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Natural Cytokines: New Potentialities of Immunotherapy of Gastroduodenal Ulcer

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Addition of splenocytokine therapy to combined therapy of peptic ulcer accelerated healing and prolonged remission.

Key Words: *extracorporeal connection of donor spleen; splenocytokine therapy; splenoperfusate; splenopide*

The philosophy of therapy of peptic ulcer is complex and largely depends on its views on the etiology and pathogenesis. Many protocols of conservative therapy with histamine H_2 receptors, proton pump inhibitors, cytoprotectors, and antibiotics are known. Despite introduction of standard protocols of the drug therapy, every year about 6000 patients die from consequences of inadequate treatment of this condition.

The use of porcine donor spleen and splenic preparations, containing bioactive substances and natural cytokines (short-distance immune response mediators) is an intensely developing trends in the treatment of many diseases associated with impairment of the barrier properties in the whole body and tissues against the background of immunodeficient states [1,2,10]. The use of donor spleen and its preparation leads to local acceleration of reparative processes and systemic activation of cell immunity and nonspecific defense factors.

MATERIALS AND METHODS

In the search for more effective methods for the treatment of peptic ulcer, refractory ulcers, we used several variants of splenocytokine therapy (SCT).

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A total of 230 patients (18-67 years; 57% patients aged 20-40 years; 173 men and 57 women) were observed. The patients were divided into 5 groups: 4 main groups received SCT in addition to combined therapy, and one control group. Group 1 consisted of 22 patients; traditional therapy was supplemented by extracorporeal connection of porcine donor spleen (ECCDS) and later the patients received splenoperfusate (SP) orally. Group 2 consisted of 9 patients with nonhealing ulcers (for 89.6±12.3 days), in whom traditional treatment was supplemented with ECCDS and transendoscopic injections of splenopide around the ulcerative defect. The disease in this group was more refractory, with concomitant disorders. Group 3 (30 patients) received a complex of antiulcer SP therapy: intravenously (10 patients) and orally (20 patients). Group 4 consisted of 88 patients; combined therapy was supplemented by transendoscopic injections of splenopide around the ulcers. Group 5 (control) consisted of 80 patients receiving traditional conservative therapy, 20 of these receiving placebo orally.

Combined therapy of all patients was carried out according to standard eradication protocols.

Splenocytokine therapy included extracorporeal connection of porcine donor spleen, oral and intravenous administration of SP, and transendoscopic injections of splenopide around the ulcers.

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ECCDS was carried out under conditions of the operation room. After special treatment, the spleen was connected via the veno-venous contour under conditions of total or local heparin treatment. Perfusion was carried out using a roller pump (20-30 ml/min during 40-50 min).

Splenoperfusate for intravenous and oral treatment was prepared by the standard method; 400 ml SP was drip-infused intravenously. The course consisted of 5-7 infusions at 2-3-day intervals.

Oral SP treatment: 100 ml SP 1 h before meals (twice a day at 12-h intervals); 30 min before SP, the patients were injected (intramuscularly) with 1 ml 0.1% atropine. The duration of treatment was 14-21 days.

Transendoscopic injections around ulcerative defects were carried out using immunomodulator splenopide (Institute of Transplantology and Artificial Organs; Registration Certificate R No. 001938/01-2002 of December 19, 2002). This preparation is a mixture of bioactive peptides isolated from porcine and cattle spleens, containing native cytokines (IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ, granulocytic macrophagal CSF). After detection of the ulcer defect during fibrogastroduodenoscopy it was washed with 0.25% procaine solution, after which 2 ml (40 mg) preparation was injected into the submucous layer with a needle injector into 3 sites of ulcer edges; the treatment was carried out at 1-2-day intervals. No complications or side effects were observed.

Indications for SCT are as follows: refractory ulcers; frequent exacerbations (2 and more annually); decreased total and immunological resistance; contraindications to surgical treatment of peptic ulcer. SCT was also carried out as preoperative treatment in patients with indications for surgical treatment of peptic ulcer.

One-third patients (32.3%) had ulcer history longer than 10 years. 101 patients of the main groups (44.1%) had ulcers that did not heal for a long time. Ulcers not healing within 1.5-2 months (duodenal ulcers) and 2.5-3 months (peptic ulcers) of drug therapy were considered not healed for a long time (refractory).

On admission all patients presented with clear-cut clinical manifestations of peptic ulcer exacerbation. The leading syndromes in the clinical picture were pain (in all cases) and dyspepsia (in 83.6%).

Fibroesophagogastroduodenoscopy was carried out in all patients before the study; this method detected the morphological substratum of the disease: chronic ulcer in the stomach or duodenal bulb. The ulcer was located in the stomach in 74 (32.3%), in the duodenal bulb in 146 (63.8%) patients, and 10 (3.9%) had ulcers of both location.

Ulcer size varied from several millimeters to 3-4 cm; oval and round ulcers predominated. Deep and crater-like ulcers with solid edges were detected in 67% patients.

Acid-producing function of the stomach was evaluated by intragastric pH-metry.

Histological studies of biopsy specimens of the gastroduodenal mucosa, collected in the periulcerous zone, from the bottom and edges of ulcer defect were carried out.

Inflammatory changes in the gastroduodenal mucosa were classified into 3 degrees [6] according to the severity of lymphoplasmacytic infiltration of the mucosal lamina propria.

The severity of chronic duodenitis was evaluated as described previously [13].

The effect of SCT on humoral nonspecific defense factors was evaluated. Serum bactericidal acti-

TABLE 1. Comparative Data on the Efficiency of Ulcer Eradication Therapy

Treatment protocol	Day of disappearance of subjective manifestations of peptic ulcer	Day of healing	Day of refractory ulcer healing
Traditional drug therapy+SCT			
splenopide injections around ulcer	4-6	12-14	12-15
ECCDS+SP orally	4-6	17-18	20-24
ECCDS+injections	4-5	10-12	12-14
SP intravenously+SP orally	5-8	18-20	24-30
Standard protocols of treatment for ulcer			
de-nol+trichopol+tetracyclin	10-12	22-25	No healing
quamatel+clacide+trichopol	10-12	20-22	No healing
ranitidine+trichopol+amoxicillin	12-13	21-24	No healing
omeprasole+de-nol+trichopol+tetracyclin	7-9	18-20	No healing
traditional drug therapy+injections of other drugs around ulcer	6-8	14-16	No healing

vity (SBA) was evaluated by modified photonephelometric method [8], serum complement by hemolytical titration by 50% hemolysis [7], β-lysins by photonephelometric method [3], and lysozyme by modified photonephelometrical method [4]. The function of B-immunity system was evaluated by serum levels of IgA, IgM, IgG as described previously [12]. Cell immunity was evaluated by blood counts of total lymphocytes, T-lymphocytes, B-lymphocytes, and T-lymphocyte subpopulations. They were studied by the rosette formation test [11]. *Helicobacter pylori* were detected by histological method (Giemza staining) and by urease method [9].

RESULTS

The efficiency of splenotherapy was evaluated by clinical, endoscopic, morphological, and immunological parameters.

Pain syndrome disappeared on days 3-4 in the majority of patients receiving splenotherapy, dyspepsia disappeared on days 5-6. In controls these clinical manifestations of peptic ulcer exacerbation were arrested on days 9-14.

Endoscopic control was carried out before treatment, after disappearance of clinical syndromes of peptic ulcer (days 7-10), on days 14-17 and 21-30. In patients subjected to transendoscopic injections around the ulcer the healing was controlled during every session of injections.

Stage I transformed into stage II on days 5.0±1.5 in patients receiving SCT (after 2-3 injection sessions). Perifocal edema and hyperemia became less pronounced by this term, inflammatory border decreased, the edges of ulcer defect flattened. The ulcers shrank due to migration of the ulcer crater edges towards the center and convergence of the folds. Ulcer bottom was cleansed from deposit. Inflammation in the gastroduodenal mucosa persisted (Fig. 1).

The endoscopic picture of "red cicatrix" corresponded to transformation of stage II into stage III (on days 10±2, 4-5 sessions of injections). Ulcerative defect was presented by a fine linear cicatrix with solitary granulations at the edges. Inflammation of the gastroduodenal mucosa decreased (Fig. 2). Control endoscopy on days 35±3 showed transformation of stage III into stage IV. Fibrogastroscopy showed no ulcer.

Ulcers in the main groups healed on days 12-15 on average, clinical symptoms disappeared and ulcers healed more rapidly in the groups receiving transendoscopic injections of splenopide around the ulcers (Table 1).

The layer of necrotic mass gradually thinned, signs of inflammation in the mucosa disappeared (neutrophil, plasmacyte, macrophage counts decreased). As reparative processes progressed, the granulation tissue at the bottom of the ulcer grew and the epithelium "crawled" from ulcer edges towards the center. Analysis of biopsy specimens from sites of former ulcer defect of controls showed persisting lymphoplasmacyte infiltration, dysregeneration, and replacement of granulation tissue by coarse connective tissue fibers. No pronounced degenerative and inflammatory changes were detected in biopsy specimens from patients receiving SCT.

Study of acid-producing function of the stomach after treatment in the main groups showed 40% elevation ($p<0.05$) of pH in hyperacidity cases.

By the beginning of treatment serum bactericidal activity and lysozyme were suppressed in all patients. The complement values were less decreased. The level of β-lysins surpassed the normal.

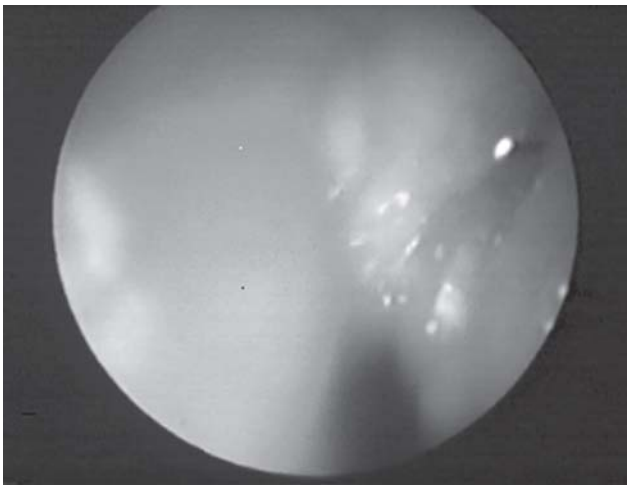

Fig. 1. Ulcer defect (1.5×1.0 cm) with edematous edges at the upper contour of the duodenal bulb and closer to its posterior wall. The bottom is filled with fibrin. Pronounced deformation of the bulb.

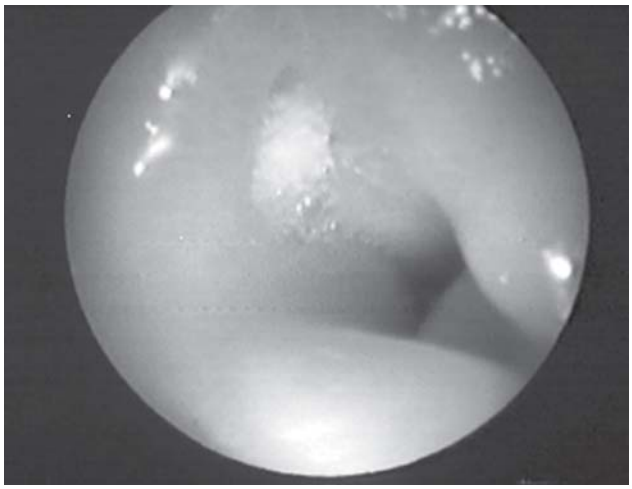

Fig. 2. Surface ulcer defect (0.4×0.3 cm), day 8, session 4, without clear-cut borders, with slight uneven fibrin in the center.

TABLE 2. Dynamics of Factors of Nonspecific Defense in the Main Groups

Group	Lysozyme, g/ml	β-Lysins	SBA, %	Complement, Unit/ml
Control (donors)	17.0±0.5	37.7±1.9	75.7±0.4	50.9±0.8
Main (uncomplicated ulcers)				
before therapy	15.5±0.2	46.2±1.1	56.8±1.0	50.0±1.4
day 4	21.7±0.3***	36.2±1.0***	37.0±1.3***	58.5±1.5*
by discharge	19.2±0.2***	37.3±0.5***	79.4±1.8***	54.10±0.84*
Main (refractory ulcers)				
before therapy	13.5±0.8	52.0±1.9	49.5±2.7	44.0±1.3
day 4	17.5±0.8*	46.6±1.8**	63.1±3.4***	48.5±1.3*
by discharge	18.3±0.7*	37.2±3.3**	68.0±3.5**	49.5±3.3*

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the corresponding values before therapy.

TABLE 3. Cell Immunity Parameters in Peptic Ulcer Patients before and after SCT

Parameter	Group			
	uncomplicated duodenal ulcers	refractory duodenal ulcers	uncomplicated gastric ulcers	refractory gastric ulcers
Absolute lymphocyte count/1 μl				
Total count	2473.78±252.20 2075.68±240.40	2241.62±187.87 2138.44±135.22	2603.88±591.45 1967.76±548.86	1846.94±170.00 1940.86±146.72
T	1482.59±145.20 1247.52±122.32	1363.40±234.38 1205.78±126.35	1644.36±430.02 1256.22±386.52	958.78±79.86 1055.94±70.52
B	598.78±58.86 509.34±59.30	512.24±50.48 505.69±63.12	594.36±144.85 469.94±142.62	402.44±31.24 440.86±20.62
T-active	1074.56±108.88 887.63±104.12	961.20±80.22 901.78±70.26	1071.52±284.62 947.78±272.74	709.68±126.25 848.81±60.22
T-helpers	804.14±82.85 676.78±84.10	586.68±44.58* 566.58±42.32	970.90±232.64 751.36±226.42	431.20±42.64* 439.64±40.00*
T-suppressors	256.12±32.68 214.89±33.20	362.24±31.88 275.73±36.97*	266.82±50.89 197.22±46.78	332.12±26.36 303.68±24.88*
Relative lymphocyte count/1 μl				
T	61.38±2.14 61.48±1.38	51.88±1.40** 60.20±1.49**	61.34±2.26 62.25±2.12	52.06±0.86** 57.88±1.93*
B	25.02±2.18 25.52±0.86	23.34±1.06 24.08±1.08	22.74±0.94 22.31±1.08	22.16±1.24 24.76±1.34
T-active	44.14±1.364 44.68±0.56	43.26±1.04** 44.02±1.96**	47.30±0.74 47.78±0.78	41.48±0.84** 43.32±0.66**
T-helpers	30.34±1.04 34.20±1.38	26.46±1.03** 32.56±1.18**	36.74±0.88 37.56±0.88	23.44±1.06** 26.89±0.86**
T-suppressors	13.38±1.40 10.58±1.48	16.62±0.94** 11.84±0.52**	10.54±0.42 10.32±0.48	18.06±0.38** 14.76±0.54**

Note. Numerator: parameters during exacerbation; denominator: after healing. *p<0.05, **p<0.01 compared to uncomplicated ulcers.

Before discharge the nonspecific defense values were high, approaching the normal levels: SBA 79.4±1.8%, complement 54.1±0.84 U/ml, lysozyme 19.2±0.2 g/ml, approacing the normal; β-lysine decreased to the normal level (37.3±0.5%) indirectly indicating attenuation of inflammatory processes (Table 2).

The increase in IgG concentration was most pronounced on admission to hospital. By discharge blood IgG concentration decreased significantly from 16.9±1.5 to 11.9±1.1 g/liter (p<0.05). The concentration of IgA virtually did not change (3.1±0.6 initially and 3.7±0.2 g/liter after therapy). The concentration of

IgM tended to decrease (from 1.72±0.37 to 1.43±0.28 g/liter, differences are insignificant).

Cell immunity status was evaluated by the content of lymphocyte subpopulations (Table 3).

Exacerbation of peptic ulcer is associated with suppression of cell immune response (Table 3), particularly manifest in chronic resistant ulcers: the count of T-helpers markedly decreased, while that of T-suppressors increased. Positive shifts were observed after SCT: the counts of T- and B-lymphocytes increased significantly, that of T-suppressors decreased.

Delayed results of treatment of peptic ulcer were followed up for 1-3 years in 58 patients of the main groups and in 38 controls. Relapses were much more incident in controls in comparison with the patients receiving splenotherapy. The incidence of relapses during the first year was 5% in the main groups and 40% in the control group. During two years the incidence of relapses was 20% in the main groups and 65% in the control group. After 3 years the incidence of relapses reached 25% in the main groups and 84% in controls.

The results suggest that "defense" factors (local and general immunity, regeneration) should be taken into consideration during treatment of gastroduodenal ulcer. SCT actively and favorably modulated regeneration and immunity processes, accelerating ulcer healing and improving its quality. Transendoscopic injections of splenopide around the ulcers proved to be most convenient and effective method of SCT.

Hence, SCT is an effective, pathogenetically justified method of ulcer eradication.

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