Inhibition of cell mediated cytotoxicity by sulphasalazine: effect of in vivo treatment with 5-aminosalicylic acid and sulphasalazine on in vitro natural killer cell activity

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Abstract

Decreased cell mediated cytotoxicity occurs frequently in inflammatory bowel disease, particularly in patients with active disease. It is not clear, however, whether this decrease is caused by the disease or is a consequence of the medical treatment. In this study we evaluated the effect of in vivo treatment with 5-aminosalicylic acid and sulphasalazine on the in vitro natural killer cell activity in five patients with inflammatory bowel disease in remission and in four healthy control subjects in a double blind randomised crossover trial preceded and separated by four weeks of treatment with placebo. The natural killer cell activity was significantly impaired in 67% (six of nine subjects) after four weeks' sulphasalazine treatment and tended to be related to subjects with a slow acetylator phenotype. In contrast, 5-aminosalicylic acid treatment caused only a marginal reaction in the natural killer cell activity in 22% (two of nine subjects). The inhibitory effects were found to be reversible since the decreased natural killer cell activity was completely restored after placebo treatment in all subjects. In conclusion, in vivo treatment with sulphasalazine inhibits the in vitro natural killer cell activity and this seems to be mediated by the sulphapyridine moiety. This phenomenon may contribute to the low natural killer cell activity found in patients with active inflammatory bowel disease.

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Natural killer cells have a role in many aspects of the inmmunological defence system. Their major function is induction of cytolysis of malignant and virus infected cells.[1] Besides their involvement in cellular immune mechanisms, they also play a part in the humoral immune response, as shown by the interaction with B cells in the regulation of the immunoglobulin synthesis.[2] With regard to cellular cytotoxicity, several studies have shown that natural killer cell activity is impaired in patients with inflammatory bowel disease.[3-6] Moreover, in some studies this impairment was found to be related to the disease activity.[7 8] The major problem with the interpretation of results obtained in patients with active disease, however, is that it is usually impossible to determine whether disease activity or the effect of medical treatment is the cause of impaired natural killer cell activity.

Sulphasalazine is a commonly used drug for the treatment of inflammatory bowel disease and

rheumatoid arthritis. Its mode of action is still unclear, although it has been shown to possess both antibacterial and immunosuppressive activity. The active moiety seems to be 5-aminosalicylic acid in inflammatory bowel disease and sulphapyridine in rheumatoid arthritis.[9-11] The immunosuppressive activity of the drugs has been illustrated by inhibition of phagocyte chemotaxis, oxygen metabolite production, leukotriene production, and lymphocyte proliferative activity in vitro.[12-15] Moreover, sulphasalazine and sulphapyridine were recently shown to inhibit in vitro cellular cytotoxicity of both peripheral blood and intestinal mononuclear cells.[16-18] It is still unclear, whether in vivo treatment with sulphasalazine has a similar inhibitory effect on natural killer cell activity. Since deranged cell mediated cytotoxicity does not seem to be involved in the pathogenesis of inflammatory bowel disease,[5 6] inhibition of natural killer cell activity by sulphasalazine may result in insufficient clearing of infected and malignant cells.

In this study we evaluated the effect of 5-aminosalicylic acid and sulphasalazine treatment on the in vitro activity of peripheral blood natural killer cells in five patients with inflammatory bowel disease in remission and in four healthy control subjects.

Material and methods

STUDY DESIGN

We studied five patients with inflammatory bowel disease (one with Crohn's disease and four with ulcerative colitis) in remission and four healthy volunteers in a double blind randomised crossover trial. Both drugs were administered orally, 5-aminosalicylic acid at a dose of 500 mg four times a day and sulphasalazine at a dose of 1000 mg four times a day for four week periods preceded and separated by four week placebo treatment periods (double dummy design). The

TABLE I Natural killer cell activity of patients and control subjects after four weeks' treatment with 5-aminosalicylic acid (5-ASA), placebo, and sulphasalazine (SASP)

	5-ASA	Placebo*	SASP
Controls (n=4)	62·5 (6·3)†	59·2 (5·9)	26·2 (18·3)
Patients (n=5)	52·5 (9·1)	74·4 (4·8)	40·2 (18·2)
All (n=9)	56·9 (5·7)	67·6 (4·4)	34·1 (12·4)‡

*Mean percentage cytotoxicity of the two placebo periods.
†Mean (SEM) percentage cytotoxicity.
‡p<0·05 significance of differences compared with the placebo.

drugs and matching placebo's were supplied by Tramedico-Falk (The Netherlands). At the end of each four week period blood was taken and the disease activity was determined by the Harvey and Bradshaw index[19] from diary cards maintained by the patients during the whole period. The four healthy volunteers had no physical disorders and were taking no medication.

CYTOTOXICITY ASSAY

Mononuclear effector cells were obtained by Ficoll-Hypaque density gradient centrifugation of heparinised peripheral blood. The cells were washed and resuspended in Roswell Park Memorial Institute 1640 culture medium supplemented with 10% heat inactivated fetal calf serum, 2 mM glutamine, and antibiotics and after counting were brought to a concentration of $2 \cdot 5 \times 10^6$ cells/ml. The viability of the cells always exceeded 97%. The target cells used in the cytotoxicity assay were the K-562 erythroleukemia cell line cultured in the same culture medium. The K-562 cells were labelled with 100 μCi/5×10^6 cells of sodium[51]-chromate and used in a concentration of 1×10^5 cells/ml. The cytotoxicity assay was performed in triplicate at a 50:1 effector-target ratio in a volume of 200 μl. After 18 hours incubation, the supernatants of the 96 microwell plates were harvested (Skatron supernatant harvesting system). The released label was counted in a gamma counter. Spontaneous release was determined by incubation of the targets alone and maximal release by incubation of the target cells with saponin. Cytotoxicity was determined by the following formula:

$$\text{Cytotoxicity (\%)} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100$$

The reproducibility of this assay was good as illustrated by the intra-assay coefficient of variance ($2 \cdot 8\% \pm 0 \cdot 5\%$) and the inter-assay coefficient of variance ($4 \cdot 2\% \pm 1 \cdot 4\%$) determined by triplicate determinations of five subjects at two different time points.

Serum samples obtained at the end of each period were analysed for sulphasalazine, sulphapyridine, and acetyl-sulphapyridine by a colori-

metric assay after hydrolysation and extraction of the serum as previously reported.[20] The acetylator phenotype of each subject was determined by the percentage acetyl-sulphapyridine v sulphapyridine + acetyl-sulphapyridine in the serum. When less than 30% of sulphapyridine was acetylated the subject was considered to have a slow acetylator phenotype.[21]

STATISTICAL ANALYSIS

All results are expressed as mean (SEM). Statistical significances of the differences were evaluated by the Student's t test or the separate variance analysis when the standard deviations were statistically different.

Results

The natural killer cell activity was similar in patients and control subjects at the end of the placebo periods (Table I). The mean cytotoxicity values of the two placebo periods were identical in patients and controls (combined 64·6 (5) v 68·5 (5·7)). Moreover, the reproducibility of the natural killer cell activity determinations, expressed as the correlation coefficient of the two placebo periods from patients and controls, was good ($r = 0 \cdot 95$, $p < 0 \cdot 02$).

The natural killer cell activity after the sulphasalazine period was significantly decreased both in patients and controls compared with the mean of the two preceding placebo periods ($p < 0 \cdot 05$, Table I). In contrast, 5-aminosalicylic acid did not affect the natural killer cell activity in the control subjects and only marginally in the patients. The natural killer cell activity was impaired – that is, below the lowest placebo value (46·2%) – after sulphasalazine treatment in six (67%) subjects (three patients and three controls) (Fig (a)), but was reduced in only two subjects (one patient and one control) after 5-aminosalicylic acid treatment (Fig (b)). All patients remained in remission during the different treatment periods. Thus, the changes in the natural killer cell activity were not attributable to the disease activity according to the Harvey and Bradshaw index (Table II). Neither the concentration of sulphasalazine nor of its metabolites in serum were found to be related to the change in natural killer cell activity or the resulting natural killer cell activity at the end of the sulphasalazine treatment period (Table III). The percentage of subjects with an impaired natural killer cell activity after sulphasalazine treatment tended to be higher, however, in the slow acetylator group (80%, four of five) than in the fast acetylator group (50%, two of four).

Discussion

Treatment with sulphasalazine suppresses natural killer cell activity of peripheral blood cells in vitro, which seems to be more prominent in subjects with a slow acetylator phenotype. In contrast, 5-aminosalicylic acid had no significant effect on natural killer cell activity.

Studies on natural killer cells in inflammatory bowel disease indicate that these cells probably do not play a role in the pathogenesis of the

Natural cell activity of patients ●........● and controls ●____● after in vivo treatment with sulphasalazine (a) or 5-aminosalicylic acid (5-ASA) (b) compared with the preceding placebo period.
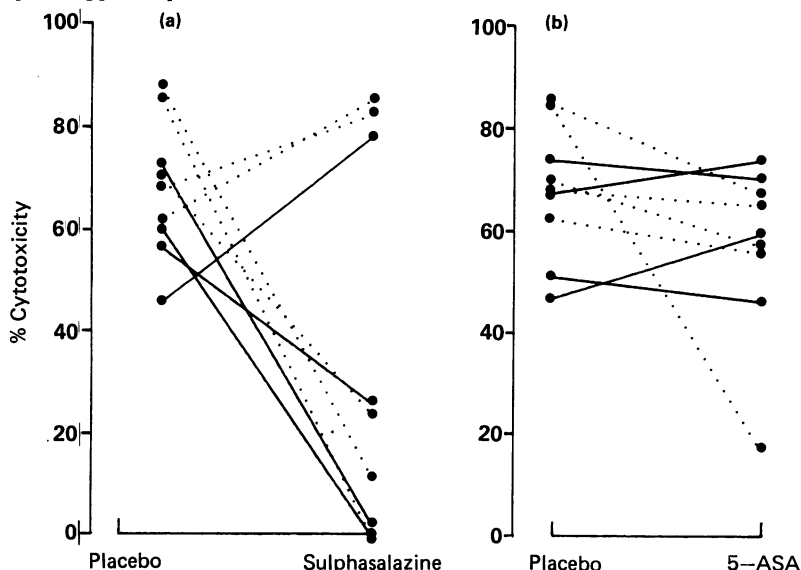
TABLE II   *Disease activity of patients with inflammatory bowel disease according to the Harvey and Bradshaw index during the different periods of treatment.*

Patient	Placebo	5-ASA	Placebo	SASP
1	2	0	0	0
2	2	2	5	0
3	0	0	2	0
4	1	1	0	2
5	0	0	0	1

5-ASA=5-aminosalicylic acid; SASP=sulphasalazine.

disease.[5][6] Peripheral blood natural killer cell activity is usually low in inflammatory bowel disease[3-8] and the activity and number of natural killer cells in the intestinal wall are often so low that they are unlikely to lyse and destroy normal autologous epithelial cells.[4][5] Natural killer cells do, however, take part in the immunological homeosteasis, and inhibition of their activity may have detrimental effects on the immunological defence mechanisms against infection and malignant disease. Previous studies on the effect of sulphasalazine and 5-aminosalicylic acid on natural killer cell activity were performed by addition of these drugs in vitro to the assay system.[16-18] These studies showed unequivocally the inhibition of natural killer cell activity by sulphasalazine and sulphapyridine but not by 5-aminosalicylic acid. In this study we showed that in vivo administration of sulphasalazine to patients with inflammatory bowel disease or controls also resulted in decreased natural killer cell activity. This effect was reversible, as shown after four weeks of treatment with placebo. Since this inhibitory effect is hardly observed with 5-aminosalicylic acid, it is probable that the sulphapyridine moiety of sulphasalazine is mainly responsible for the inhibition of natural killer cells in vivo, which may also explain the higher frequency of inhibition in the slow acetylator subjects. Interestingly, sulphapyridine was also found to be the active component in the inhibition of the production of oxygen metabolites by granulocytes, indicating that this moiety seems to possess the most prominent immunosuppressive feature.[13] The fact that sulphasalazine, and to a much lesser extent 5-aminosalicylic acid possesses natural killer cell inhibitory effects does not exclude disease activity as another cause of impaired natural killer cell activity in inflammatory bowel disease. We found no relation between prednisolone treatment and decreased natural killer cell activity in a previous study of patients with

inflammatory bowel disease[8] and MacDermott et al[4] found low natural killer cell activity in untreated active inflammatory bowel disease.

In conclusion, treatment with sulphasalazine, in contrast to treatment with 5-aminosalicylic acid, can inhibit natural killer cell activity in vitro. This treatment may contribute to impaired natural killer cell activity in some patients. The decreased natural killer cell activity in patients with inflammatory bowel disease, however, is one of the many immunological changes that occur during the inflammatory process and are probably secondary to the disease.

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TABLE III   *Serum concentrations of sulphasalazine (SASP) and its metabolites and natural killer (NK) cell activity after four weeks' treatment with SASP*

	SASP*	Ac-SP*	SP*	% Ac-SP† (phenotype)	NK before‡	NK after‡
Patient 1	11	22	77	22 (slow)	68·1	82·4
Patient 2	72	26	46	36 (fast)	62·4	85·3
Patient 3	10	44	50	47 (fast)	87·7	11·1
Patient 4	18	57	279	17 (slow)	69·9	23·4
Patient 5	3	23	100	19 (slow)	85·4	-1·0
Control 1	2	48	23	68 (fast)	45·7	78·1
Control 2	0	37	13	74 (fast)	56·3	25·6
Control 3	4	6	58	9 (slow)	59·9	-0·6
Control 4	0	6	20	23 (slow)	72·3	1·8

*SASP, acetyl-sulphapyridine (Ac-SP), and sulphapyridine (SP) in µmol/l.
†Determined by Ac-SP/(Ac-SP+SP), between parentheses acetylator phenotype.
‡% Cytotoxicity.