used in veterinary practice. It is a highly purified semi-synthetic substance, obtained by the chemical polymerization of natural N-acethylmuramylohydrolase found in the albumen of chicken eggs. Preclinical trials in vitro have revealed that lysozyme dimer, depending on its concentration, is able to enhance the production of IFN-α and modulate the synthesis and release of IL-2, interleukin-6 (IL-6) and TNF-α by cultured human lymphocytes stimulated by concanavalin A (Con-A). The in vitro trials show that lysozyme dimer stimulates the phagocytic activity of leukocytes isolated from cow's milk and blood. Our preclinical in vivo studies investigated the dose-dependent immunotropic effects of lysozyme dimer using various experimental models. Immunomodulating or immunocorrecting effects of lysozyme dimer were defined on inbred strain of mice stimulated with thymus-dependent antigen, i.e. sheep erythrocytes (SRBC), or pharmacologically suppressed by the administration of high dose of cyclophosphamide (200 mg/kg) or hydrocortisone (125 mg/kg), or subjected to acute stress (immobilization stress). The article presents the current state of knowledge on the immunomodulatory properties of lysozyme dimer under conditions of stimulation or suppression of the immune system. We review what is known specifically from preclinical trials about the adjuvant action of lysozyme dimer on the primary and secondary humoral response of SRBC-immunized mice as well as about its immunocorrective effect.

Keywords: lysozyme dimer, primary and secondary humoral immune response, T and B lymphocytes, immunomodulation, immunocorrection, mice

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1. Introduction

Understanding the real functions of the immune system which go far beyond purely defensive tasks, elevated it to the rank of one of the three most important body integration systems. The immune system together with the endocrine and nervous systems form a neuro-hormonal-immune network. This triad constitutes an overarching and functional relationship that is responsible for and determines the health balance of the organism, i.e. its homeostasis.

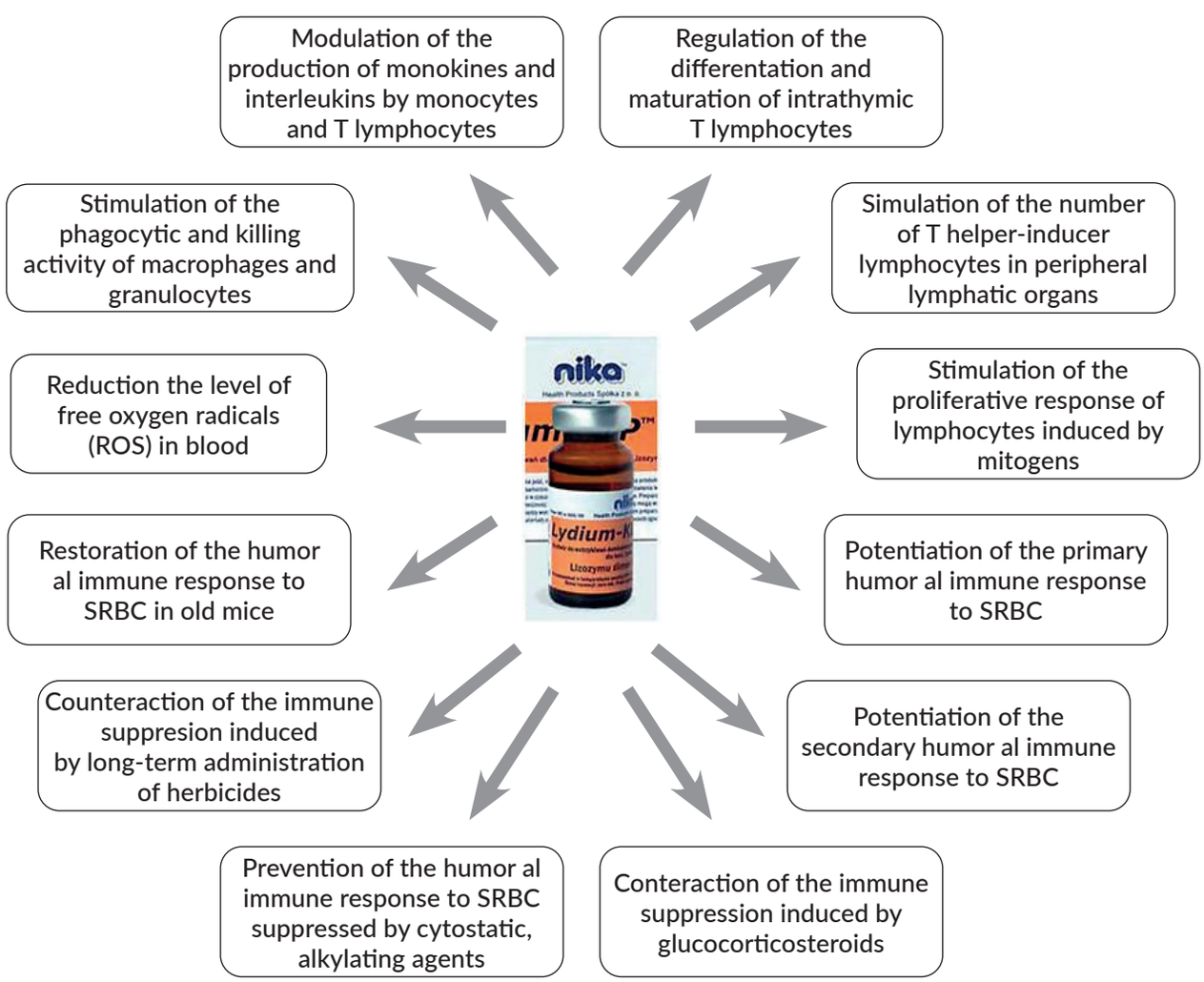
In addition to the defensive function based on the ability to recognize, destroy and eliminate foreign structures or abnormal endogenic elements, the immune system is responsible for ensuring the protection and tolerance to the body's own normal tissues and is also obliged to exert protrophic influences that aim at supporting the regeneration processes of its own recognized tissues. The ability of performing these three basic functions of the immune system (defense, tolerance and regeneration) is conditioned by the successful cooperation of immunocompetent cells and their

products including monokines, cytokines, neuropeptides, growth factors and biogenic amines. Therefore, the challenge of pharmacological control over these endogenous immune mechanisms has become a very important issue in both experimental and clinical veterinary and medical research in recent years.

Introduction of pharmacological immune response modulators, of both natural origin (*Propionibacteria*, thymus hormones, biostimine, *evening primrose* extract and chitosan) and those artificially synthetized (levamisole, isoprinozin, azimexon), has contributed to a significant progress in the treatment of many diseases of various etiologies. This is especially true for acquired secondary immunodeficiencies which may manifest as increased incidence of infections, occurrence of recurrent and/or opportunistic infections as well as the occurrence of allergic, autoimmune and neoplastic diseases. The effect of an immunomodulatory drug (biological response modifier) includes both the enhancement and suppression of the immune response. The direction and effect of an immunomodulator depends on the immune status of the body as well as the dose, route and frequency of administration.

Lysozyme dimer is a highly purified semi-synthetic substance, obtained by the chemical polymerization of natural N-acethylmuramylohydrolase found in the albumen of chicken eggs. It is the active ingredient of Lydium-KLP (Nika Health Products), a biological response-modifying drug used in veterinary practice (Fig.1). Preclinical trials *in vitro* have revealed that lysozyme dimer, depending on its concentration, is able to enhance the production of IFN-α and modulate the synthesis and release of IL-2, interleukin-6 (IL-6) and TNF-α by cultured human lymphocytes stimulated by concanavalin A (Con-A) [Klein & Kiczka, 1994]. The *in vitro* trials also show that lysozyme dimer stimulates the phagocytic activity of leukocytes isolated from cow's milk and blood [Kiczka et al.,

Fig. 1. Immunotrophic properties and activities of lysozyme dimer (preparation of Lydium-KLP, Nika Health Products)



1994]. Moreover, the results obtained *ex vivo* show that lysozyme dimer is also able to modulate the synthesis and release of IL-1 by murine peritoneal macrophages stimulated *in vitro* with lipopolisaccharide from *E. coli* (LPS). It was found that the modulating effect of the drug depends on the dose as well as the number of consecutive doses administered [Obmińska-Mrukowicz et al., 2002]. Other authors have reported that lysozyme dimer administered to pigs enhances the production of interleukin-1 (IL-1), interleukin-2 (IL-2) and interferon γ (IFN-γ) [Siwicki et al., 1997].

In the Department of Pharmacology and Toxicology, Wrocław University of Environmental and Life Sciences, there were carried out preclinical *in vivo* studies investigating the dose-dependent immunotropic effects of lysozyme dimer using various experimental models. Immunomodulating or immunocorrecting effects of lysozyme dimer were defined on inbred strain of mice stimulated with thymus-dependent antigen, i.e. sheep erythrocytes (SRBC), or pharmacologically suppressed by the administration of high dose of cyclophosphamide (200 mg/kg) or hydrocortisone (125 mg/kg), or subjected to acute stress (immobilization stress). Immunotropic effects of the lysozyme dimer were also determined on control Balb/c mice with unchanged immune system.

2. The adjuvant action of lysozyme dimer on the primary and secondary humoral response of SRBC-immunized mice.

The *in vivo* trials performed on SRBC-immunized mice confirmed the potentiating effect of lysozyme dimer on primary and secondary immune response. It has been found that lysozyme dimer potentiates the primary humoral response to SRBC in mice, resulting in an increased number of splenocytes producing haemolytic antibodies (PFC) and the total and 2-mercaptoethanol resistant anti-SRBC antibodies in serum [Dębowy et al, 1994; Obmińska-Domoradzka et al., 1994b]. The titre of 2-mercaptoethanol resistant antibodies in serum is roughly equivalent to that of IgG. The difference between the total and the 2-mercaptoethanol-resistant antibodies is due to the presence of IgM. It was confirmed that serum of non-immunized mice did not contain spontaneous anti-SRBC antibodies. In these studies, a relationship between the potentiating action of lysozyme dimer on the primary humoral response to SRBC and time- and dose-schedules with respect to antigen stimulation was observed. The adjuvant action of this drug has been found to also depend on the antigen dose. The effect of lysozyme dimer on the humoral response to SRBC in mice was determined in relation to doses (0.2, 2, 20 or 200 μg/kg) and to the time of the agent administration with respect to the antigen prior to or after SRBC immunization. It has been found that four-time exposure to lysozyme dimer at the doses of 0.2 to 20 μg/kg at 24 h intervals both prior to or after antigen stimulation at the dose of 4 x 10^8 sheep erythrocytes/mouse, was more activating the primary humoral response to SRBC than a single exposure to lysozyme dimer administered at the same doses. When the lysozyme dimer (0.2–20 μg/kg) was administered four times at 24 h intervals, either before or after SRBC immunization, the increase in the number of PFC was pronounced as compared to the single dose. An increased number of PFC was partially accompanied by an increased synthesis and release of anti-SRBC antibodies as determined on days 4, 7 and 14 after SRBC immunization. It has also been found, that the adjuvant action of a single dose of lysozyme dimer depended on the size of the dose applied and the time of the drug administration with respect to antigen stimulation. The strongest effect resulting in an increased number of PFC and the production of total and 2-mercaptoethanol resistant anti-SRBC antibodies in serum was noted after a single administration of lysozyme dimer at a dose of 2 or 20 μg/kg 2 h prior to the antigen stimulation. When the interval between lysozyme dimer (2 or 20 μg/kg) administration and SRBC immunization was prolonged to 24 h, the potentiating effect on primary humoral response was weaker [Obmińska-Domoradzka et al., 1994; Obmińska-Domoradzka et al., 1997a]. On the other hand, no significant changes in the specific humoral response in SRBC-immunized mice were observed when the dose was increased to 200 μg/kg and was administered once or four times every 24 h, before or after the antigen [Obmińska-Domoradzka et al., 1997a]. Administration of lysozyme dimer at a dose of 200 μg/kg reduced the potentiating effect of the drug on humoral response to antigen stimulation but did not induce immune tolerance in SRBC-immunized mice [Obmińska-Domoradzka et al., 1998a]. It has also been found that repeated administration of lysozyme dimer at the doses recommended in the treatment of infectious diseases in animals (2–20 μg/kg) does not lead to the development of the immune tolerance to this agent. This is evidenced by the maintenance or enhancement of the adjuvant effect of the immunostimulatory dose of lysozyme dimer (20 μg/kg) administered prior to antigen stimulation on the primary humoral response in SRBC-immunized mice [Obmińska-Domoradzka et al., 1998a]. Preclinical *in vivo* trials performed on SRBC-immunized mice have revealed that the adjuvant action of lysozyme dimer also depends on the antigen dose. A single dose of lysozyme dimer (20 μg/kg) administered 2 h prior to immunization of mice with SRBC (2 x 10^6 cells/mouse) does not affect their primary humoral response to this antigen. However, administration of the same single dose of lysozyme dimer (20 μg/kg) 2 h prior to antigen injection at the higher dose of 4 x 10^8 cells/mouse en-

hances primary humoral response to SRBC increasing the number of PFC and the titers of both total and 2-mercaptoethanol resistant hemagglutinins. On the other hand, lysozyme dimer administered at a dose of 20 μg/kg four times to mice immunized with SRBC at a lower dose (2 x 10^6 cells/mouse) potentiates the humoral response to SRBC, resulting in an increased number of PFC on day 7 after immunization and increased level of the total and 2-mercaptoethanol resistant anti-SRBC antibodies on days 4, 7 and 10 after priming [Obmińska-Domoradzka i wsp. 1998b]. When mice were immunized with SRBC at a higher dose of 4 x 10^8 cells/mouse and lysozyme dimer was administered four times at a dose of 20 μg/kg, the potentiating effect of the drug on humoral response occurs faster and is stronger resulting in an increased number of PFC on days 4 and 7 and an increased level of anti-SRBC antibodies on days 4, 7, 10 and 14 after priming [Obmińska-Domoradzka et al., 1997b].

Studies determining the effect of lysozyme dimer on the secondary humoral response of SRBC-immunized mice were performed in two experimental models. In the first model mice were immunized twice with an interval of 14 days: the initial dose of antigen injected intraperitoneally was 2 x 10^6 sheep erythrocytes/mouse and the next dose of 4 x 10^8 sheep erythrocytes/mouse was administered 14 days after priming [Obmińska-Domoradzka et al., 1998b]. In the second model mice were immunized twice with SRBC (4 x 10^8 cells/mouse *i.p.)* at a 30-day interval [Obmińska-Domoradzka et al., 1997b]. Lysozyme dimer at a dose of 20 μg/kg was administered intraperitoneally once, 2 h prior to immunization or four times at 24 h intervals, i.e. 2 h before and then 24, 48 and 72 h after antigen stimulation, according to the following experimental protocol: (i) after priming, (ii) after challenge, (iii) after priming and challenge. The results obtained in these studies show that lysozyme dimer administered at a dose of 20 μg/kg is also able to potentiate the secondary humoral response in SRBC-immunized mice. The effect depends on the time of exposure to the drug in relation to the priming and to the challenge as well as on the number of doses applied [Obmińska-Domoradzka et al., 1997b, Obmińska-Domoradzka et al., 1998b].

It has been also found that, irrespective of the experimental model taking into account the antigen dose and the time interval between the priming and the challenge, the strongest effect in potentiating the secondary humoral response to SRBC seems to result from lysozyme dimer administration at a dose of 20 μg/kg after the challenge. On the other hand, if the interval between immunizations was 14 days, the exposure to four doses of lysozyme dimer was more activating than a single administration of the drug to SRBC-challenged mice. The potentiating effect of the drug, irrespective of the number of doses applied, was observed until day 7 after challenge. The administration of lysozyme dimer, both after the priming and the challenge, has been found to extend the potentiating effect of the drug on the secondary humoral response to 14 days which is manifested by increased production of anti-SRBC antibodies [Obmińska-Domoradzka et al. 1998b].

It has also been shown that if the time interval between SRBC immunizations is 30 days, there is no correlation between the number of consecutive administrations of lysozyme dimer (20 μg/kg) and the intensity of the secondary humoral response to SRBC. The administration of lysozyme dimer after priming, irrespective of the number of doses applied, does not affect the secondary humoral response in SRBC-immunized mice. Lysozyme dimer administered once or four times to SRBC-challenged mice potentiates the stimulating effect of the antigen resulting in the increased number of PFC and increased level of the total and 2-mercaptoethanol resistant anti-SRBC antibodies. The potentiating effect of the drug, irrespective of the number of doses applied, was short-lived. The production of anti-SRBC antibodies was not found to be increased on day 14 after the challenge [Obmińska-Domoradzka et al., 1997b].

The presented studies allowed to determine the most optimal dose of lysozyme dimer, i.e. 20 μg/kg, which exerts the strongest adjuvant effect against the model thymus-dependent antigen (SRBC). The above dose is used in veterinary treatment in the target animal species. Studies on the adjuvant effect of lysozyme dimer have provided the basis for the use of this drug in *pro-host* therapy or to support the anti-bacterial therapy of infectious diseases. They may also serve as the starting point for research on the possibility of using lysozyme dimer to augment the immune response after vaccination of target animal species.

Currently, it is assumed that the signal initiating the activation of B lymphocytes to produce antibodies in response to thymic-dependent antigen is directly related to the stimulation by interleukin-1 (IL-1) of the immunocompetent inducing-helper ($CD4^+$) T cells which are consequently able to produce lymphokins IL-4 and IL-5 inducing the growth and differentiation of B lymphocytes [Santarlasci et al., 2013].

In our study on non-immunized mice it has been shown that the administration of lysozyme dimer once and four times at a dose of 20 μg/kg increases the percentage of single-positive mature $CD4^+$ thymocytes which may indicate the accelerating effect of the drug on the maturation and differentiation process of T lymphocytes in the thymus [Obmińska-Mrukowicz et al., 2002].

The administration of lysozyme dimer once or four times at 24 h intervals at a dose 10 times lower, i.e. 2 μg/kg, did not change the percentage of immature $CD4^+CD8^+$ thymic cells (double-positive cells) and mature $CD4^+$ and $CD8^+$ thymocytes (single-positive cells). At the same time, some changes in the percentage of splenocytes T and mesenteric lymph node T cells has been found. Administration of lysozyme dimer once and four times at a dose of 20 μg/kg has been found to the increase the percentage of $CD3^+$ (Pan-T cells) and $CD4^+$ (helper/inducer T cells) splenocytes. On the other hand, no significant changes in the percentage of T splenocyte subpopulations has been observed when the dose was decreased to 2 μg/kg, irrespectively of the number of doses applied. Exposure to four doses of lysozyme dimer (2 and 20 μg/kg) has been found to increase the percentage of $CD3^+$ mesenteric lymph node cells with corresponding increases in the percentage of $CD4^+$ and $CD8^+$ cells. Moreover, a single injection of lysozyme dimer at the doses of 2 and 20 μg/kg has not affected the percentage of $CD3^+$, $CD4^+$ and $CD8^+$ mesenteric lymph node cells.

These studies on T cell populations of central and peripheral lymphoid organs have been carried out by flow cytometry (FACS Becton-Dickinson) using monoclonal antibodies (mAb) coupled with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) (BioSource) according to the manufacturer's instructions. It has been shown that the effect of lysozyme dimer depended on the size of the dose and the number of consecutive administrations [Obmińska-Mrukowicz et al., 2002].

3. Immunocorrective effect of lysozyme dimer

The demonstration of the stimulating effect of lysozyme dimer on the number of T lymphocytes with a supporting-inducing function, i.e. the population of cells showing the highest degree of immunological competence, may indicate an immunocorrective effect of this drug which is supported by numerous studies published by our group. Administration of lysozyme dimer prior to the induction of pharmacological immunosuppression by a single high dose of cyclophosphamide (200 mg/kg) has been shown to partially or completely counteract the suppressive action of cyclophosphamide in the model of murine humoral immune response to SRBC [Obmińska-Domoradzka et al., 1997a].

Cyclophosphamide, an alkylating agent used in cancer treatment and autoimmune diseases, is also used in experimental immunopharmacology to induce immunosuppression and estimate the immunocorrecting action of drugs or substances with potential immunomodulatory properties [Braun & Hariss, 1981].

Cyclophosphamide does not possess alkylating activity *in vitro* but is converted into two biologically active metabolites in the macroorganism. Bryniarski *et al.* (2009) displayed that incubation of peritoneal macrophages of mice with these alkylating metabolites leads to the increase in the synthesis and release of IL-6 and IL-12 but it also leads to the decrease in the production of IL-10 and TGF-β by the cells. It is considered that the final action of cyclophosphamide metabolites on the cell activity depends on such factors as the concentration of the metabolites, duration of exposure, actual cell metabolism, cell genetic equipment as well as the *in vitro* or *in vivo* model used in the experiment (Bryniarski et al., 2009; Pukhalski et al., 1991).

On the contrary, it is known that B lymphocytes are very susceptible to the suppressive action of cyclophosphamide (Willers & Sluis, 1975) which is probably associated with a longer lifespan of B lymphocytes as compared with T cells (Mahiou et al., 2001). Although cyclophosphamide suppresses T cells (Marcinkiewicz et al., 1994) and macrophages (Miyauchi et al., 1990) as well, this effect seems to be less pronounced as compared to B cells. Hemendinger and Bloom (1996) observed a significant reduction in the number of differentiating B lymphocytes and increase in apoptosis in these cells using the chicken embryo model system. On the other hand, T lymphocytes were much more resistant to the suppressive effect of cyclophosphamide. Misra and Bloom (1991) reported that cyclophosphamide at the doses of 50-100 mg/kg caused B cell depletion, whereas for T lymphocytes, this effect was reported at the dose of 200 mg/kg. B cell mitosis was inhibited at the 5 mg/kg dose of cyclophosphamide while a similar effect in the case of T cells was observed at a dose of 50 mg/kg of this immunosuppressant. Besides, the authors observed approximately two-fold higher levels of cyclophosphamide and its metabolites in the bursal tissue in comparison with the thymus.

It has been found that cyclophosphamide can also modulate humoral response in SRBC-immunized mice in a dose-dependent manner (Obmińska-Domoradzka et al., 1994a).

The results obtained in this study show that a single dose of cyclophosphamide (200 mg/kg) injected 6 days prior to SRBC (4×10^8 cells/mouse *i.p)* decreased the number of PFC and diminished the production of total and 2-mercaptoethanol resistant anti-SRBC haemagglutinins. The suppressive effect of cyclophosphamide was observed for 10 days. Lysozyme dimer at a dose of 20 μg/kg, administered twice, i.e. 2 and 6 days prior to cyclophosphamide, or three times, i.e. 2, 4 and 6 days prior to cyclophosphamide, has been found to completely counteract the immunosuppres-

sive effect of this agent on the humoral response after SRBC stimulation. On the other hand, the administration of lysozyme dimer at a dose of 20 μg/kg once, i.e. 2 or 4 or 6 days prior to induction of immunosuppression with cyclophosphamide, only partially restored the animal's ability to respond to SRBC. However, the administration of lysozyme dimer at a dose of 2 or 20 μg/kg after the administration of cyclophosphamide has not been found to have any protective effect on the humoral response to SRBC [Obmińska-Domoradzka et al., 1997a].

Earlier studies by Schwarze (1977) show that the efficiency of humoral response suppressed by the administration of cyclophosphamide can be restored by the administration of a substance that exerts a stimulating effect on the helper-inducer T cell subpopulation. The studies conducted by our group have shown that lysozyme dimer is a factor increasing the percentage of this population of T cells [Obmińska-Mrukowicz et al., 2002].

The trials conducted *in vivo* on non-immunized and SRBC-immunized mice have confirmed the restorative effect of lysozyme dimer administered to mice prior Lysozyme dimer at a dose of 20 μg/kg was administered to non-immunized mice once or four times at 24 h intervals prior to hydrocortisone injection or once 2 h prior to SRBC, or four times at 24 h intervals after antigen stimulation in SRBC-immunized mice. It has been found that hydrocortisone injection at a dose of 125 mg/kg to non-immunized mice decreased the percentage of immature $CD4^+CD8^+$ thymic cells with corresponding increase in the percentage of mature $CD4^+$ and $CD8^+$ thymic cells. In addition, the percentage of $CD3^+$ and $CD4^+$ mesenteric lymph node cells was reduced. Administration of hydrocortisone (125 mg/kg) to SRBC-immunized mice decreased the number of splenocytes producing haemolytic antibodies (PFC) and diminished the production of the total and 2-mercaptoethanol resistant anti-SRBC haemagglutinins. Lysozyme dimer treatment prior to high hydrocortisone dose administration (125 mg/kg) partially counteracted the immunosuppressive action of the steroid. The protective action of the drug was expressed by the accelerated regeneration of the percentage of immature $CD4^+CD8^+$ thymocytes, $CD3^+$ and $CD8^+$ splenocytes as well as the mesenteric lymph node cells. The strongest immunocorrective effect has been noted after four injections of lysozyme dimer at a dose of 20 μg/kg. Lysozyme dimer administered to SRBC-immunized mice after their exposure to pharmacological immunosuppression has been found to lead to partial restoration of PFC and their capability of producing anti-SRBC haemagglutinins. The restorative action of lysozyme dimer did not depend on the number of doses applied (Obmińska-Mrukowicz & Szczypka, 2004). Moreover, the immunocorrective effect of lysozyme dimer was also observed in a study performed on old mice (12 months old) in which the humoral response after immunization with SRBC was significantly reduced compared to the group of young mice (8-12 weeks old). Exposure to four doses of lysozyme dimer (20 μg/kg) has been found to partially restore the primary humoral response to SRBC impaired by the advanced age (Obmińska-Domoradzka & Szczypka, 2001). The studies of Rymuszka et al. (2004) have shown that lysozyme dimer is also useful in restoration of mitogenic response of T and B lymphocytes and the metabolic activity of neutrophiles in atrazine-immunocompromised rabbits. Atrazine is a widely used herbicide that can be applied before and after planting to control broadleaf and grassy weeds. Some studies have shown that atrazine can disrupt the normal immune system function, enhancing the risk of infectious diseases [Hooghe et al., 2000; Rooney et al., 2003].

The modulating and immunocorrecting properties of lysozyme dimer (KLP-602) are also evidenced by numerous studies performed on fish. The preparation administered before or after experimental intoxication with chemical agents, e.g. chemotherapeutic agents or pesticides, corrected the impaired immune activity by stimulating the non-specific cellular and humoral defense mechanisms (Terech-Majewska et al., 2004; Rymuszka et al., 2005; Rymuszka & Siwicki, 2003). It has been shown that the injection of lysozyme dimer at a dose of 10 and 100 μg/kg to trout experimentally infected with *Aeromonas salmonicida* has the ability to stimulate cellular and humoral defense mechanisms, leading to a significant reduction in fish mortality (Siwicki et al., 1998). The authors of the study also showed that the mortality of fish with experimentally induced furunculosis was 85%, while after a single injection of lysozyme dimer it decreased to 45%, and after double administration it was only 25%. The obtained results indicate the possibility of practical use of lysozyme dimer to correct secondary deficiencies in the immune response caused by suppressive factors.

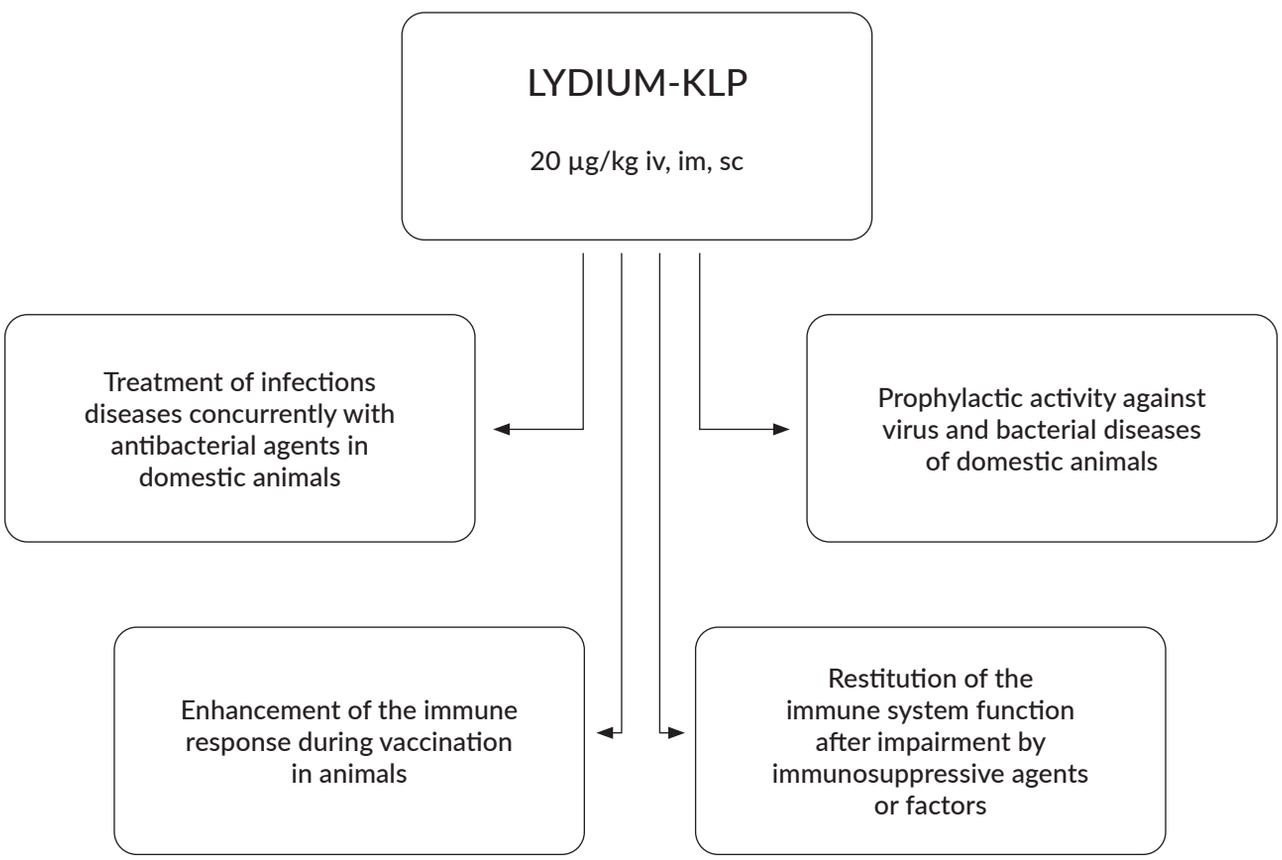
The mechanisms of the adjuvant and immunorestorative actions of lysozyme dimer are still unknown. It seems quite likely that the immunomodulatory effect of the drug is related to the activation of mononuclear cells to synthesize and release cytokines such as IL-1, IL-2 and IL-6, which was demonstrated in *in vitro* studies on mononuclear cell cultures obtained from human blood. The *in vitro* studies carried out on the cultured human lymphocytes stimulated by concanavalin (Con A) have shown that lysozyme dimer is able to modulate the synthesis and release of both IL-2 and IL-6 in a concentration-dependent manner [Klein & Kiczka, 1994]. *In vivo* studies have also found that, depend-

ing on the dose (2 or 20 μg/kg) and the number of consecutive administrations (single and four times administration), lysozyme dimer has the ability to modulate IL-1 release by peritoneal macrophages in mice stimulated *in vitro* with lipopolysaccharide (Obmińska-Mrukowicz et al., 2002). It is highly likely that the protective or adjuvant action of lysozyme dimer can be attributed to the activation of T lymphocytes and monocytes through the cytokine cascade (IL-1, IL-2, IL-6, TNF-α and INF-α) enhanced by this drug [Klein & Kiczka, 1994; Siwicki et al., 1997].

4. Conclusions

The results of studies on the immunomodulatory properties of lysozyme dimer obtained in laboratory animals indicate the possibility of practical use of this drug for pharmacological protection of immunohomeostasis in animals in the period of increased incidence of viral and bacterial infections. Moreover, lysozyme dimer may be applied to enhance the immune response during vaccination and for the restitution of the immune system function after impairment by immunosuppresive drugs or due to environmental factors (Fig.2).

Fig. 2. Clinical application of lysozyme dimer (preparation of Lydium – KLP, Nika Health Products) at a dose 20 μg/kg in domestic animals.



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