Original article

Reduced levels of complement C3 after peros administration of ornidazole in normal volunteers

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SUMMARY

Ornidazole, [a-(chloromethyl)-2-methyl-5-nitroimidazole-1-ethanol], has recently been succesfully used in the treatment of active Crohn's disease. Although the initial results were encouraging, the mode of its action is largely unknown. The present study was undertaken in order to see if ornidazole has any influence on the immune status, since immunity plays a significant role in the pathogenesis of inflammatory bowel disease, especially in Crohn's disease. Ten healthy volunteers received orally 20 mg of ornidazole per Kg of body weight daily for eight days in two divided doses. The following immunological parameters were examined before, two and eight days after the administration of the drug: serum complement (C3 and C4), serum immunoglobulins (G, A and M), absolute number of peripheral lymphocytes, total B-lymphocytes (CD19+), total T lymphocytes (CD3), T helper-induced CD3+CD4 positive, T suppressor-cytotoxic (CD3+CD8 positive), Natural Killer cells (CD3-, CD16+, CD56+), Killer cell (CD8+CD58 positive) (lymphocyte activation index CD38) and subpopulation of B lymphocytes (CD20+ and CD5+). Other parameters examined were serum electrophoresis, number of platelets and number of white blood cells. The estimation of lymphocyte subpopulations was achieved by flow-cytometry technic in total peripheral blood using direct immunofluorence method with monoclonal antibodies of double color (double color immunofluorence). Ornidazole significantly reduced the levels of complement 3 after two and eight days of the peros administration, indicating that exclusive activation of the alternate pathway of complement activation occurs. However, other immune parameters showed no significant changes. It is concluded that ornidazole affects part of immune consistuents of normal volunteers if it is administered orally at a dose of 1000 mg per day for eight days. Further studies are needed in order to clarify the possible similar effects of the drug on the immune system of patients with inflammatory bowel disease.

Key Words: Cellular Immunity, Humoral Immunity, Nitroimidazoles, Ornidazole, Metronidazole, Crohn's disease, Inflammatory bowel disease

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INTRODUCTION

Metronidazole, a nitroimidazole derivate, has successfully been used in the treatment of patients with active Crohn's disease of the small and large bowel, especially in patients with concurrent perianal involvement.[1,2] Ornidazole (a-chloro-methyl-2-methyl-5-nitroimidazole-1-ethanol), a drug with similar molecular structure and biological actions to metronidazole, has recently been found to be effective in patients with active Crohn's disease.[3,4] It has also been used as a maintenance treatment in these patients with promising results.[5] Although the initial results of the administration of ornidazole on patients with Crohn's disease were encouraging, the mode of its action is completely unknown. Metronidazole has been reported to reduce the number of microabscesses in the inflammed mucosa[6] and to influence the lym-

phocyte proliferation kinetics and leucocyte-endothelial cell adhesions both *in vitro* and *in vivo* .[7-13]

The aim of this study was to test the hypothesis that ornidazole could influence the immune system. To investigate this, we examined several immunological parameters of normal people before and after the administration of the drug in pharmaceutical doses.

MATERIALS AND METHODS

Ten normal volunteers, participated in the study. There were six men and four women aged 43±4 years. All subjects were absolutely healthy. More specifically, none was suffering from any kind of autoimmune disease and none was receiving drugs of any kind. All subjects received 20 mg of Ornidazole per Kg of BW per day in two divided doses for a total period of eight days. The estimation of the immunological parameters two and eight days after the administration of the drug was based on the theoretical assumption that accumulation of the drug at the end of the eigthth day could have a different influence on lymphocyte subpopulation, compared to the influence achieved after a shorter period of administration.

The following immunological parameters were examined before, two and eight days after the administration of the drug: serum complement (C3, C4), serum immunoglobulins (G, A and M), absolute number of peripheral lymphocytes, subpopulation of T-cells (total T cells, (CD3), T-helper-inducer (CD3+CD4 positive), T-suppressor-cytotoxic (CD3+CD8 positive), NK cells (CD3, CD16+, CD56+), Killer cells (CD8+CD57 positive) and subpopulation of B-lymphocytes (CD20+ CD5+). The estimation of subpopulations of T and B lymphocytes was achieved by flow-cytometry in total peripheral blood using direct immunofluorence method with monoclonal antibodies of double color (double color immu-nofluorence). Total serum globulin levels and number of peripheral white blood cells were estimated by the usual methods. Study's protocol was approved by the Ethic's committee of our hospital.

Statistical analysis was performed using the SPSS/PC software program. The effects of the drug were analyzed using one-way analysis of variance (ANOVA). Following the one-way ANOVA, Scheffe multiple range tests were used for multiple comparisons among different period groups, i.e. before the administration of ornidazole, and on the second and eighth day. Figures represent mean±SD on every tested group.

RESULTS

No abnormalities in the liver and renal function tests were noticed. No abnormalities in other heamatological parameters were found at the end of the trial.

Table 1 shows the values of serum immunoglobulins, the absolute number of peripheral lymphocytes, the number of white blood cells, the results of serum electrophoresis and the values of total serum immunoglobulins before, two and eight days after the administration of ornidazole. No significant differences were observed.

Table 2 shows the results of the estimation of T and B lymphocyte subpopulations before, two and eight days after the administration of the drug. No significant differences were observed on either the second or eigth days after treatment.

Table 3 shows the results of serum estimation of complement C3 and C4. As indicated in the table, Scheffe multiple range tests revealed that statistically significant differences in the values of C3 serum complement were observed after comparison of the values of C3 before the administration of the drug and eight days after $\{F(2,12)=6.33,\ P<0.013\}$. However, differences in the values of C4 complement were not significant.

DISCUSSION

The results of the present study show that ornidazole significantly reduces the levels of complement C3 if it is administered for eight days at a dose of 20 mg/Kg of BW per day for 8 days. (The administration of the drug for no more than eight days was decided because we considered a longer period of administration in healthy volunteers as unethical). This reduction was more prominent and became statistically highly significant after eight days. To the best of our knoweledge, this reduction of the levels of C3 complement after administration of ornidazole has never been described before.

However, other immunological parameters showed no abnormality. The drug did not show any positive or negative influence on the values of subpopulations of T and B lymphocytes. Moreover, ornidazole did not have any influence on the absolute number of peripheral lymphocytes, the number of peripheral white blood cells and platelets and the level of total serum immunoglobulins.

The nitroimidazoles metronidazole and ornidazole have succesfully been used in the treatment of active Crohn's disease.[1-5] However, the exact mechanism of their action remains largely unknown. It is well known that

Table 1. Hematochemical and immunological parameters before, after 2 and 8 days of ornidazole treatment (means ± SD)

Parameter	Before	after 2 days	after 8 days	F (2,12)	P	
Serum electrophoresis						
	Albumin (%)	62.6±3.9	62.6±3.5	62.7±4.1	0,01	NS
	a1 globulin (%)	3.4±0.55	3.4±0.38	3.48±0.5	0,04	NS
	a2 globulin (%)	8.3±0.95	8.2±0.92	7.8±1.1	0.30	NS
	globulin (%)	12±0.84	11.6±0.9	11.4±1	0.57	NS
	globulin (%)	13.8±2.9	14±2.7	14.6±2.9	0,12	NS
Total Globulins (g/dl)	2.6±0.43	2.5±0.36	2.6±0.41	0,05	NS	
Serum immunoglobulins						
	IgG (g/l)	12.7±3.33	12.4±3.15	12±2.58	0,08	NS
	IgA (g/l)	2.31±0.71	1.97±0.55	2.3±0.64	0,47	NS
	IgM (g/l)	2.41±2.65	2.43±2.69	2.47±2.75	0,01	NS
PBL (/mm3)	1757±406	1859±500	1870±396	0,10	NS	
WBC (/mm3)	6536±802	6472±385	6224±932	0,24	NS	
Platelets (/mm3)	232800±19791	235200±6942	238600±15175	0,19	NS	
ESR (mm1111)	15.2±6.9	14.0±5.5	13.2±7.3	0,11	NS	

PBL = Absolute number of peripheral lymphocytes, WBC = White blood cells, ESR = Erythrocyte Sedimentation Rate, *NS = No Significant Differences*

Table 2. B and T lymphocyte subpopulations before after 2 and 8 days of ornidazole treatment (means ± SD)

Parameter	Before	after 2 days	after 8 days	F	P
CD3	74.6±8.4	75.8±8.6	74.6±10.3	F(2,12)=0.03	NS
DD19	8.4±4.8	7.8±3.1	8.2±4.9	F(2,12)=0.03	NS
CD4	41.8±7.27	41.8±5.5	40.2±7.9	F(2,9)=0.03	NS
CD8	38.5±2.2	39±4.5	36.3±7.2	F(2,9)=0.25	NS
CD4/CD8	2.8±2.2	3.8±1.7	2.2±1.9	F(2,9)=0.61	NS
CD3+CD4	41.3±7.2	41.8±7.2	43.2±9.9	F(2,10)=0.07	NS
CD3+CD8	33.3±7.6	30.8±6.6	31.5±6.9	F(2,6)=0.11	NS
CD57+CD8	14.8±5.5	15.6±6.3	13.2±5	F(2,10)=0.24	NS
CD3+CD16+56	11±5.5	16.3±4.5	13.4±6.9	F(2,10)=0.81	NS
CD4+CD38	21.2±6	20.4±6.4	22±6.8	F(2,12)=0.08	NS
CD8+CD38	13±3.6	14.4±3.4	13.6±3.2	F(2,10)=0.17	NS
CD20+CD5	7.8±2.8	9.4±4.4	8.8±2.4	F(2,12)=0.29	NS

NS = No significant differences

both drugs exhibit a strong antibacterial action against anaerobs. Metronidazole taken orally has been shown to increase the mitogenic response to phytohemagglutinin (PHA) in a dose-response fashion as well as to block the inhibitory effect of histamine on lymphocyte proliferation.[7] The same was also found in *in vivo* studies[9,10] indicating a possible immunostimulatory action. Moreover, metronidazole can promote the epithelization process[8] and reverse the leucocyte adherence and emigration responses elicited by indomethacin in *in vitro* experiments.[11] The latter could explain the beneficial effects of metronidazole on intestinal inflammation. However, older descriptions claimed that metronidazole could negatively influence cellular immunity.[13]

It is well known that activation of the complement system causes accelerated metabolism of the activated components. It is generally accepted that low concentrations of C4 indicate that classic pathway activation has occurred. On the other hand, decreased levels of C3 suggest that rather intense activation of either pathway is

Table 3. C3 and C4 complement serum levels before, after 2 and 8 days of ornidazole treatment (mean ± SD)

	Serum Complement	
	C3 (g/l)	**C4 (g/l)**
Before	0.96±0.09* *	0.26±0.05
After two days	0.84±0.09*	0.24±0.09
After eight days	0.76±0.09* *	0.2±0.07
F(2,12)	6.33	0.87
P	P<0.01)	NS

**=Statistically significant differences between subgroups in Sheffe Tests*

occuring and this decrease, if found to be associated with normal levels of C4 (as in the case of administration of ornidazole), indicate that exclusive activation of the alternate pathways is occuring.[14]

In Crohn's disease an increased rate of synthesis and catabolism of C3 has been observed, implying activation of complement sequence, even though serum levels of C3 are not depressed. The fractional catabolic rate and synthesis rate of C3 are increased in patients with Crohn's disease.[15] Significantly raised levels of serum C3 in patients with Crohn's disease were found in another study.[16] In this study the levels of C3 were lower in patients with inactive than in patients with active disease. Substantially elevated plasma C3c in Crohn's disease suggests hypercatabolism of C3, that is involvement of complement reactions.[17] It has been found that the mean C3 concentration in jejunal fluid concentration of patients with Crohn's disease was higher compared to normal controls[18] and this was attributed to stimulated synthesis of complement by activated intestinal monocytes and macrophages. It has recently been proposed that there is a locally regulated production of complement in the intestine of patients with Crohn's disease, as cells expressing complement genes have been identified in the intestinal wall.[19]

It is well accepted that gastrointestinal inflammation is attenuated by antibiotics active against anaerobic bacteria (such as ornidazole) and is accelarated by luminal bacterial overgrowth. It is possible therefore, that the mode of action of these drugs could be related either to their action against the gut anaerobs or to the immune system or both. We think that further studies, both on normal people and patients suffering from inflammatory bowel diseases are needed, in order to further clarify this interesting matter.

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