

# Cytostatic Influence of Thioproline on Peripheral Lymphocytes of Healthy Persons and Non-Hodgkin's Lymphoma Patients

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## A. Introduction

Thiazolidine-4-carboxylic acid (Thioproline, Norgamem) is an agent of possible antitumorigenic effect [1, 2, 7]. This drug seems to induce a "reverse transformation" of pathological cells by restoring contact inhibition in cell cultures [2–6]. No evidence of a cytotoxic effect of Thioproline has so far been observed [2, 4, 9]. In our previous work we demonstrated that Thioproline exerts strong inhibition of DNA synthesis in normal lymphocytes [8]. In this study we present experiments performed in normal as well as in non-Hodgkin's lymphoma (NHL) lymphocytes treated by Thioproline *in vitro*.

## B. Materials and Methods

*In vitro* experiments on normal and NHL peripheral blood lymphocytes (PBLs) were carried out and the influence of different Thioproline concentrations was investigated by:

1. [<sup>3</sup>H]Thymidine uptake into normal PBLs stimulated in culture with phytohemagglutinin (PHA)
2. [<sup>3</sup>H]Thymidine uptake into pathological lymphocytes without adding PHA according to spontaneous incorporation of indicator
3. Suppressor activity of both groups of lymphocytes measured in the presence of Thioproline using ConA and [<sup>3</sup>H]thymidine

4. Intracellular concentration of cAMP and cGMP
5. Trypan blue test evaluation of cell viability

We used the lymphocytes of 20 healthy persons (first-time blood donors) ranging from 18 to 40 years in age and lymphocytes obtained from 20 NHL patients (11 with lymphocytic lymphoma, 6 with centroblastic-centrocytic and immunoplasmocytic lymphoma, and 3 with acute lymphoblastic leukemia). Peripheral blood lymphocytes from healthy donors and PBLs from NHL patients were isolated on Lymphoprep (Nyegaard, Norway). A quantity of  $2 \times 10^5$  PBLs were cultured for 72 h (37°C, 5% CO<sub>2</sub>) with PHA (only normal PBLs) (Wellcome) together with Thioproline in four different concentrations calculated on the basis of the therapeutic doses according to blood volume (5, 20, 40, 80 µg) [2, 9]. The drug was diluted to 0.02 ml and incubated with examined lymphocytes (density of lymphocytes in suspension  $0.2 \times 10^6$ /ml) in a minimum essential medium (MEM) (0.2 ml-containing glutamine and antibiotics). Investigated lymphocytes were also incubated simultaneously with PHA 24 h before or after Thioproline intake. [<sup>3</sup>H]Thymidine uptake was evaluated as counts per minute of isotope extracted from the lymphocytes and expressed as a percentage of the control value (without drug). Lymphocyte suppressor activity was defined as [<sup>3</sup>H]thymidine uptake ratio of ConA lymphocyte culture stimulation and ConA stimulation in culture after 24 h. Intracellular level of cAMP and cGMP was measured using cAMP and cGMP RIA Kits (Amersham, UK).

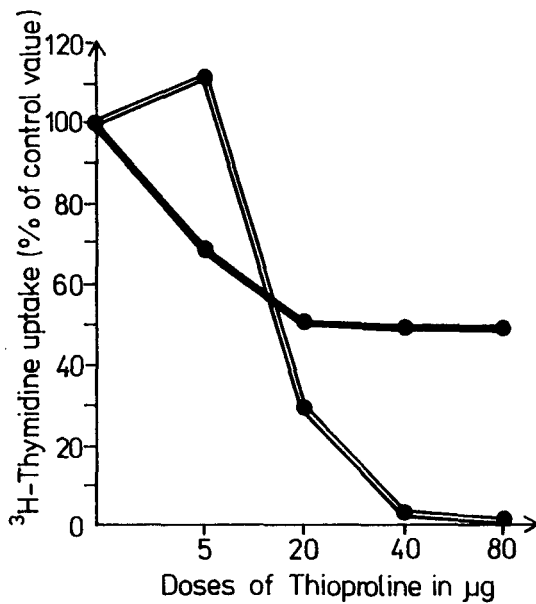
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### C. Results

Of the normal and pathological PBLs used in the experiment, Thioproline caused dose-dependent inhibition of [<sup>3</sup>H]thymidine uptake (except at the lower concentration of 5 μg), where increased uptake of [<sup>3</sup>H]thymidine was observed (Fig. 1). Similar results were obtained by normal PBL incubation pretreated in culture with Thioproline 24 h before the addition of PHA and in a second group pretreated with PHA 24 h be-

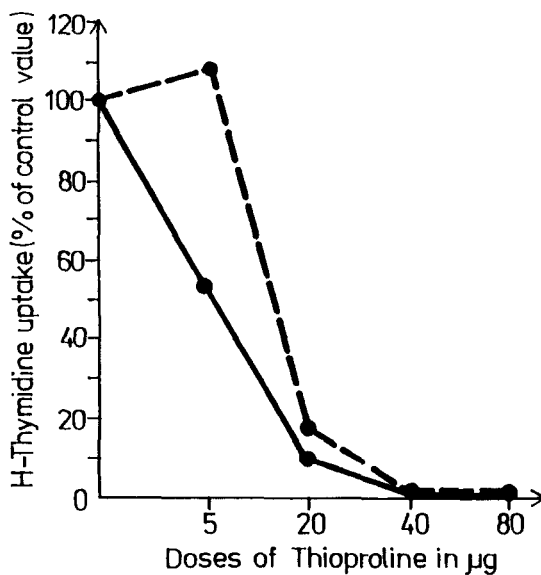
fore the addition of Thioproline (Fig. 2). A viability test of PBLs after 24 and 48 h incubation with Thioproline showed a great decrease in viability of pathological lymphocytes. No significant decrease in normal lymphocyte viability was observed (Fig. 3). At a higher concentration, 80 μg, Thioproline caused a reduction in the intracellular level of cAMP and cGMP within normal PBLs as well as in NHL PBLs (Fig. 4). Thioproline has no effect on the suppression activity of normal and NHL PBLs (Fig. 5).



—○— obtained from 20 healthy donors  
—●— obtained from 20 NHL patients

Thioproline showed dose dependent inhibition of <sup>3</sup>H-Thymidine uptake into pbl.

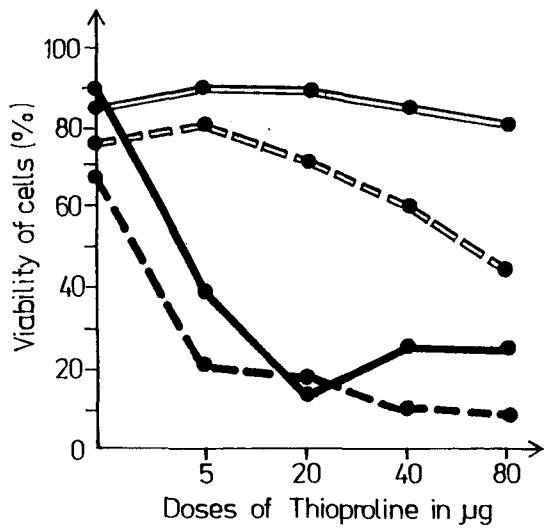
Fig. 1. Influence of Thioproline on [<sup>3</sup>H]thymidine uptake in PBLs (mean values)



a - -○- - pretreated with PHA 24 hrs. before Thioproline  
b —●— pretreated with Thioproline 24 hrs. before PHA.

Lymphocytes of normal blood donors pretreated with PHA or TP demonstrated low incorporation of <sup>3</sup>H-Thymidine in following measure.

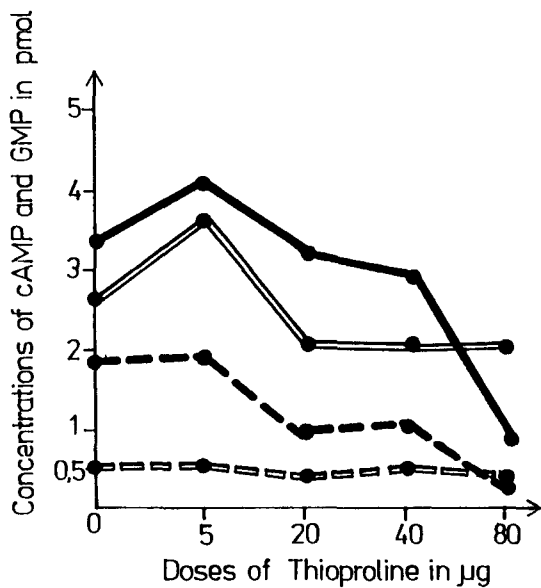
Fig. 2. Influence of Thioproline on PBLs (mean values) obtained from 20 healthy donors stimulated in culture with PHA



—○— normal pbl after 24 hrs. } incubation with TP  
 - - □ - - normal pbl after 48 hrs. }  
 —●— NHL pbl after 24 hrs. } incubation with TP  
 - - ● - - NHL pbl after 48 hrs. }

Thioprolone caused great decrease in viability of pbl obtained from NHL patients.

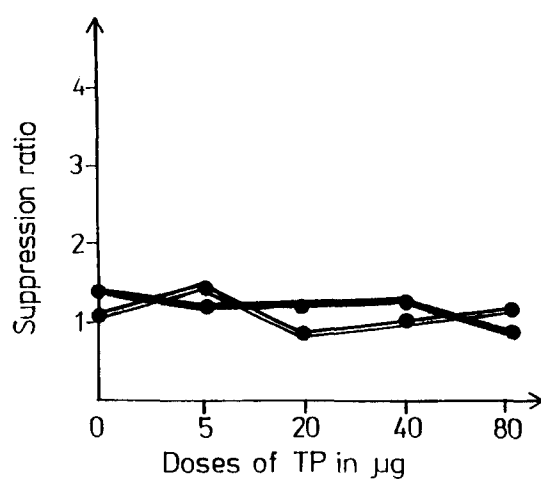
**Fig. 3.** Viability test of PBLs after 24 and 48 h incubation with Thioprolone (TP) (mean values of 20 experiments)



—○— cAMP level in normal pbl  
 —●— cAMP level in NHL pbl  
 - - □ - - cGMP level in normal pbl  
 - - ● - - cGMP level in NHL pbl

High dose of TP caused lowering of cAMP and GMP level in both groups of pbl.

**Fig. 4.** Influence of Thioprolone (TP) on intracellular level of cAMP and cCMP (mean values of 20 experiments)



—○— obtained from 20 healthy donors  
 —●— obtained from 20 NHL patients

TP showed no significant effect on suppressor activity of pbl.

**Fig. 5.** Influence of Thioprolone (TP) on suppressor activity of PBLs (mean values)

## D. Conclusions

Thioprolin caused in vitro dose-dependent inhibition of [<sup>3</sup>H]thymidine uptake into normal and NHL PBLs. At the highest concentration Thioprolin decreased the intracellular level of cAMP and cGMP in examined PBLs. Thioprolin has no effect on the suppression activity of normal and NHL PBLs. Inhibition of [<sup>3</sup>H]thymidine uptake into NHL PBLs seems to be caused by a cytotoxic mechanism.

## References

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