Effect Of Ampicillin Sodium On Immune Response

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Abstract

Penicillins are B-lactam antibiotics widely used in clinical practice and they induce many allergic reactions, which can be either cellular or humoral. B-lactams are able to interact covalently with serum proteins, forming immunogenic conjugates and their metabolism can provoke a number of different antigenic determinants that have been implicated in the specificity of the immunological response. In the present study, Ampicillin Sodium (17.5mg/kg), a semi-synthetic amino penicillin was used to study its effect on immune response by measuring different immune parameters like total serum protein concentration, passive haemagglutination, total leucocyte and total lymphocyte count. The antigen used was sheep RBC. It was observed that there was no significant change in total serum protein content and antibody titre. Ampicillin caused a slight increase in total leukocyte count and slight decrease in total lymphocyte count. It can be concluded that Ampicillin does not adversely affect the specific immune response in normal rabbits.

INTRODUCTION

The immune system is known to be involved in the etiology and pathophysiological mechanism of several disease [12]. The function and efficiency of the immune system may be influenced by many exogenous and endogenous factors resulting in either immunosuppression or immunostimulation [34].

Studies have indicated that various antibiotics interfere with immune response [56]. Antibiotics like clindamycin and netilmycin administered at therapeutic doses to rabbits caused a significant inhibition of phagocytosis [6]. Lomefloxacin, Ofloxacin and cipropfloxacin had no effect on immune response [789]. Erythromycin, colistin and chloramphenicol markedly depressed humoral response, resulting in decreased TIg concentration in mice [10111213].

Ampicillin is a bactericidal agent which adheres to bacterial penicillin-binding proteins, inhibiting bacterial cell wall synthesis. The present study has been focused on the effect of ampicillin on immune response [14].

METHODOLOGY

EXPERIMENTAL ANIMALS

New Zealand White Rabbits (6 months old) were used for the study. They were kept under standard laboratory conditions. The animals were housed in polypropylene cages and were fed with standard rabbit chow and water ad libitum. Ethical committee in accordance with animal experimentation and care has approved all animal procedures.

Rabbits were randomly divided into two groups, with each group containing six animals. Group I served as control which received 0.5ml of antigen, SRBC intramuscularly on the first day of the experiment. Group II rabbits were administered with ampicillin sodium at a dose of 17.5mg/kg body weight, iv twice daily for a period of 4 days and 0.5ml of SRBC im on the first day.

COLLECTION OF SHEEP RED BLOOD CELLS (SRBC)

The area under the external jugular vein of sheep was carefully shaved avoiding any abrasions. The area was cleaned with 70% alcohol and 20ml of blood was withdrawn. The anticoagulant used was acid citrate solution. Later SRBC was washed with phosphate buffer solution (PBS) three times at 150g for 10minutes. A 1% suspension of the packed SRBC was then prepared.

SELECTION OF DOSE

The rabbit dose was calculated on the basis of surface area ratio [15].

IMMUNIZATION PROCEDURE

The rabbits were divided into two groups-control and test. Ampicillin sodium at a dose of 17.5mg/kg, iv and SRBC

(0.5 ml, im) were administered to the test group. The control animals were administered with the antigen, SRBC (0.5ml, im). Blood was withdrawn from the marginal ear vein prior to the administration of drugs and on 1 st , 7 th , 14 th , 21 st and 28 th day after immunization [8] . It was centrifuged, serum separated and stored at -40ºC. The serum obtained was used for the estimation of antibody titre [16] , total serum protein [1718] , total leucocyte count [19] and total lymphocyte count [19] .

PASSIVE HAEMAGGLUTINATION

The sera collected were diluted two times in each subsequent well of the haeagglutination plate and equal volume of 1% SRBC was then added to each well and later incubated at 37 ºC for a period of 2 hours. The agglutination titre was read after overnight incubation. If the cells form a continuous carpet on the base, it was considered as positive agglutination and if no agglutination has occurred, the cells formed a button at the bottom [16] .

PROTEIN ASSAY

The total protein content was determined by using Biuret method. Bovine serum albumin (BSA) was used as the standard. Absorbance was measured at 545nm. A graph was plotted with concentration of protein on x-axis and absorbance on y-axis. The concentration of the test was calculated from the graph [1718] .

TOTAL LEUCOCYTE COUNT

Fresh blood was pipetted out using a WBC pipette under aseptic conditions. The white blood cells were counted by non- automated (manual) cell count technique [19] .

TOTAL LYMPHOCYTE COUNT

Blood samples were collected in heparinized vials and were diluted in equal proportion with phosphate buffer solution. About 10 ml of diluted blood was carefully layered over 2.5ml Histopaque 1077 in round bottomed tube conical centrifuge tube. It was then centrifuged at 1800 rpm for 30minutes and the layer of lymphocytes at interface was taken out and washed three times with PBS as follows.

1500rpm × 15
1200rpm × 12
1000rpm × 10

The lymphocytes were re-suspended in PBS and the cell number was determined with a Neubauer improved haemocytometer. Later the lymphocytes were diluted with PBS to give a final concentration of 1×10^{6} /ml.

The mean value was expressed was mean ± SE. Statistical analysis was done by using students ‘t’ test. P value less than 0.05 were considered significant [19] .

RESULTS AND DISCUSSION

Successful anti-microbial therapy requires normal functioning of the immune system in the host. Studies have indicated that various antibiotics interfere with immune response. In the present study, we have evaluated the effect of ampicillin sodium on different immune parameters like passive haemagglutination, total serum protein, total leucocyte count and total lymphocyte count.

Passive haemagglutination is a measure of humoral immune response. The active haeagglutination test detects antibodies to red blood cell antigens. By binding different antigens on to the red cell surface covalently or non-covalently, the test can be extended to detect antibodies to antigens other than those found on red cells [9] . In our study, it was found that there was no significant difference in antibody titre in the group in which antigen was given and the group in which antigen was given in combination with ampicillin sodium (Fig.1 & 2).

Figure 1

Fig.1. Lower view of haemagglutination test plate

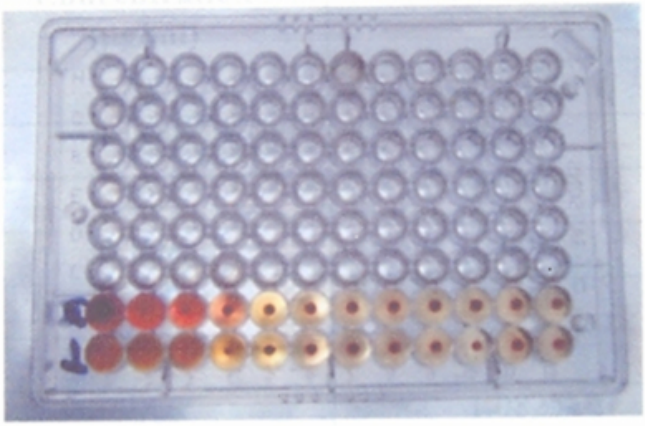
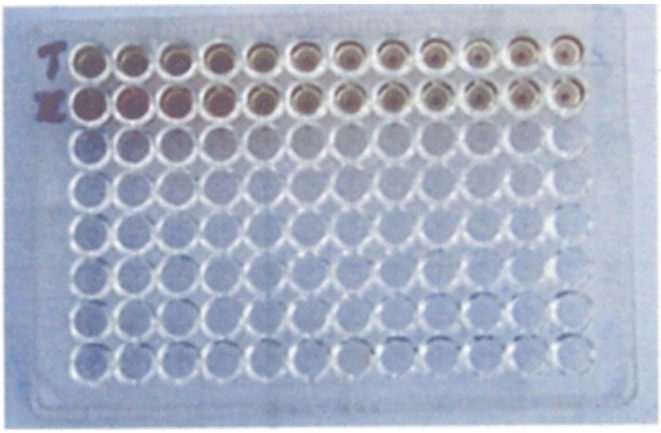


Figure 2

Fig.2. Upper view of Haemagglutination test plate



The measurement of protein concentration is mainly used to calculate the level of purity of a specific protein. When treated with dilute $CuSO_4$ in alkaline medium, a purple colour was obtained. This is the basis of biuret test and is widely used for identification of proteins and peptides [10][11]. In our study, total serum proteins did not differ significantly (P 0.05) between treated and control groups (Table.1).

Leucocytes (white blood cells) are nucleated cells that are involved in the defense mechanism of the body. They act as immunocytes and maintain the body immunity [12]. Ampicillin sodium caused a slight increase in the total leucocyte count when compared with the control group but there was no significant (P 0.05) chance to point out.

Lymphocytes mediate the immunologic responses of the body. Among the 2 different categories of lymphocytes ie., T-lymphocytes and B-lymphocytes, the earlier mediate cellular immunity which is concerned with transplant rejection and reject of tumour cells. The latter mediate humoral immunity by producing antibodies, which are concerned with protecting the body against bacterial, viral and other infections [12]. In the study, it was observed that there was a slight decrease in the lymphocyte percentage in the test group when compared to the control but there was no significant (P 0.05) difference between test and control groups (Table.1).

Figure 3

Table.1 Effect of ciprofloxacin on various immune parameters

Parameters		Time interval in Days				
		0	7	14	21	28
TIg (mg/ml)	A	35.45±3.23	39.61±3.02	42.82±3.40	45.24±3.34	48.86±3.58
	B	38.71±3.14	40.00±3.65	44.16±3.11	46.96±3.22	50.20±3.86
TSP (g/100ml)	A	4.04±0.34	4.12±0.40	4.12±0.40	4.16±0.42	4.24±0.46
	B	4.08±0.39	4.10±0.42	4.12±0.44	4.20±0.44	4.30±0.45
TLC (per cubic mm)	A	8840±0.72	8912±0.72	9110±0.66	9366±0.60	9548±0.76
	B	8710±0.70	8934±0.78	9332±0.72	9444±0.68	9564±0.70
DLC(%)	A	52.20±3.38	56.32±3.44	57.44±2.98	59.64±3.45	64.52±3.50
	B	52.14±3.53	55.28±3.64	57.88±3.10	60.30±3.86	63.27±3.74

A : Antigen control
B : Antigen + Ampicillin sodium
TIg- Total serum immunoglobulin levels
TSP-Total serum protein
TLC-Total leucocyte count
DLC-Lymphocyte percentage

CONCLUSION

It has been found that the treatment of rabbits with ampicillin sodium at a dose of 17.5 mg/kg did not significantly alter the immune response. Thus the drug has got no effect on humoral and cell-mediated immune response to the antigen. Hence, from the above results it can be concluded that ampicillin at therapeutic dose will not interfere with the immune response.

References

1. Dale MM, Foreman JC, Fan TPD. Introduction to the immunology and pathology of host defence mechanisms. Textbook of Immunopharmacology: Blackwell scientific Publication, Oxford 1994.p.1.
2. Chirigos MA. Immunomodulators: current and future development and application. Thymus 1992;19:7.
3. Blanca M, Vega JM, Garcia J et al. Allergy to Amoxycillin with good tolerance to other penicillins; study of the incidence in patients allergic to betalactams. Clin Exp allergy 1990;20:475-81.
4. De Weck AL. Drug Allergy, immunotherapy, immune complexes and anaphylaxis. Curr Opin Immunol 1990;2:548-57.
5. Dewdeny JM. Immunology of the antibiotics. In: Sela M ed. The antigens.New York : Academic press,1977:114-122.
6. Sheng FC, Frieschlag J,bBackstrom B, Kelly D, Busuttil RW. The effect of in vivo antibiotics on neutrophil (PMN) activity in rabbits with peritonitis. J Surg Res 1987;43:239.
7. Iordanova AI, Smolkind TV, Nikitin AV. Multifactor

analysis of the action of norfloxacin on the humoral immune response. Antibiot Khimioter 1995;40:16.
8. Iordanova AI, Navashin PS, Smolkina TV, Nikitin AV. Effect of lomefloxacin on the synthesis of antibodies to vaccine EV fraction I and haemaglutinins. Antibiot Khimioter 1996;41:44.
9. Jayakumar K, Honnegowda, Krishnappa G, Sastry KNV, Narayana K. Effect of ciprofloxacin on specific immune response in rabbits. Indian J Exp Biol 2002; 40:111-4.
10. Nicolas R, Amurrio C, Lewden S, Camarero M, Cisterna R. Effect of erythromycin treatment on specific immunologic response in mice. Arch Immunol Ther Exp 1989;37(3-4):405-13.
11. Levine BB, Redmond AP, Fellner MJ, Voss HE, Levytska V. Penicillin allergy and the heterogeneous immune responses of man to benzyl penicillin. J Clin Invest 1966;45:1895-906.
12. Ferrente A, Rowan-Kelly B, Thong YH. Suppression of immunological responses in mice by treatment with amphotericin B. Clin Exp Immunol 1979;38:70.
13. Biglino A, Foajanrno B, Pollono AM, Gioannini P. Effect of erythromycin on the immune response and interferon production. Immunopharmacol 1987;14:101.
14. Pharmacist Drug HandBook. Springhouse Corporation Pennsylvania 2001.p.141-3.
15. Ghosh MN. Fundamentals of Experimental Pharmacology.3rd ed. Kolkatta:Hilton and Co;2005.p.192.
16. Alton GG, Jones LM, Peitz DE. Laboratory techniques in Brucellosis. WHO Monograph Series 55.
17. David W, Burden, Donald B, Whitney. Biotechnology: Proteins to PCR: 51.
18. Gornel AG, Barduril CJ, David MM. Determination of serum proteins by means of biuret reaction. J Biol Chem 1949;177:751.
19. Pal GK, Pravathi P. Text Book of Practical Physiology 2nd ed. Orient Longman Private limited;2005.p.55-57,76.

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