α-Mercaptopropionyl-glycine influence on the in vitro proliferative response of human lymphocytes

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Summary. The effect of α-mercaptopropionyl-glycine on the in vitro proliferative response of healthy subjects' lymphocytes was studied. Low doses of the drug enhanced spontaneous lymphocyte blastigenesis but had no effect on the PHA-induced blastic response. With increasing concentrations of α-mercaptopropionyl-glycine inhibition of ^{3}H-thymidine incorporation was found in unstimulated as well as in PHA stimulated lymphocytes.

Sulfhydryl compounds are widely distributed in animals, plants and microorganisms. α-mercaptopropionyl-glycine, derived from the chemical condensation of thiolactic acid with glycine, is a drug which has been shown to have various biological functions. Among these are extensive detoxification, intensification of enzyme activities, interaction with cystine to form a mixed disulfide, and liver protection, all of which are presently applied in the treatment of various diseases[1-3].

Some properties of sulphydryl compounds are related to immunocompetence. To date, particular interest has been directed toward its influence on the B-dependent system (depolymerization of 19S IgM[4,5], decreased rheumatoid factor titer[6], and increased primary antibody response[7]). Regarding the influence of thiols on T-immune function, Binderup et al.[8] recently reported that D-penicillamine enhances the functional activity of rat peritoneal macrophages, and Igarashi et al.[9] have found that 2-mercaptoethanol induces, in murine spleen cell culture, the polyclonal activation of killer T-cells. Monna et al.[10] reported that α-mercaptopropionyl-glycine depresses the blastigenesis of the lymphoid elements of guinea-pigs sensitized to tuberculosis antigens. Neverthless, the effects of the sulfhydryl compounds on human T-dependent immunity have not yet been sufficiently investigated.

The aim of this study was to evaluate the effect of α-mercaptopropionyl-glycine on the in vitro proliferative response of healthy subjects' lymphocytes.

Material and methods. Venous blood was obtained from 22 healthy human volunteers who showed a normal blood picture and did not have a history of allergy, infection, medications or immunizations. Their ages ranged from 21 to 47 years.

Lymphocytes were separated by density-gradient sedimentation[11] with Lymphoprep (Nyegaard & Co. As, Oslo). The cells were washed 3 times in Hanks' balanced salt solution and resuspended in TC medium 199 (Difco Laboratories, Detroit, USA).

Lymphocyte cultures have been previously described in detail[12,13]. Briefly, mitogen cultures were prepared in 16×95 mm sterile tubes by adding 1×10^6 lymphocytes to 1 ml solution containing TC medium 199, supplemented with 20% autologous plasma, 100 U of penicillin, 100 µg of streptomycin and 50 µl of PHA-M (Difco). The cultures were incubated at 37 °C in CO_2 atmosphere for 72 h. Spontaneous lymphocyte transformation was evaluated in identical cultures where PHA was omitted. α-Mercaptopropionyl-glycine was dissolved in TC medium 199 and 0.1 ml was added to the cell cultures at varying concentrations (0; 10; 50; 100; 250; 500; 1000 µg/10^6 lymphocytes). All cultures were performed in triplicate.

Cell viability was tested by the trypan blue exclusion test after 72 h of culture; 94% of the cells were viable.

DNA synthesis was quantified by incorporation of 1 µCi of ^{3}H-thymidine (Cea Ire Sorin, sp. act. 26 mCi/mM) for the last 12 h of culture. The radioactive DNA protein was

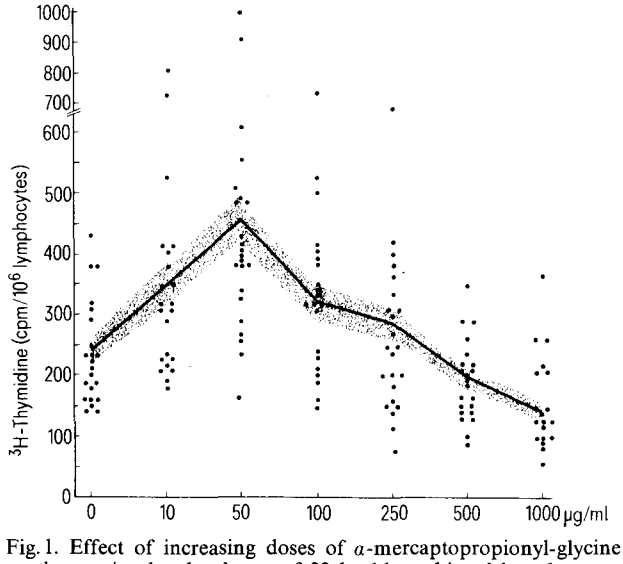

Fig. 1. Effect of increasing doses of α-mercaptopropionyl-glycine on the unstimulated cultures of 22 healthy subjects' lymphocytes. The mean values±SE were as follows: without drug: 238±18; 10 µg/ml: 354±35; 50 µg/ml: 457±41; 100 µg/ml: 337±29; 250 µg/ml: 277±27; 500 µg/ml: 196±14; 1000 µg/ml: 149±15. The shaded area = SE.

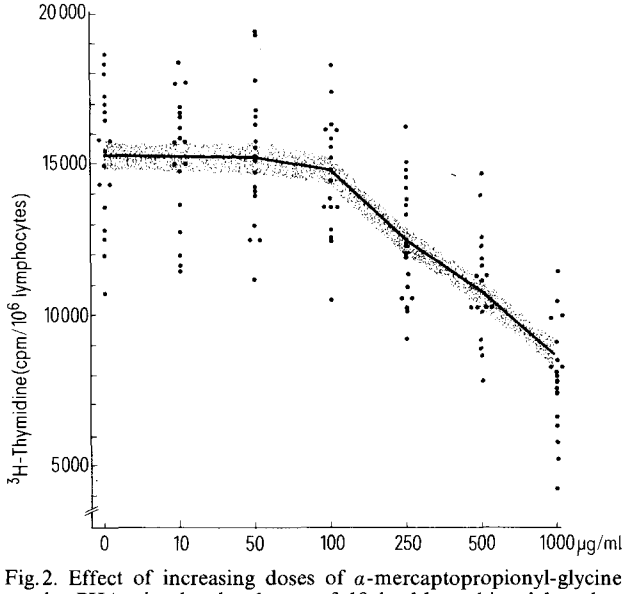

Fig. 2. Effect of increasing doses of α-mercaptopropionyl-glycine on the PHA-stimulated cultures of 18 healthy subjects' lymphocytes. The mean values±SE were as follows: without drug: 15318±537; 10 µg/ml: 15259±522; 50 µg/ml: 15316±539; 100 µg/ml: 14737±467; 250 µg/ml: 12622±490; 500 µg/ml: 10962±427; 1000 µg/ml: 8076±454. The shaded area = SE.

transferred to a scintillation vial with scintillation liquid, and the ct/min were determined in a Packard Liquid Scintillation Counter. The results are expressed as the mean ct/min/10^6 lymphocytes of triplicate cultures±SE. Student's t-test was used to estimate the significance.

Results and discussion. When low doses of α-mercaptopropionyl-glycine (50 µg/ml) were added to lymphocyte cultures of healthy subjects, a significant ($p < 0.001$) stimulation of lymphocyte blastigenesis was found (figure 1). The dose-response curve shows that ^{3}H-thymidine incorporation by lymphocytes cultured in the presence of the drug was nearly twice that of control cultures. With increasing concentrations of α-mercaptopropionyl-glycine, radioisotope incorporation progressively decreased. At doses of 100 µg/ml, a slight degree of stimulation was still noted. However, this effect subsequently diminished and at doses of 500 and 1000 µg/ml a marked inhibition of the blastic response was observed ($p < 0.001$).

Figure 2 refers to the influence of α-mercaptopropionyl-glycine on mitogen stimulated cultures. Low doses had no effect on the blastic activity, whereas higher concentrations (500 and 1000 µg/ml) caused a definite inhibition ($p < 0.001$).

α-Mercaptopropionyl-glycine potentiates enzymes that have a −SH radical, such as coenzyme A. It induces several reactions that lead to the production of succinic acid, acetic acid and other intermediate compounds, thereby furnishing cellular energy[14]. However, high doses of thiolic compounds have an antagonistic action with respect to the enzymatic activities dependent on pyridoxal-phosphate[15]; recently, this effect has been confirmed with particular regard to lymphocytes[16]. One can therefore hypothesize that α-mercaptopropionyl-glycine interferes with the receptors that prime the mechanisms preceding DNA duplication. As described above, at low or high doses respectively, activation or inhibition of the enzymes involved in DNA synthesis may be a further possibility.

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An alkaline protease in the delayed hypersensitivity skin lesions in the guinea-pigs[1]

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Summary. An alkaline hemoglobinolytic protease was extracted from the delayed hypersensitivity skin lesions induced by bovine γ-globulin as an antigen in the guinea-pig. The enzyme was heat-labile and inhibited by thiol-blocking reagents. The mol.wt was more than 100,000 and optimal pH around 9.

The mechanisms of delayed hypersensitivity reactions (DHR) are the focus of much current interest. However, the underlying biochemical reaction is still unknown. While lymphokines are thought to be putative soluble mediators of DHR[2,3], their relevance to in vivo events in cellular hypersenitivity is still unclear.

In a series of experiments to elucidate chemical agents in vivo sites of DHR, we have reported on the presence of macrophage-chemotactic factors in DHR skin sites in guinea-pig skin[4-6]. The present report adds to these reports the presence of an alkaline protease (ALPS) in the skin lesions of DHR.

Materials and methods. As previously reported[5,6], Hartley guinea-pigs, 300–500 g of both sexes, were sensitized by injecting into 4 foot pads 10 µg of bovine γ-globulin (BGG, Armour, Kankakee, Illinois, USA) emulsified in complete Freund's adjuvant. 7 days later, the animals were injected intradermally with 10 µg/site of BGG in the flanks of the animals in 20 sites. Precipitating serum antibody to BGG could not be demonstrated in any of the animals tested at this time. Inflamed skin sites, 24-h-old, were excised and dried in acetone according to the method of Hayashi et al.[7]. The extract, obtained from the skin acetone powder with 67 mM phosphate buffer, pH 7.4 (1 g/10 ml) for 4 h, was precipitated by ammonium sulfate (0–80% saturation) and the precipitate was redissolved in 10 mM phosphate-buffered 0.15 M NaCl, pH 7.4, and dialyzed against the same buffer for 16 h.

Proteolytic activity was measured utilizing ^{3}H-acetylated hemoglobin as a substrate[8]. Gel filtration was performed using Sephadex G-100 (Pharmacia, Uppsala, Sweden).

Results and discussion. 2 pH optima of the proteolytic activities were obtained at acidity and alkalinity in the inflamed skin extract (figure 1). It was of interest to note that the proteolytic activity in extracts of inflamed skin sites was 3 times as high as that of extracts from control unchallenged skin in sensitized animals at pH 9. The extract was found to contain mainly heat-labile and thiol protease active at pH 9 (table).

The extracts were subjected to Sephadex G-100 filtration. Main ALPS activity eluted in the void volume and was separated from acid protease activity which was retarded. The mol.wt of ALPS was thus estimated to be more than 100,000. The ALPS had optimal activity around pH 9. As