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Serum IgG and IgM Levels in Ranitidine Treated Healthy Volunteers

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SUMMARY

The effects of ranitidine on serum immunoglobulins, IgG and IgM levels was observed in healthy adult male volunteers, subjected to antigenic stimulation by tuberculin skin test. Ranitidine did not alter the serum IgG and IgM levels significantly when administered for 14 days.

INTRODUCTION

Histamine is known to down regulate the immune response after antigenic stimulation[1]. With the delayed hypersensitivity reactions this is partially caused by stimulation of the histamine H_2-receptors on T-suppressor (Ts) and cytolytic-T (Tc) cells[2,1]. The normal lytic function of Tc-cells is suppressed while the Ts-cells are stimulated to release the histamine induced suppressor factor (HSF) which inhibits other cell functions i.e. T-helper cell proliferation[1]. The production of HSF is abrogated by culture in medium containing a histamine H_2-receptor blocker but not by the addition of a histamine H_1-receptor blocker[1].

H_2-receptor antagonists, cimetidine and ranitidine have been documented to modulate the immune system in various animal and clinical trials[3-11].

Cimetidine inhibits the immune suppression mediated by Ts-cells[12]. The same effect has been observed with ranitidine[10]. T-cells also affect the ß-cell proliferation and release of immunoglobulins. The interaction between the T-helper cells and the ß-cells is essential for efficient production of immunoglobulins[13]. It appears that regulation of T-cells would affect the ß-cell function i.e. production of immunoglobulins.

Histamine appears to cause immuno-suppression after antigenic stimulation through its action on T-suppressor cells which express H2-receptors and not H1-receptors[1]. It has been documented in various trials that H2-receptor antagonists, cimetidine and ranitidine modulate the T-cell responses. Therefore it is possible that these drugs would also affect serum immunoglobulin levels, though indirectly i.e. only T-suppressor cells have H_2-receptors[2,1].

White and Ballow[7] reported increase in serum immunoglobulins levels in patients with common variable hypogammaglobulinemia after treatment with cimetidine. Pryzbulowski et al.[14] reported alterations in the IgG, IgM and IgA levels during treatment with ranitidine in patients of peptic ulcer. Nielson et al.[10] have also reported increase in the antibody response to tetanus toxoid ($P<0.01$) after ranitidine administration.

This study was therefore designed to verify the effect of ranitidine on serum IgG and IgM levels in healthy adult male volunteers when subjected to antigenic stimulation i.e. tuberculin skin test.

PATIENTS AND METHODS

Thirty three adult healthy male volunteers were randomly allocated to treatment in this single-blind placebo controlled trial over 15 days. Strict inclusion criteria were observed and only healthy individuals with similar socio-economic backgrounds, who were on no medication, between 18 to 25 years of age were admitted to the trial after ethical approval and informed consent had been obtained.

The individuals were randomly divided into two groups; placebo controlled group and the active treatment group (Table 1). During the trial the individuals were subjected to antigenic stimulation by administering tuberculin skin test. This was done to induce histamine release and activate the T-cells, which would interact with the β-cells as well as other cells.

The tuberculin skin test (Monotest) using multiple puncture technique (Heaf test) was administered twice during the trial i.e. day 5 and day 12. The 'kit' is manufactured by Pasteur Mereiux of France and contains purified tuberculin equal to 30,000,000 IU min in its centesimal composition.

The blood samples of both groups were taken on day 0 and day 15. Disposable syringes of 26 gauge needle were used to draw 2 cc of blood. After centrifugation in the laboratory, the sera were stored in the serum storage tubes in the deep freezer till the determination of serum IgG and IgM levels was done.

Determination of serum IgG an IgM levels was done using radial immunodiffusion method. The immunodiffusion plates "Nor-partigen[R] IgG-HC" and "NOR-Partigen[R]-IgM" are manufactured by Behringwerke AG, Marberg Germany. The kits are approved by the Paul-Ehrlich-Institut, Federal Office for Sera an Vaccines, Germany.

Each pack of "Nor-partigen[R] IgG-HC" and "NOR-Partigen[R]-IgM" consists of one 12-well plate. It contains monospecific antiserum to human IgG/s-chain and human IgM/μ-chain in a ready-for-use agarose-gel layer. The antiserum is obtained by immunization of rabbits, sheep and goats.

After obtaining the immunodiffusion plates from a local distributor, they were kept at a temperature of +6°C in the refrigerator of the laboratory. The temperature was strictly controlled. After opening, the tests were performed on the same day on one plate, all wells were utilized in one sitting.

Ranitidine (Zantac[R]) and placebo tablets resembling ranitidine tablets, were supplied for the purpose of this research by Glaxo Laboratories (Pvt) Limited. One tablet of Zantac[R] contains 150 mg of ranitidine as the hydrochloride.

A 300 mg oral dose of ranitidine (Zantac[R]) taken twice a day (2 tablets in the morning, 2 in the evening) was decided upon. This was in accordance with the dosage used in earlier studies[15] as well as those frequently used in gastric diseases[16].

The placebo tablets were similarly administered, 2 in the morning and 2 in the evening to the placebo controlled group (Group 1) (Table 1).

Determination of serum IgG and IgM

After all the samples had been obtained determination of serum IgG and IgM was carried out. Using the tablet provided for the purpose, the cover of aluminium container was pulled off. Plastic container was then removed and the opened plate was allowed to stand for 5 minutes at room temperature for evaporation of any condensed water which might have penetrated into the cells. The stored serum samples were taken out from the deep freezer and kept at room temperature for about 15 minutes to allow them to thaw. The volume of serum required per well was 5 μl (0.005 ml). This was obtained using a special suction micropippete. Procedure A (table of calibration values/1 control serum) was employed according to the instructions in the literature of the kit. For checking the accuracy of the kit, control serum provided along with the kit was introduced into well 1. Wells 2 to 12 in each plate were then filled by the specimens to be examined after introduction of the specimens the plates were allowed to stand tightly closed at room temperature.

Evaluation

After expiration of a diffusion period of 2 days for IgG and 5 days for IgM, the diameters (D) of the precipitates were measured using a scaled magnifying glass against a black background with lateral illumination. The corresponding assay results were ascertained by reading the values from the appended table of calibration values for the precipitate ring diameters measured.

Statistical analysis

The mean difference in the levels between day 0 and day 15 within each group was evaluated in the first step. Then this mean difference in the levels achieved in the active treatment group (Group 2) was compared to that achieved during placebo administration. Statistical significance of the mean absolute difference within each group was evaluated by applying paired student's t-distribution test and statistical significance of the mean absolute difference between the two groups (Group 2 vs 1) was evaluated by applying single-tailed student's t test.

RESULTS

Group 1

Seventeen healthy volunteers received placebo for 14 days. One volunteer developed fever on 8th day. He was dropped from the trial. Another was dropped due to non-compliance. The serum IgG and IgM levels in this group registered a statistically significant increase in their levels ($P<0.01$ and $P<0.005$) due most probably to tuberculin skin tests. This increase, however, remained within normal range and limits.

Group 2

Sixteen volunteers were allocated to this group. One of them developed diarrhoea on the 2nd day of treatment. He was dropped. Another developed epigastric discomfort, nausea and flatulence on the sixth day. He was also dropped.

The serum IgG and IgM levels determined at day 0 and day 15 registered a statistically highly significant increase ($P<0.01$ and $P<0.005$) similar to that seen in the placebo controlled group (Group 1). The increased levels remained within normal range and limits as seen in the placebo-controlled group (Group 1).

Table 1: Treatment groups.

	DAYS															
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Group 1 Placebo-controlled	Blood sample	P	P	P	P	P T:L	P	P	P	P	P	P	P T:R	P	P	Blood sample E
Group 2 Ranitidine trated	Blood sample	R	R	R	R	R T:L	R	R	R E	R	R	R	R T:R	R	R	Blood sample E

R, Ranitidine; P, Placebo; E, Evaluation of induration; T, Tuberculin test; L, Left fore-arm; R, Right fore-arm.

The treatments the groups received during study

The tuberculin reaction was interpreted 72 hours after administration

Table 2: Mean Serum Immunoglobulin Levels (IgG & IgM) (g/l) Standard Deviation

Group	*n*	*Levels*		*Day 0*	*Day 15*	*Mean absolute difference*
1.	15	IgG	(IFCC)	10.328±3.951	11.120±4.093	0.792±0.520
			(Behring)	11.227±4.296	12.085±4.448	0.858±0.566
		IgM	(IFCC)	1.304±0.331	1.514±0.358	0.210±0.099
			(Behring)	1.813±0.463	2.144±0.496	0.331±0.136
2.	11	IgG	(IFCC)	10.558±3.115	11.756±3.602	1.198±1.147
			(Behring)	11.476±3.386	12.778±3.914	1.302±1.247
		IgM	(IFCC)	1.275±0.390	1.574±0.447	0.299±0.117
			(Behring)	1.771±0.512	2.187±0.619	0.416±0.161

A statistically highly significant increase in serum IgG and IgM levels in both groups 1 and 2 ($P<0.005$). On comparison (Group 4 vs 1) the increase in the levels is statistically insignificant.

On group comparison (Group 2 vs 1) the mean absolute differences in serum IgG and IgM levels, between day 0 and day 15, were statistically insignificant (see results).

DISCUSSION

This study shows that ranitidine in therapeutic doses, does not significantly alter serum IgG and IgM levels in healthy adult male individuals when administered for 14 days. However both the levels, serum IgG and IgM, registered a statistically significant increase within the groups. This was most probably due to antigenic stimulation by the tuberculin skin test. The levels remained within normal range and limits in both groups. The increase seems to be a normal physiological response due to the interaction between T and β lymphocytes[17]. Since ranitidine modulates T cell activity, it was expected that the β cells would also be affected resulting in increase in the immunoglobulin levels. Contrary to the previous findings of Nielson et al.[10] who reported a positive effect of ranitidine on antibody response to tetanus toxoid, and Przybylowski et al.[14] who reported a tendency for an increase in the serum IgG and IgM levels in 25 patients of duodenal ulcer treated with ranitidine for 4 weeks, we could not get the same effect after ranitidine administration for 14 days. Therefore we conclude that ranitidine does not significantly alter serum IgG and IgM levels when administered for 14 days to healthy adult male volunteers subjected to antigenic stimulation. Greater and significant effect is seen on the cell-mediated immune response (tuberculin reaction) rather than on serum IgG and IgM levels. This is probably due to the fact that ranitidine does not affect β cells directly. Ranitidine blocks the histamine H2-receptors present on T-suppressor cells[1,8] preventing the histamine induced dampening of the immune response and thus modulating the delayed hypersensitivity reactions. But then T-cells regulate β cell activity[13]. Since this effect is indirect for ranitidine therefore, whether higher dosage of ranitidine for extended period of time is required to register any effect on the humoral immunity i.e. serum IgG and IgM levels[14], can be elucidated further investigation of this drug in comprehensive controlled clinical trials.

REFERENCES

1. Rocklin RE, Beer DJ. Histamine and immune modulation. Adv Intern Med 1983; 28: 225-51.
2. Melmon KL, Rocklin RE, Rosenkranz RP. Autacoids as modulators of the inflammatory and immune response. Am J Med 1981; 71: 100-106.
3. Gifford RRM, Ferguson RM, Vos BV. Cimetidine reduction of tumour formation in mice. Lancet 1981; 1: 638-39.
4. Hansbrough JF, Zapata-Sirvent RL. Shackford SR, Hoyt D, Carter WH. Immunomodulating drugs increase resistance against sepsis in traumatized mice. J Trauma 1986; 26: 625-30.
5. Jorizzo JL, Sams WM, Jegasothy BL, Olansky AJ. Cimetidine as an immunomodulator: Chronic mucocutaneous candidiasis as a model. Ann Intern Med 1980; 92: 192-95.
6. Tonnesen H, Knigge U, Blow S, et al. Effect of cimetidine on survival after gastric cancer. Lancet 1988; 2: 990-92.
7. White WB, Ballow M. Modulation of suppressor cell activity by cimetidine in paitents with common variable hypogammaglobuinemia. N Engl J Med 1985; 312: 198-202.
8. Hast R, Bernell P, Hansson M. Cimetidine as an immune response modifier. Med Oncol Tumor Pharmacother 1989; 6(1): 111-13.
9. Mertens WC, Bramwell VH, Gwadry-Sridhar F, Romano W, Banerjee D, Lala PK. Effect of indomethacin plus ranitidine in advanced melanoma in patients on high dose interleukin-2. Lancet 1992; 340(8816): 397-98.
10. Nielson HJ, Hammer JH, Moesgaard F, Heron I, Kehlet H. Ranitidine improves post-operative suppression of antibody response to preoperative vaccination. Surgery 1992; 111(1): 69-73.
11. Witkamp L, Velthius PJ, Verhaegh ME, et al. An open prospective clinical trial with systemic ranitidine in the treatment of psoriasis. J Am Acad Dermatol 1993; 28(5): 778-81.
12. Sahasrabudhe DM, McCune CS, O'Donnell RW, Henshaw EC. Inhibition of suppressor T-lymphocytes (TS) by cimetidine. J Immunol 1987; 138: 2760-63.
13. Ganong WF, Golub ES, Green DR, et al. Review of Medical Physiology. 16th Ed. Appleton & Lange, 1993: 473-80.
14. Przybylowski J, bartelik S, Szrajer J, Kolus D. Changes of IgA, IgG and IgM immunoglobulins and of 10 other plasma proteins in patients with duodenal ulcer treated with ranitidine. Wied Lek 1993; 46(2): 9-13.
15. Nielson HJ, Svenningsen A, Moesgaard F, et al. Ranitidine improves certain cellular immune responses in asymptomatic HIV-infected individuals. J Acquir Immune Defic Syndr 1991; 4(6): 577-84.
16. Meuwissen SGM, Klinkenberg-Knol E. Treatment of reflux oesophagitis with H2-receptor antagonists. Scand J Gastroenterol 1988; 23: 201-10.
17. Afoke AO, Eeg-Olofsson O, Hed J, Kjellman NI, Lindblom B, Ludvigsson J. Seasonal variation and sex differences of circulating macrophages, immunoglobulins, and lymphocytes in healthy school children. Scand J Immunol 1993; 37(2): 209-15.

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