

The immune-modulating role of Potassium iodide on an ANA-Ease ELISA mode taking a positive sera for ANA (Anti-nuclear Abs) of the IgG isotype

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Abstract:

Background : Selective *in-vivo* anti-fungal action of Potassium iodide (KI) is an enigma, but circumstantial evidences strongly indicate some immune mechanism. **Aim :** To demonstrate *in-vitro* immune-modulating role of KI. **Materials & methods :** 31 sample (patient sera) collected for the detection of ANA (Anti-nuclear Abs) of the IgG serotype. Taking the positive sample, for each time ,KI solution (0.4%) was given at a different step (before the addition of conjugate, substrate , before & after the addition of doubly diluted patient sera) of the assay procedure, to note any significant change in the final O.D. reading, which is directly proportional to the antibody activity in the sample. Procedure repeated for five times & the average result was considered. Procedure repeated with inert carbon particles showed no visible change in O.D. value. **Results :** KI when introduced *after* addition of the doubly diluted patient sera ,the graphical interpretation showed that with increasing 2-fold serial dilution of patient sera, the corresponding O.D. read at 450 nm, almost showed a steady fall. **Conclusion:** KI probably imparts it's immune-modulating action by structural reformation of Ig molecule, explained in terms of affinity, that decreases proportionately with increasing 2-fold serial dilution of patient sera, with steady fall in the O.D. reading. Such immune-modulation may be related with therapeutic efficacy of this drug when used for clinical conditions, particularly, when no direct anti-microbial role of iodide is established.

Key words: Potassium iodide, immune-modulator ,ELISA, Serial dilution, O.D (Optical Density.)

I. Introduction

Potassium iodide (KI) is an agent which has no *in-vitro* antimicrobicidal action¹, yet has long been used for treating some bacterial or fungal diseases probably due to enhancement of specific immunoprotection mechanism by some unknown pathway².

Oral KI therapy is found to effect specific lysis of those fungi in tissues having histological hallmarks of some eosinophilic immune deposit^{3,4} around them in the form of Splendore-Hoeppli (SH) bodies^{5,6,7,8}. It also alters the course of Lepra reaction & some type III immune complex diseases.

All these indicate a need for experimental evidences of immune-modulating role of the drug.

Aim s & Objective of the study :

To study & interpret the level of interaction of KI with the antibody-antigen complex, conjugate & substrate, on an ANA-Ease ELISA mode so as to demonstrate & reinforce the *in-vitro* immune-modulating role of Potassium Iodide (KI) to predict their presumptive influences on *in-vivo* protection

II. Methods & Materials

Out of the 31 sample (patient sera) collected for the detection of ANA (Anti-nuclear Ab)of the IgG serotype, one sample (no.12) was found to be positive. Taking the positive sample, the assay was done.

- 1) Patient positive sera diluted to two-fold serial dilution with sample Diluent as 1/50, 1/100up to 1/6400, respectively.
- 2) 100µl from each of the respective doubly diluted patient sera was dispensed in to it's corresponding well & 100µl of standard positive control dispensed to the well kept for control.
- 3) Incubated for 20 minutes at room temperature.
- 4) After 20 minutes, the well contents were aspirated and washed with wash buffer for four times
- 5) 100µl of conjugate dispensed into each well & incubated for 20 minutes at room temperature.
- 6) After 20 minutes, the well contents were discarded & carefully washed for four times with wash buffer.
- 7) 100µl of TMB substrate dispensed into each well & incubated for 10 minutes.
- 8) 100µl of stop solution dispensed into each well. To allow equal reaction times, the stop solution has been added to the wells in the same order as the TMB substrate.
- 9) The optical density (OD) of each well was read at 450nm in a micro plate reader within 10 minutes.

Optical density is directly proportional to the antibody activity in the sample.

In our study, for each time (total for 4 times) KI solution (0.4%) was given at a different step (before the addition of conjugate, substrate , before & after the addition of doubly diluted patient sera) of the assay procedure, keeping the remaining steps same, to note any significant change in the final result so obtained. Procedure repeated with inert carbon particles showed no visible change in O.D. value.

III. Results

Out of the 31 sample (patient sera) collected for the detection of ANA of the IgG serotype, **sample no.12 was found to be positive.**

Sample No.	O.D.	Sample No.	O.D.	Sample No.	O.D.	Sample No.	O.D.
1.	0.330	9.	0.067	17.	0.146	25.	0.154
2.	0.130	10.	0.089	18.	0.119	26.	0.267
3.	0.110	11.	0.125	19.	0.221	27.	0.162
4.	0.150	<u>12.</u>	<u>2.470</u>	20.	0.118	28.	0.164
5.	0.180	13.	0.304	21.	0.147	29.	0.160
6.	0.540	14.	0.392	22.	0.054	30.	0.449
7.	0.220	15.	0.102	23.	0.046	31.	0.231
8.	0.160	16.	0.165	24.	0.113	Positive control	<u>1.447</u>

Taking the positive sera (sample no. 12), the assay was done.

In study A : KI was introduced **before the addition of the conjugate**. i.e., before step 5 of the assay procedure, keeping the remaining steps same.

In study B : KI was introduced **before the addition of the substrate**. i.e., before step 7 of the assay procedure, keeping the remaining steps same.

In study C : KI was introduced **before the addition of the doubly diluted patient sera**. i.e., before step 1 of the assay procedure, keeping the remaining steps same.

In study D : KI was introduced **after the addition of the doubly diluted patient sera**. i.e., before step 3 of the assay procedure.

In study E : **Inert Carbon particles** was introduced **after the addition of the doubly diluted patient sera**. i.e., before step 3 of the assay procedure.

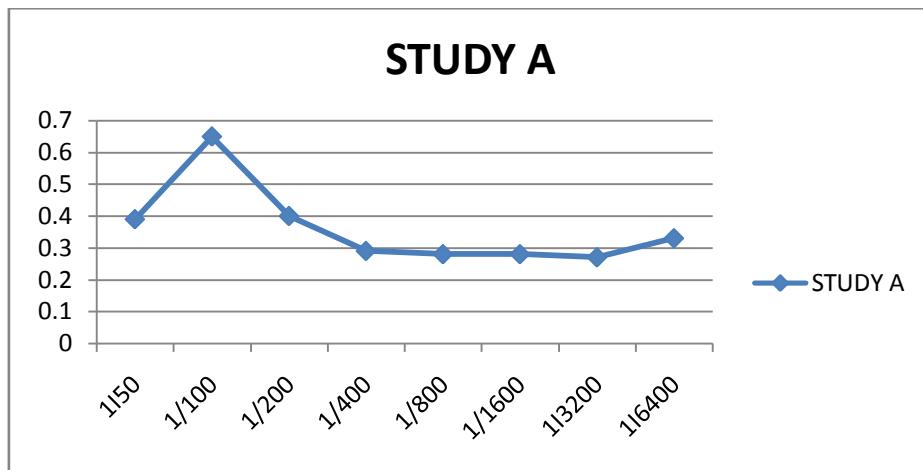
After completing the procedure of each study , the optical density (OD) of each well were read at 450nm. in a micro plate reader within 10 minutes.

Optical density is directly proportional to the antibody activity in the sample.

Each study(i.e., A,B,C,D) was repeated for five times & average reading was considered.

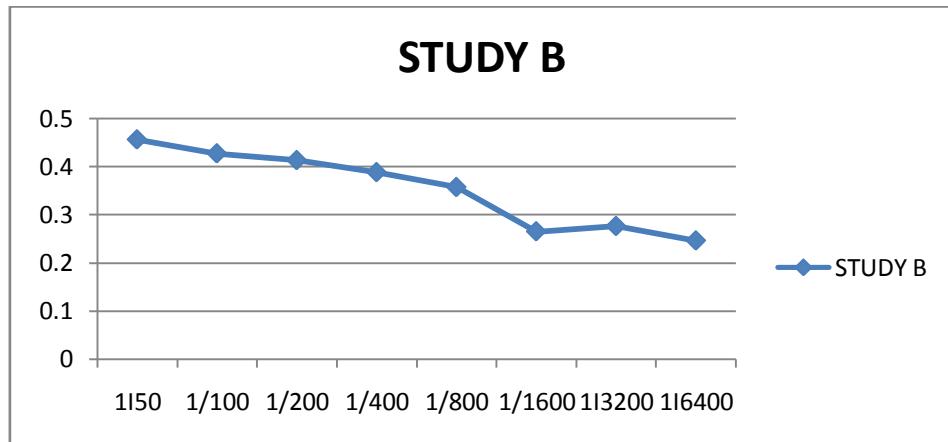
2 fold Dilution Of Ab →		1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
O.D. read at 450n.m.	Study A →	0.390	0.650	0.400	0.290	0.280	0.280	0.270	0.330
	Study B →	0.456	0.427	0.413	0.388	0.357	0.265	0.276	0.246
	Study C →	0.370	0.610	0.380	0.270	0.240	0.270	0.250	0.270
	Study D →	0.446	0.409	0.390	0.378	0.343	0.281	0.240	0.220
	Study E →	0.340	0.580	0.350	0.240	0.210	0.240	0.220	0.240

Study A:



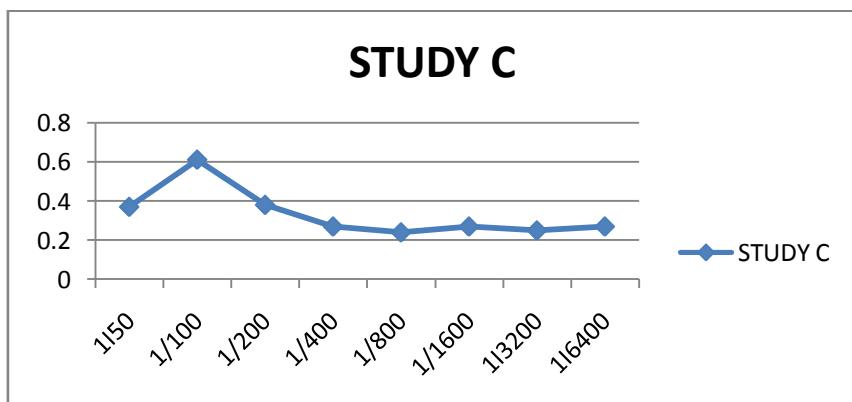
Here, with increasing 2-fold serial dilution of patient sera, the corresponding O.D. read at 450 nm, showed a initial peak followed by a rapid fall & then reached a plateau state , with a slight rise at the end. This uneven curve is of no definite pattern, indicating no significant interaction of KI with the sera &/or conjugate, with addition of KI at this stage of the reaction.

Study B :



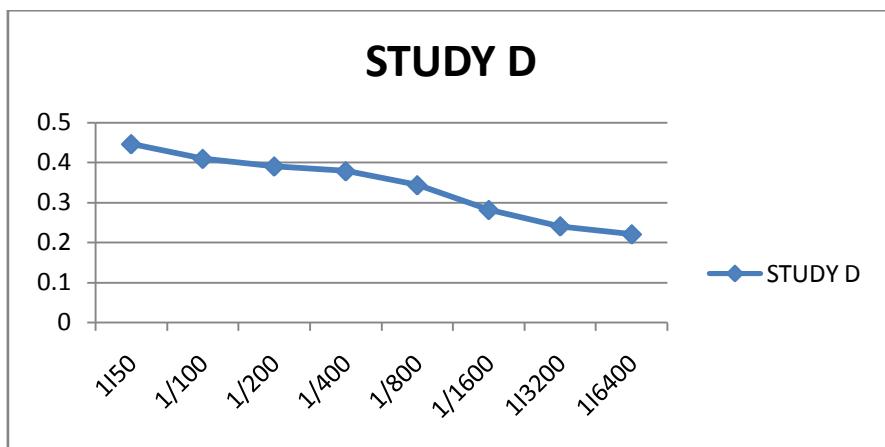
Here, with increasing 2-fold serial dilution of patient sera, the corresponding O.D. read at 450 nm, almost showed a steady fall. This steady decline of the curve indicates that there must have been a level of interaction with KI & the substrate, with addition of KI at this stage of the reaction. This is insignificant from our study point of view.

Study C :



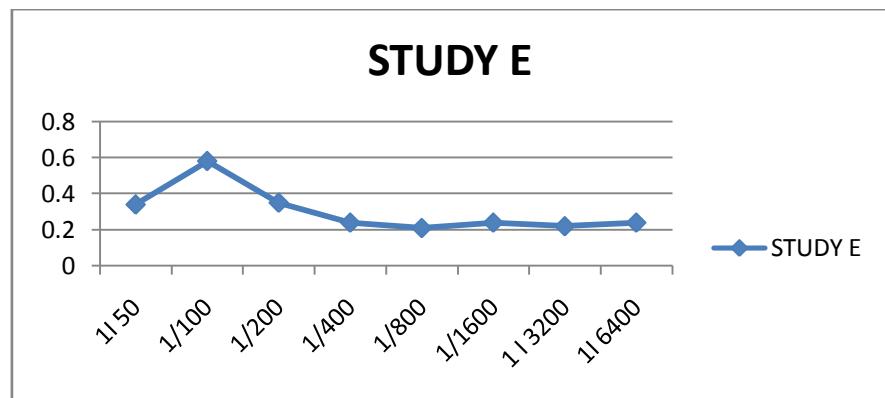
Here, with increasing 2-fold serial dilution of patient sera, the corresponding O.D. read at 450 nm, showed an initial peak followed by an uneven fall & rise. This uneven curve reflects no definite pattern, indicating no significant interaction of KI with the Antigen coated in the well, with initial addition of KI before the start of the assay procedure.

Study D :



Here, with increasing 2-fold serial dilution of patient sera, the corresponding O.D. read at 450 nm, almost showed a steady fall. This steady decline of the curve indicates that there must have been a level of interaction with KI & the added sera, with addition of KI at this stage of the reaction. This is significant from our study point of view.

STUDY E:



Here, with increasing 2-fold serial dilution of patient sera, the corresponding O.D. read at 450 nm, showed an initial peak with sharp fall followed by an almost steady line parallel to the base line. This uneven curve reflects no definite pattern, indicating no significant role of Carbon particles unlike KI.

IV. Discussion

Urabe, *et al.* (1969) believed⁹ that the therapeutic effect of KI was mediated through fungicidal effect of molecular iodine formed by the human body. Although KI appears to be particularly effective in those conditions where neutrophils predominate, the drug has an inhibitory effect on neutrophilic chemotaxis¹⁰. So, effect of KI on neutrophils cannot be a mechanism of action of the drug against selective infections. In fact, if any specific immune-protection mechanism is potentiated by some interaction in presence of KI, this would boost-up host- defense in general & would be a marvel drug for all varieties of microbial infections in immunocompetents.

Klebanoff *et al.* (1967)¹¹ hypothesized that iodine –induced enhanced intra-phagocytic killing was due to iodine- myeloperoxidase complex formation. The hypothesis is based on observation of *in-vitro* bactericidal activity against *Escherichia coli*, but in practice, it is neither effective against any *E coli* infections nor any intracellular bacterial infections. KI is effective against localized form of infections of some selective low virulence microbes only & paradoxically poorly effective against their systemic infections².

Human volunteer study by Rex & Bennett (1990)¹² disapproved the role of enhanced phagocytosis by KI-treated macrophages & pointed towards the need of a realistic hypothesis, because all previous hypothesis indicate wide -spectrum antimicrobial action of KI , which is not true & fail to explain why such is of very selective in nature, particularly in diseases with Splendore-Hoeppli phenomenon e.g. Sporotrichosis, Actinomycosis, Entomophthoramycosis , Chromoblasomycosis & some mycetomas¹³. Some other clinical conditions related to Arthus phenomenon e.g., nodular vasculitis, erythema nodosum¹⁴, Sweet's syndrome¹⁵. (Horio *et al.* 1980) were successfully treated by oral KI, when drug started at early onset.

Smith et al.(1998) reported¹⁶ a case of Sweet's syndrome in which KI was tried successfully after oral prednisolone and sequential addition of dapsone and minocycline all failed to produce clinical benefit E.J. Schulz(1976) found¹⁷ that, twenty-four of twenty-eight patients with erythema nodosum and sixteen of seventeen patients with nodular vasculitis responded to treatment with potassium iodide.

KI probably has got an immune-modulating role as evident from several *in-vitro* immunological reactions such as in immune reactions with incomplete Abs¹⁸, Complement mediated lysis of specific microbes¹⁹.

Visible immunological reactions depends on avidity & affinity of Antigen Antibody binding. Bovine Albumin(BA) caused visible agglutination of red cells with Anti-Rh D sera containing the incomplete IgG¹⁹. Probable explanation could be that addition of a polar molecule , such as BA in the suspending medium increases its ability to dissipate the repulsive force between the red cells. . The cells approach each other closely enough for an IgG molecule to span the gap, thus increases the avidity of the reaction resulting visible agglutination¹⁹

KI like BA , enhances agglutination reaction between Rh positive cell & Coombs positive sera¹⁹. It may also modify the outcome of Complement fixation dependant cytolysis either enhancing or retarding the reaction¹⁵.

KI probably imparts it's immune-modulating action by structural reformation of Ig (Immunoglobulin) molecule, explained in terms of avidity¹⁵ & sometimes by affinity. (Avidity refers²⁰ to the strength of association between multiple antibody binding sites and multiple antigenic determinants & Affinity refers¹⁶ to the strength of association between one antigenic determinant and one antibody binding site.).

The present model dealt with the study & interpretation of the level of interaction of KI with the antibody-antigen complex, conjugate & substrate, on an ANA-Ease ELISA mode taking a positive sera for ANA (Anti-nuclear Abs) of the IgG isotype. When KI was introduced after the addition of the doubly diluted patient sera (i.e., before step 3 of the assay procedure) ,the graphical interpretation showed that with increasing 2-fold serial dilution of patient sera, the corresponding O.D. read at 450 nm, almost showed a steady fall. . This is significant from our study point of view. We know that the optical density (O.D.) is directly proportional to the Ab activity in the sample. Here the Ag is fixed & coated to the well of the micro titre plate & the patient sera containing Ab is relatively mobile & so here the structural reformation of Ig molecule can be explained in terms of affinity following the addition of KI. KI probably enhances the affinity of the Ab towards the Ag molecule significantly so as to influence O.D. reading before the addition of the conjugate in the next step, after a sufficient time gap.. This level of affinity decreases proportionately with increasing 2-fold serial dilution of patient sera, thus, showed a steady fall in the O.D. reading. Here, KI probably imparts it's immune-modulating action by structural reformation of Ig (Immunoglobulin) molecule, explained in terms of affinity.

V. Conclusion

Such immune-modulation may have some relation with therapeutic efficacy of this drug when used for clinical conditions ,particularly ,when no direct anti-microbial role of iodide is established.

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