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Stimulation of human neutrophil migration by aurothioglucose and thioglucose

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Abstract

Migration of human neutrophils was enhanced by aurothioglucose in a concentration-dependent manner. Migration was especially pronounced when the drug was present in the lower compartment of the Boyden chamber, suggesting that the enhancement of migration was chemotactic rather than chemokinetic. Thioglucose followed the same pattern of stimulation as the related gold compound, but the enhancement at the maximally stimulating concentration was less; at higher concentrations thioglucose inhibited random migration.

Introduction

Aurothioglucose has been used effectively for the treatment of rheumatoid arthritis [1, 2]. When compared to aurothiomalate, it produced less side effects and a higher percentage of improvement [1]. The mechanism by which aurothioglucose and other gold compounds exert their beneficial effect is unclear.

Neutrophils are suspected to be important mediators in inflammatory conditions because they are able to migrate to the site of inflammation where they may release inflammation-promoting substances. Modulation of chemotactic migration by a drug might, therefore, influence the inflammatory process.

Recently, we found that some sulphur-containing anti-rheumatic compounds were able to stimulate migration of neutrophils [3, 4]. In this study we considered the effect of aurothioglucose on human neutrophil migration to determine whether the drug possessed either activating or inhibitory properties with regard to this function.

Materials and methods

Isolation of neutrophils

Human neutrophils were isolated from the venous blood of healthy volunteers by using dextran sedimentation followed by centrifugation over Ficoll-Isopaque, and hypotonic haemolysis of contaminating erythrocytes. Isolated neutrophils were resuspended in a medium consisting of 140 m*M* NaCl, 5 m*M* KCl, 10 m*M* glucose, 20 m*M* Hepes (pH = 7.3), and 0.5% bovine serum albumin.

Chemotaxis

Cell migration was measured with the Boyden chamber technique, as modified by Zigmond and Hirsch [5]. The two compartments of the chamber were separated by a cellulose acetate Millipore filter with a pore size of 3 μm. Neutrophils were placed in the upper compartment of the chamber, followed by incubation for 40 min at 37 °C. After migration, the filters were fixed and stained and the

distance travelled in micrometers into the filter was determined according to the leading front technique [5]. The assays were carried out in triplicate and the migration distance of the neutrophils was determined at five different filter sites.

Results

Aurothioglucose had a strong enhancing effect on neutrophil migration when it was present in the lower compartment of the Boyden chamber. The enhancing effect increased from 10^{-5} to 10^{-4} *M* at higher concentrations the enhancing effect diminished (Fig. 1).

The effect of thioglucose on random migration resembled that of aurothioglucose (Fig. 1). The maximal enhancement also occurred at a concentration of 10^{-4} *M*, but was lower than that of aurothioglucose. At a concentration (in the lower compartment of the Boyden chamber) of 10^{-3} *M*, thioglucose inhibited random migration.

Enhancement of random migration also occurred when either aurothioglucose or thioglucose was present in both compartments of the Boyden chamber, but the effect was small as compared with the situation when the drugs were present only in the lower compartment. Under conditions where random migration (no drug) was 48.7 ± 1.1 μm, with aurothioglucose (100 μ*M*), migration was 52.9 ± 1.9 μm, while with the same concentration of thioglucose, the response was 52.3 ± 1.4 μm.

Aurothioglucose-induced potentiation of migration was strongly inhibited by methylene blue, and completely inhibited by LY83583, two inhibitors of guanylate cyclase. Aurothioglucose caused a moderate enhancement of cGMP level in neutrophils; the level increased from 3.97 ± 0.62 to 6.44 ± 0.53 pmol cGMP/10^7 cells after exposure to 100 μ*M* aurothioglucose for 2 min.

Discussion

Both aurothioglucose and thioglucose produced a marked stimulating effect on neutrophil migration. Because the effect was small when the drugs were present in both compartments, and large when they

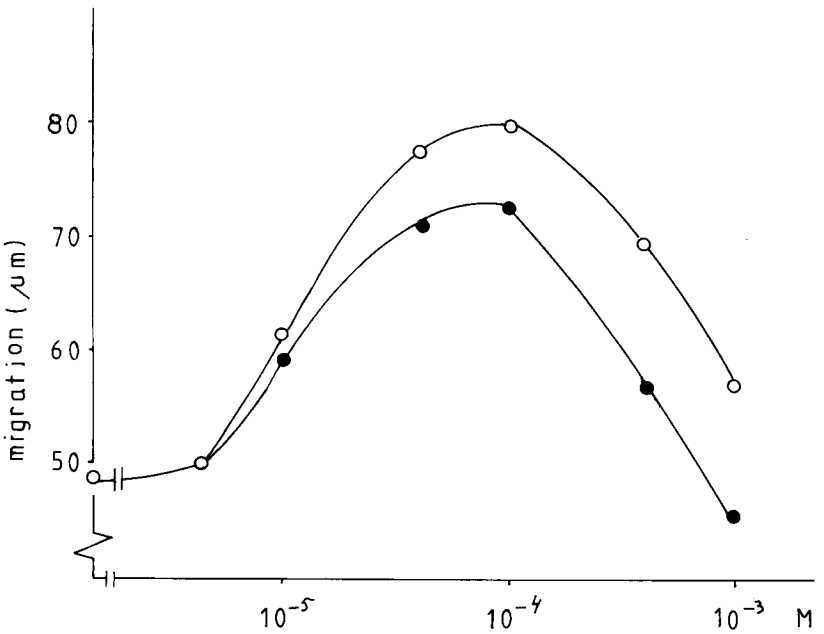

Figure 1
Effect of increasing concentrations of aurothioglucose (○), or thioglucose (●), on neutrophil migration. The drugs were present in the lower compartment of the Boyden chamber, and the concentrations at the site of the moving cells is, thus, between zero and the indicated concentration. Values given are the means of three experiments (15 determinations).

were present in the lower compartment only, we conclude that the enhancing effect may be chemotactic rather than chemokinetic.

The effect of aurothioglucose resembles those of some other anti-rheumatic drugs, namely D-penicillamine, captopril, and tiopronin, which equally have potentiating effects on neutrophil migration [3, 4]. These agents all increase the cGMP level in the neutrophil. The effect of aurothioglucose on the level of cGMP, and the inhibitory effects of methylene blue and LY83583 strongly point to a possible involvement of cGMP in the stimulating effect of aurothioglucose.

Whether the stimulating effect of aurothioglucose on neutrophil migration is of importance for the beneficial effect of the drug in arthritis, or plays a role in one of the many adverse effects of the drug remains to be determined. In general, it is assumed that inhibition of neutrophil functions is benevolent in inflammatory conditions. The activating effect of aurothioglucose does not fit in this view. It is conceivable, however, that the stimulation of cGMP metabolism results in the generation of other substances which are not necessarily related with migration, and contribute to the therapeutic effect.

References

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