ORAL NEUTROPHIL ALTERATION TEST IN DRUG ALLERGY

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The neutrophil alteration test (NAT), developed in 1962, is used in the diagnosis of various infections and allergic diseases [1, 2]. However, the NAT requires blood to be taken, and for that reason it may present certain difficulties. At the same time, we know that many neutrophils, which play an active part in antimicrobial protection, migrate through the epithelial layer on to the surface of the oral mucosa from the blood [3]. Many receptors for IgG IgM, and the C3 component of complement are present on the membrane of neutrophils in the oral cavity [4], evidence of the high functional activity of these cells.

The aim of this investigation was to study the possibility of using the oral NAT for the diagnosis of drug allergy.

EXPERIMENTAL METHOD

The teeth were first carefully cleaned and the mouth rinsed with drinking water. Next, for 1 min the mouth was rinsed with Hanks' solution, pH 7.4, in a volume of 25 ml. Next, 70 mg of sodium citrate was added to the washings and the mixture shaken until the salt had completely dissolved. The mixture was centrifuged at 2000 rpm for 10 min and the supernatant poured off. The residue was resuspended in Hanks' solution, the suspension was stood vertically for 2-3 min, and the supernatant was then transferred into another tube. The cell suspension was diluted with Hanks' solution to a concentration of 600-900 cells/mm^3 and divided into 5 samples, each containing 150 μl of suspension. Hanks' solution was added in a volume of 50 μl to each of two tubes (control), and to the resulting three tubes were added 50 μl of Hanks' solution containing the drugs for testing (penicillin, streptomycin, thiazole, and aspirin) in different concentrations (experiment). The contents of the tubes were thoroughly mixed and incubated at 37°C for 1 h. Before the cells were stained the contents of the tubes were again mixed and then one drop of acridine organge in a dilution of 1:20,000 was added to each tube.

A drop of the mixture from each tube was applied to a slide and a vital preparation was made, and was examined without delay under the luminescence microscope. Neutrophils (100) were counted in a field of vision and the percentage of altered cells calculated. For each patient the difference (a) between the percentage of deformed cells in the experiment and control was determined. The result of testing was considered to be positive if $a \geq 16$, negative if $a \leq 10$, and doubtful for a = 10-15.

EXPERIMENTAL RESULTS

After observations of the oral neutrophils in the luminescence microscope the cells were divided into the following groups: a) round neutrophils with homogeneous red granules and green luminescence of the nucleus; b) oval neutrophils with palely stained, nonhomogeneous granules within the cytoplasm, and round leukocytes with an indistinct intracellular structure, and leukocytes with a dull fluorescence stain; c) ameboid leukocytes with vacuoles in the nucleus and cytoplasm, with a damaged membrane, macro- or microleukocytes, leukocytes with nonhomogeneous cytoplasm; d) red leukocytes; e) enlarged, green leukocytes. Leukocytes of groups *a* and *b* were regarded as normal neutrophils, those of groups *c, d,* and *e* as altered neutrophils.

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During testing of 213 patients with drug allergy and 89 clinically healthy individuals, the oral NAT was positive in 68% and 7% of cases, respectively.

The number of positive results of the test also was analyzed in relation to the clinical type of drug allergic reaction. In the group of patients with reactions of anaphylactic shock type combined with urticaria, positive results of the test were found in 82% of cases, and in patients with reactions of purely shock type, in 38% of cases.

The test also reflects the severity of the diseases. For instance, in the presence of a severe degree of immediate type reactions positive values of altered neutrophils were found in 82% of cases, but in 66% of cases with a weak degree of these reactions.

Not a single positive case was found in the group of subjects who had never taken the drugs, although there were some doubtful results.

The suggested method of assessment of the state of the oral neutrophils by luminescence microscopy is a noninvasive procedure, it is easy to perform, and yields results in the course of a short time (90 min).

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