THE POSSIBLE EFFECTS OF B-ADRENERGIC BLOCKERS ON THE IMMUNOLOGICAL REACTIONS

By

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INTRODUCTION

B-adrenergic blocking agents have received major attention because of their utility in the management of cardiovascular disorders (Prichard et al., 1980). Furthermore, it was reported that some patients suffering from rheumatoid arthritis or other connective tissue disorders had improved following propranolol therapy for co-existent hypertension or angina pectoris (Moore et al., 1978).

Fleir (1971) reported that, the human peripheral lymphocytes have adrenergic sensitivity corresponding to alpha and beta receptors; alpha-adrenergic stimulation associated with augmentation, and beta with inhibition of lymphocyte transformation. Also lymphoid tissue is known to possess a high content of norepinephrine presumably related to its sympathetic

(Hadden, 1971). Furthermore, B-adrenoceptor blockers, may have immunomodulatory properties. Such a possibility was suggested by animal studies showing that propranolol can inhibit mitogenic activation of lymphocytes (Henderson et al., 1981), and can enhance antibody production in mice (Nakazawa et al., 1976).

The present study was conducted to assess the effects of beta-adrenergic blockers, propranolol and pindolol on the immune response in immunized mice with sheep red blood cells (SRBCs), also they were compared with hydrocortisone as a standard immuno-suppressive agent and all were compared with immunized control mice. The test drugs have different pharmacological properties to declare if these different properties

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have a role in the possible immunological effects or not.

**Material and Methods**

**Animals Used and Drug Treatment:**

Mice used throughout the study were divided into five groups, the first group served as unimmunized control group, consists of 7 mice and each animal received sterile saline intraperitoneally (I.P) daily for 3 consecutive days in doses of 0.3, 0.4 and 0.6 ml respectively. The second group of 7 mice served as immunized control one and each animal received only the antigen (SRBCs 5% in RPMI medium) I.P. for 3 consecutive days in dose of 0.3, 0.4 and 0.6 ml respectively. The other three groups for the 3 test drugs, the 3rd group for receiving hydrocortisone hemisuccinate in the dose of 40 mg/kg body weight I.P. for 5 consecutive days, the 4th group for receiving propranolol in the dose of 2 mg/kg B.Wt. I.P for 5 consecutive days and the 5th group for receiving pindolol in the dose of 2 mg/kg B.Wt I.P. for 5 successive days. Each one of the last three groups was subdivided into 3 subgroups each contained 7 mice. The 1st subgroup for giving the test drug before antigen immunization, the 2nd subgroup for giving the test drug with antigen simultaneously and the 3rd subgroup for giving the test drug after antigen immunization.

**Sample Collection and Preparation:**

After decapitation of animals the blood was collected and put in incubator at 37°C for one hour then centrifugated to separate the serum for determination of total serum immunoglobulins and immunoglobulin G(IgG). The serum kept frozen until assay.

The spleens were aseptically removed from mice then each spleen was placed in 5 ml. sterile RPMI 1640 medium containing foetal calf serum 10% and penicillin streptomycin 2%. These spleens were prepared for plaque forming cells assay.

**Methods Used:**

The methods which were chosen to assess the immunological effects of test drugs were.

(I) Quantitative estimation of immunoglobulins:

The method was the single radial immunodiffusion technique described by Fahey and McKelvey (1965) and
Mancini et al., (1965), for quantitative estimation of total globulin and IgG.

(2) Plaque forming cells (PFcs) Technique:

This Technique for detecting the antibody forming cells numbers in spleen representing the cellular immune response. This Technique was devised by Jerne et al., (1963) and Fauci and Pratt (1976).

RESULTS

I- Effect of The Test Drugs on The Serum $\gamma$ globulin and Immunoglobulin G (IgG) in Sensitized Mice:

As illustrated in tables (1, 2 and 3) and figures (1 and 2) there was a highly significant increase in serum $\gamma$ globulin and IgG levels in sensitized control group as compared with unsensitized control group.

(A) Effect of hydrocortisone:

As shown in table (1) and Figures (1 and 2) the administration of hydrocortisone hemisuccinate before, with or after antigen immunization produced a very highly significant reduction of the mean $\gamma$ globulin and IgG levels ($P<0.001$) as compared with sensitized control group.

(B) Effect of propranolol:

As illustrated in table (2) and Figs.(1 and 2), the administration of propranolol produced a highly significant reduction ($P<0.01$) of $\gamma$ globulin and significant reduction ($P<0.05$) of IgG level when it was given before immunization and produced a highly significant reduction of both $\gamma$ globulin level ($P<0.01$) and IgG level ($P<0.005$) when it was given with antigen simultaneously. But when it was given after immunization it produced significant reduction of both $\gamma$ globulin and IgG level ($P<0.05$) as compared with the immunized control group.

(C) Effect of pindolol:

As illustrated in table (3) and Figs (1 and 2), the administration of pindolol produced a very high reduction of $\gamma$ globulin level ($P<0.001$) and significant reduction of IgG level ($P<0.05$) when it was given before and with antigen immunization. But, when it was given after immunization, produced highly significant reduction of $\gamma$ globulin level ($P<0.005$) and significant reduction of IgG ($P<0.05$) as compared with sensitized control group.

II- Effect of The Test Drugs on Plaque Forming Cells (PFcs) in Spleen of Sensitized Mice.
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1973).

In the present study, hydrocortisone produced the best reduction in Ψ globulin and IgG levels when it was given with antigen simultaneously. This effect is supported by Taniguchi and Tada (1971) who reported that cortisone treatment before immunization showed temporary accelerated antibody formation, whereas treatment during antigen immunization was moderately suppressive. Also Malinodan et al., (1970) reported that cortisone elicit its maximum suppressive activity when administered just prior to antigen.

(B) Effect of Propranolol:

In the present study the best result was obtained when the drug was given with antigen concurrently. Furthermore, it is clear from the previous data that this immunosuppressive effect is less than those of propranolol.

The immunosuppressive effects of propranolol could be attributed to effect of propranolol on the B-adrenergic receptors on murine lymphocytes which is stimulated with antigen to produce antibodies representing all five major immunoglobulin classes (Strom and Corptenter, 1960 and Sell et al., 1970). Furthermore, the increase of norepinephrine levels in spleen of mice after SRBCs immunization in vivo shares in the antibody response (Besedovesky et al., 1979 and Sanders & Munson, 1984). So propranolol by blocking B-adrenoceptors can prevent the subsequent events. This explanation is supported by Bennes et al., (1968) who reported that propranolol reduced antibody formation more than tenfold when it is given 5 days before antigen injection in rats and it produced marked depression of cAMP level in spleen of sensitized mice.

In addition, the suppressive effect of propranolol may be due to its membrane-stabilizing effect where it appears to block action potentials in conducting tissue by a series of events, which in turn hyperpolarizes the membrane through the effect of free Ca+2 on K+ fluxes (Porzig, 1975 and Szasz et al., 1977). This changes in the ionic environment around critical sites could mediate the inhibition of lymphocyte capping by propranolol, where the capping of membrane molecules provides a useful model for detecting the transduction across the cell membranes of signals generated by antigen binding indicating active response of the cell (Ashman, 1973 and 1980).

The best results when the drug
antibody response to myophosphoryl at
agen (35%9). The evaluation of serum
erter 6 days from the first dose of anti-
sensitized control group was 34.33%
the count of PECs in spleen of
sensitized mice with S.RBCs
plaque forming cells count in spleen

II. Effect of the Test Drugs on

formation in vitro.
that myophosphoryl inhibited antibody for
by Pearnman (1979), who studied also
the reduced antibody formation and
"he" (1969), who reported that epineph-
the previous data, it is obvious
from the previous data, it is obvious
that the effect on X-globulin level is more
Ferrari after antigen immunization.
ol level but produced a highly significant
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ol level and significant reduction of X-glob-
correlated with antigen production a very
reduction of propionaldol either before or on-
In the present study, the adnimis-

(c) Effect of Propionaldol:

and Carpentier (1980).

level produced by hydrocortisone.
level reached, but did not reach the
mice group but did not even to the
be below those of the minimum-
strong, that the reduced X-globulin level
of propionaldol was so
regard the comparison with hydrocorti-

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the cellular level is possible with the hemolytic plaque technique. The technique as described initially by Jerne et al. (1963) enumerates only cells releasing high hemolytic efficiency IgM antibodies, IgM PFcs reach maximum numbers on days 4-5 from the first dose of antigen (pierce et al., 1971) while IgG PFcs usually are detected later and reach maximum numbers later than do IgM-PFcs (Sell et al., 1970).

This antigenic stimulation of IgM-PFcs may be attributed to B-adrenoceptors stimulation in spleen cells and due to sympathetic neurotransmitter release and subsequent action on immunocompetent cells. This view can be supported by Bese-dove by et al. (1979) who reported that after SRBcs immunization in vivo, the level of norepinephrine in the rat spleen is increased. Moreover, the B-adrenoceptors have been identified to be the receptor responsible for the enhanced IgM antibody response produced by norepinephrine in mouse spleen cells immunized by SRBcs in vitro (Sanders and Manson, 1984). Also cAMP enhances antibody formation in vivo and in vitro where it increases the number of antibody forming splenic cells in mice immunized with SRBcs (Hadden et al., 1979 and Strom and Carpenter, 1980).

A) Effect of Hydrocortisone:

In the present study, hydrocortisone produced a very highly significant reduction of PFcs count % in mice immunized with SRBcs when it was given either before, with or after immunization.

Corticosteroids, following in vivo administration, produced redistribution of the circulating peripheral leucocytes with increased numbers of neutrophils and decreased numbers of monocytes lymphocytes and oesinophils. Moreover, lymphocyte with Fc receptor for IgM are exquisitely sensitive to the redistribution effect in vivo corticosteroids. Furthermore, both in vivo and vitro corticosteroids can alter the complex regulatory balance of helper and suppressor inleuences as well as alter both accessory and effector cells function (Cupps et al., 1982).

In general, corticosteroid impaired cellular immunity through inhibiting the migration of T-cells to the site of antigen disposition and blocking the local interaction between lymphocytes and monocytes (Parillo and Fauci, 1979).

B) Effect of Propranolol:

In the present study, the adminis-
mumps suppresses agglutination of PFCs to SRBCs in vitro. Moreover, CAMP increases the number of anti-RBC antibodies forming splenic cells in sensitized mice. The effect of propional is more or less similar. The antibody response produced by mouse spleen cells sensitized by SRBCs in vitro is higher than that of spleen cells sensitized in vivo. The enhanced IgM antibody response is responsible for the enhanced IgM antibody response. The increased IgM level in the plasma of propional-administered mice may be attributed to the increased production of PFCs. The increased IgG level in the plasma of propional-administered mice may be explained by the increased production of PFCs. The increased IgM level in the plasma of propional-administered mice may be explained by the increased production of PFCs.
Table (1): Effect of hydrocortisone hemisuccinate on serum γ globulin and IgG levels (mg/100ml) in sensitized mice (7 animals in each group).

<table>
<thead>
<tr>
<th></th>
<th>CONTROL GROUPS</th>
<th>TEST GROUP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>unsensitized group</td>
<td>Sensitized group</td>
</tr>
<tr>
<td>Mean of γ globulin</td>
<td>1268.33 ± 176.229</td>
<td>1600 ± 223.696</td>
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<tr>
<td>± S. D.</td>
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<tr>
<td>Mean of IgG</td>
<td>790.000 ± 126.39</td>
<td>1066 ± 154.96</td>
</tr>
<tr>
<td>± S. D.</td>
<td></td>
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</table>

P: Significance of difference between the means of the test drug and the sensitized control group.
P< : Significance of difference between the means of the sensitized control group and unsensitized control group.

Table (2): Effect of propranolol as serum γ globulin and IgG levels (mg/100ml) in sensitized mice (7 animals in each group).

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### Table (4): Effect of Hypocretinergic Hypothalamic on the HPA axis in mice in the spleen of sen.

<table>
<thead>
<tr>
<th>p</th>
<th>Mean ± S.D.</th>
<th>TEST GROUP</th>
<th>CONTROL GROUP</th>
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<tbody>
<tr>
<td>&gt; 10.0% &gt; 1.0%</td>
<td>197 ± 17.7</td>
<td>2, 3 subgroups</td>
<td>2, 3 subgroups</td>
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</table>

### Table (5): Effect of B-adrenergic etc.

<table>
<thead>
<tr>
<th>p</th>
<th>Mean ± S.D.</th>
<th>TEST GROUP</th>
<th>CONTROL GROUP</th>
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<tbody>
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<td>197 ± 17.7</td>
<td>2, 3 subgroups</td>
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</tr>
</tbody>
</table>
Table (5): Effect of propranolol on plaque forming cells in spleen of sensitized mice (7 animals in each group and subgroup).

<table>
<thead>
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<th>TEST GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitized group</td>
<td>1st subgroup</td>
</tr>
<tr>
<td>Mean ± S. D. P</td>
<td>34.33% ± 2.87</td>
<td>32.33% ± 7.174</td>
</tr>
</tbody>
</table>

P: Significance of difference between the mean of the test drug and the sensitized control group.

Table (6): Effect of pindolol on plaque forming cells in spleen of sensitized mice (7 animals in each group and subgroup).

<table>
<thead>
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<th>TEST GROUP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sensitized group</td>
<td>1st subgroup</td>
</tr>
<tr>
<td>Mean ± S. D. P</td>
<td>34.33% ± 2.87</td>
<td>31.66% ± 2.42</td>
</tr>
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</table>

P: Significance of difference between the mean of the test drug and the sensitized control group.
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REFERENCES


الملخص العربي

التأثير المحتمل لمضادات المستقبلات الإادرينالينية من نوع بيتا
في التفاعلات المناعية لحيوانات التجارب

أجريت هذه البحث لدراسة تأثير بعض مضادات المستقبلات الإادرينالينية من نوع بيتا مثل البريميترول والبندولون على التفاعلات المناعية في الفئران المستحقة مناعيًا وذلك بحث تنبيه بكرات الدم الحمراء من الأذناء.

وقد احتوى البحث على خمس مجموعات من الفئران:

المجموعة الأولى: تشمل على الفئران التي لم تستحث مناعاً.
المجموعة الثانية: تشمل على الفئران التي استحثت مناعياً وذلك بحثتنها بكرات الدم الحمراء من الأذناء في الغشاء البروتوني.
المجموعة الثالثة:
تشتمل على ثلاث مجموعات فرعية:

مجموعة (أ): تم حقنها باليبرامينول لمدة خمسة أيام ثم أستحثت مناعياً.
مجموعة (ب): تم حقنها باليبرامينول لمدة خمسة أيام مع حثها مناعياً في نفس الوقت.
مجموعة (ج): تم حقنها باليبرامينول لمدة خمسة أيام بعد حثها مناعياً.
المجموعة الرابعة: تشتمل أيضاً على ثلاث مجموعات فرعية مثل المجموعة الثانية تماماً مع إعطاء البريميترول بدلاً من اليبرامينول.
المجموعة الخامسة: تشتمل أيضاً على ثلاث مجموعات فرعية مثل المجموعة الثالثة مع إعطاء البندولون بدلاً من اليبرامينول.

وقد تم معرفة التأثير المناعي لهذه العقاقير بتعيين معدل التغير في كمية الجلوبولينات المناعية الكلية والمنوعية من نوع "ج" وأيضًا تعني معدل التغير في عدد الخلايا اللبمفارية المكونة للأجسام المضادة في الطحال.

وقد أوضح نتيجة البحث الآتي:
1. وجد أن حقن اليبرامينول في الغشاء البروتوني أحدث اختلافاً له دلالات إحصائية ملموسة جداً في كمية الجلوبولينات المناعية الكلية والمنوعية من نوع "ج" وجود الخلايا اللبمفارية المكونة للأجسام.

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