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Production of Specific Immune Serum Against the Steroid Estradiol Hormone

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Abstract: The present work is devoted to the preparation of hyperimmune sera against the steroid hormone. The effective production schedules are developed for immunization of rabbits to obtain specific hyperimmune sera against estradiol, providing a high specific immune response in 100% of the animals, a significant reduction in immunization terms and decrease in material and labor costs. Immunization schedules are based on the optimal combination of specific estradiol conjugate samples with immunoamplifiers and complete and incomplete Freund's adjuvants. To develop production process of the conjugate samples possessing antigenic properties authors have used three methods based on the activated esters technique. For obtaining highly active specific hyperimmune serum, the rabbits were immunized according to several schedules. The resulting antisera were treated using different methods: response of the immune diffusion (RID), enzyme immunoassay (EIA) and Castellani absorption method. The obtained specific antisera are a high-quality biological product to be used in the production of various immunobiological samples.

Key words: Immunization · Rabbits · Immune serum · Antigen · Antibody · Immunogen · Hapten · Conjugate · Estradiol hormone

INTRODUCTION

The ability of antibodies (AB) to form a highly specific strong immune complexes with a variety of antigens is of particular interest to specialists in the field of immunodiagnostic when designing diagnostic samples that enable to develop reliable means and methods of immunological instant diagnosis [1, 2]. Technology development of diagnostic samples involves the use of antisera obtained from blood of animals immunized with different antigens (AG). At that, their activity significantly affects the results of the study. To produce the immune serum, one has to select the optimal animal immunization schedule which depends on many factors. This is primarily physical and chemical condition of administered antigen, its activity and specificity, its dose, methods, intervals and frequency of antigen application, the total duration of immunization cycle, the use of adjuvants and immunomodifiers [3, 4]. Size, aggregation status and nativeness of antigen may influence the quantity and quality of antibodies. Small polypeptides (10 kD) and non-protein antigens, called haptens, are not immunogenic, i.e. not capable to trigger the body's immune response. It is known that the higher the molecular weight of the substance, the higher its immunogenicity. To create the immunogenicity of haptens, it is necessary to conjugate them with high-molecular carriers [5]. To improve the immunogenicity of the proteins with mass of 30-50 kD, one must either combine them together in polymeric molecule or connect with a protein carrier [6].

Based on the foregoing, the purpose of our study was to obtain highly active, specific hyperimmune sera against the hapten-estradiol.

MATERIALS AND METHODS

Preparation of the immunogen. In this work we have used the estradiol hormone produced by Sigma (USA) and bovine serum albumin (BSA) produced in Russia.

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Cyanamide (MW=42.04) produced by MP Biomedicals (France) and glutardialdehyde produced by Fluka (Switzerland) were used as linking agents. Estradiol conjugate samples with a BSA protein carrier were produced using several methods.

Immunization of Animals: Forty eight male rabbits of Chinchilla breed weighing 2-3 kg were selected as donors for the antisera against estradiol. All procedures on the experimental animals were conducted in accordance with the methodological recommendations [7]. Before immunization, blood sampling was carried out (drawing 5 ml of blood from each animal), followed by analysis of pre-immune serum for cross-reaction with obtained samples of BSA estradiol conjugate.

Testing of Antisera: The specific activity of the resulting antisera and conjugate samples of BSA estradiol was determined by response to immune diffusion (RID) according to O. Ouchterlony in 1% agar gel (Difco, USA) [8]. Reaction was performed in the standard way. The level of specific antibodies in serum was determined in an indirect competitive EIA [9,10,11]. The 96-basin polystyrene trays (Nunc, Denmark) were used as a solid phase. For carrying out EIA, the cells of the 96-basin tray for immune response were sensibilized by heterologous estradiol conjugate on phosphate-buffered saline (PBS) pH 7.2–7.4 at different concentrations (5-10 μg/ml) at 4°C nightlong. The EIA results were taken into account by using a spectrophotometer with a wavelength of 460 nm. The purification of polyclonal sera against the antibodies to carriers was performed according to Castellani method. The method is based on the adsorption of antibodies by the excess of relevant protein [12, 13]. To confirm the reproducibility and reliability of the results obtained in the study, the variation statistics methods, set out in [14, 15], were employed.

RESULTS AND DISCUSSION

Preparation of Specific Antisera: Estradiol hormone, by its chemical nature, is a hapten. It has a small molecular weight of 272.37 mM and does not have its own immunogenicity [4]. Due to the inability of using the hormone itself to produce specific immunoglobulins, the method of producing conjugate samples by cross-linking the hormone with a high-molecular carrier was employed. It is known from the literature that bovine serum albumin (BSA) is one of the most common protein carriers used to produce immunogens. The molecular weight of BSA is 67 kD, it has a high solubility in water, BSA molecule contains 59 amino-acid lysine residues, of which 30-35 residues contain primary amine groups capable of reacting, for example, in the peptide bond formation reaction [16, 17]. Protein molecules increase the probability of creating good T-cell epitopes and are more likely to become involved in the process of concentration on the surface of special AG-processing/AG-presenting cells, even at threshold concentrations of antigen [18]. To process methods for producing conjugate samples having antigenic properties authors used three methods based on the activated esters technique. To determine the amount of hapten molecules, bound to the protein, the UV – spectroscopy was used. In studies of steroid-protein conjugates as immunogens for production of anti-steroid antibodies it has been shown that at low density of haptens the antibodies are formed with low levels, moderate density is optimal, whereas the high density may inhibit the formation of antibodies. As the result of study, it was determined that the epitope density of estradiol made up 12 moles per one mole of protein (BSA), that allowed one to use the resulting conjugate to immunize laboratory animals and to prepare polyclonal antibodies specific to the original sample.

For producing highly immunized hyperimmune serum the immunization of the rabbits was performed according to several schedules. In order to enhance the immune response and reduce the possibility of tolerance, authors have used complete and incomplete adjuvants. Fourteen days before the primary immunization the animals were booster-immunized by injecting two doses of the antigen with Freund's complete adjuvant. After booster-immunizing, the antigen was administered 4-fold with an interval of 7 days in a dose of 1.0 ml (0.1 ml of AG diluted in 0.4 ml of PBS and emulsified by 0.5 ml of the incomplete Freund's adjuvant) subcutaneously in the back at several points. Fourteen days after the last injection of antigen, blood samples were taken to test the sera by RID and EIA methods. The secondary immunization was performed after 2 months in accordance with the described schedule. Using this immunization schedule we have obtained hyperimmune rabbit sera with high specific activity in immunological responses. Levels of specific antibodies to antigenic determinants of hapten-estradiol ranged from 1:800 to 1:3200 when measured in EIA and 1:2-1:4 when determined in RID.

It is important to note that the use of conjugate samples for immunization of laboratory animals entails difficulties when testing blood serum for the presence of specific immunoglobulins, since specific antibodies are

produced to a greater extent on the antigenic determinants of a high-molecular carrier as they have an overwhelming advantage. In this regard, purification of polyclonal sera was performed against antibodies to carriers. Optimal parameters of absorption were determined by titration technique. The separation of specific immunoglobulins from blood serum was performed by ammonium sulfate salting; purification was carried out by chromatography.

Summary: The first phase of research started with working out the methods of getting estradiol conjugate samples with antigenic properties. We have applied three conjugation methods based on the activated esters technique. Bovine serum albumin (BSA) with a molecular mass of 67 kD was used as the high-molecular protein carrier for conjugation to the hapten-estradiol molecules. To determine the immunogenic properties of the produced conjugate samples, we have immunized the laboratory animals by injecting the immunological samples produced by different methods. As a result of testing the obtained sera it was found that all conjugate samples have the ability to elicit an immune response. Comparison of the antigen administration schedules with and without adjuvant shows that the administration with adjuvant allows one to use the smaller doses of antigen, significantly increases the serum antibody response and reduces the tolerance risk [19,20,21]. The obtained sera were treated with different methods. When testing, the levels of antibodies, specific directly to antigenic determinants of hapten-estradiol, were quite high, ranging from 1:800 to 1:3200 in EIA and from 1:2 to 1:4 in response to immune diffusion in a mode were they have effected. Specific antisera were obtained from the animals with the highest level of antibodies.

CONCLUSIONS

The effective production immunization schedules were determined to obtain hyperimmune sera. These schedules are based on the optimal combination of BSA estradiol conjugate samples with Freund's adjuvant, providing a high specific immune response in 100% of animals.

The resulting hyperimmune sera are a high quality biological material for application in the production of various immunobiological samples.

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REFERENCES

1. Vylegzhanina, A.V., 2007. Development of EIA test system for determining dexamethasone in biological substrates. Dissertation FGU VGNKI, Moscow.
2. Hanly, W.C., J.E. Artwohl and T.B. Bennett, 1995. Review of Polyclonal Antibody Production Procedures in Mammals and Poultry. ILAR Journal, 3: 37.
3. Tyumentseva, I.S., A.E. Afanasiev, V.I. Efremenko, I.A. Bazikov and E.V. Alieva, 1999. Production process for preparing a diagnostic serum. The invention patent # 2135210. Bull. # 24 dated 27.08.1999 . Moscow.
4. Aliyeva, E.V., I.S. Tyumentseva, E.N. Afanasiev, M.P. Lavreshin, N.E. Afanasiev, E.A. Gorobets, A.A. Semircheva and A.YU. Mironov, 2008. The experience of producing antisera for diagnostic samples. Kursk scientific and practical journal "Human Health", 1: 10-13.
5. Erlanger, B.F., 1980. The preparation of antigenic hapten-carrier conjugates: A survey. Methods Enzymol., 70: 85-104.
6. Fuentes, M., J. M. Palomo, C. Mateo, A. Venteo, Sanz A.R. Fernândez- Lafuente and J.M. Guisan, 2005. Optimization of the modification of carrier proteins with aminated haptens. Journal of Immunological Methods, 307(1-2): 144-149.
7. Kuflina, S.A. and T.N. Pavlova, 1985. Euthanasia of experimental animals. Guidelines for the breeding of animals for experiments, Moscow, pp: 9.
8. Ouchterlony, O., 1949. Antigen-antibody reactions in gel. Arkiv for Kemi. Mineral. O Ged., 261(14): 1-9.
9. Popov, M.M., A.J. Tsyganenko and V.V. Minukhin, 2005. Immunology Fundamentals: Textbook. - Kharkov: "Osnova" Publishers, pp: 276.
10. Efremenko, D.V., 2006. Enzyme-immunoassay (EIA) method / Efremenko D.V. , Zharnikova I.V., Efremenko A.A., Garkusha I.V., Zhdanova E.V., Yurkina I.V., Aliyeva E.V., Afanaseva E.E. // The invention patent of the Russian Federation # 2290641, publ. 27.12.2006. Bull., # 36 pp: 5.
11. Vaneeva, L. and N. Tsvetkova, 1980. Immunoenzyme method, its present and future // Immunology, 2: 13-19.

12. Wisdom, G.B., 1995. Peptide Antigens. Wisdom G.B. Oxford University Press, Oxford.
13. Horbett, T.A., 1999. The role of adsorbed adhesion proteins in cellural recognition of biomaterials. BMES Bulletin, 23(2): 5-9.
14. Syzrantsev, P.I., 1978. Simple technique to calculate basic statistical quantities. Socialist Grain Farming, 3: 185-203.
15. Urbach V. Yu, 1975. Statistical methods in biological and medical research. Moscow: Medicine, pp: 296.
16. Devenyi, N. and J. Gergely, 1976. Amino Acids, Peptides and Proteins. Guidance on protein biochemistry and immunochemistry. Akademiai Kiado, Budapest. Book, pp: 368.
17. Wisdom, G.B., 1995. Peptide Antigens / Wisdom G.B., Oxford University Press, Oxford.
18. Fearon, D.T., 1995. The CD 19/CR2/TAPA-1 complex of B lymphocytes: Linking natural to acquired immunity / Fearon D.T., Carter R.H. // Annu. Rev. Immunol, 13: 127-149.
19. Van Den Hauwe, 2002. Simultaneous determination of betamethasone and dexamethasone residues in bovine liver by liquid chromatography/tandem mass spectrometry / Van den Hauwe 0., Castro Perez J., Claereboudt J., Van Peteghem C., Rapid Communications in Mass Spectrometry, 15: 857-861.
20. Fontalin, L.N. and L.A. Pevnitskii, 1978. Immunological tolerance, M., pp: 282.
21. Luo Y., 2003. Simultaneous analysis of twenty-one glucocorticoids in equine plasma by liquid chromatography/tandem mass spectrometry. Luo Y., Uboho C. E., Soma L. R., Guan F. Y., Rudy J. A., Tsang D. S., J. Agric. Food Chem, pp: 326.