EFFECT OF GOLD THIOMALATE ON CELL PROLIFERATION AND COLLAGEN SYNTHESIS OF SYNOVIAL CELLS IN CULTURE

RAMADASAN KUTTAN

Amala Cancer Research Centre,
Amala Nagar, Trichur - 680 553

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Summary : Human synovial cells from cases of rheumatoid and osteoarthritis were cultured and at their 3-5 passages, were treated with gold thiomalate. At early-log phase gold thiomalate arrested the proliferation of cells. However, at confluent state shere was a slight proliferation of synovial cells. This was followed by an increase in prolyl hydroxylase, collagen and protein synthesis, indicating that gold salts directly stimulate the synovial cells.

INTRODUCTION

The gold salts have been found to be particularly useful in early rheumatoid arthritis but not in burnt out cases with gross deformities and ankylosis (5). Of Gold salts in use; gold thioglucose produces necrosis of hypothalamus resulting in changed food habit and obesity (3) without any connective tissue changes (4). On the other hand gold thiomalate produces weight gain but not obesity. The weight gain has been shown to be metabolically induced (2).

The therapeutic effect of gold salts in rheumatoid arthritis has been explained mostly through its action on connective tissue. There are very few reports on metabolic changes indueed by gold salts. In the present study the metabolic effect of gold salts in cultured synovial cells has been related with its effect on connective tissue synthesis.

MATERIAL AND METHODS

Human synovial tissue was obtained, after individual consent, from patients suffering from rheumatoid arthritis (RA - Cells) and osteo arthritis (OA - Cells) during therapeutic surgery. The tissues were brought to the laboratory within 2 hr in Dulbecco's Modified Eagles medium (DME).

Explants (5 mm) were grown in the same medium containing fetal calf serum (10%) and antibioltics. Cells grown between 2-4 passages were used for the experiment (8).

Gold salts were added to the cells in fresh medium and the cell number after incubation for required days were determined by hemocytometer.

Prolyl hydrerglase was studied in the sonicates of cells (7). Collagen and non-Collagen protein synthesis was studied using cells treated with gold salts for 48 hr and after incorporation (6 hr) of H^3-proline (6). Statistical significance was determined by comparison with non-treated controls by student 't' test.

Gold thiomalate (Aureothiomalate, injectible, Merck, Sharp and Dhome) and Gold chloride (Auric Chloride; E. Merck) were used (concentrations refer to the salts).

RESULTS

Effect of Gold Thiomalate on Cell proliferation of confluent synovial cells : Effect of two concentrations of gold thiomalate on the proliferation of confluent synovial cells is seen in Table I. The drug produced a statistically significant change in the cell proliferation of RA-cells and OA-cells.

TABLE I : Effect of gold salts in synovial cell proliferation.

Cell line	*Gold thiomalate*		*Gold chloride*	
	Concentration (μg/ml)	*Cell number (million)*	*Concentration (μg/ml)*	*Cell number (million)*
RA Cells	Nil	1.65	Nil	1.65
	50	1.68	10	2.32**
	100	1.85*	25	2.80***
OA Cells	Nil	1.76	Nil	1.93
	50	1.96*	10	2.20
	100	2.20**	25	2.72***

RA - Cells from rheumatoid arthritis tissue.

OA - Cells from osteo arthritis tissue.

Each result is the average of 3 different estimations.

*Statistically significant at the level of $P<0.05$

**Significant at the level of $P<0.01$

***Significant at $P<0.001$

In order to see whether the changes were due to metallic gold or thiomalic acid, another gold salt, gold chloride was used. When gold chloride was used there was statistically

significient increase in the cell number of both cell lines indicating that the effect is due to gold.

When synovial cells were treated with gold compounds at their early log-phase they had a different effect than seen with the confluent cells, viz., there was a complete arrest of growth after initial multiplication (Fig. 1), while the control cells increased their cell number as expected.

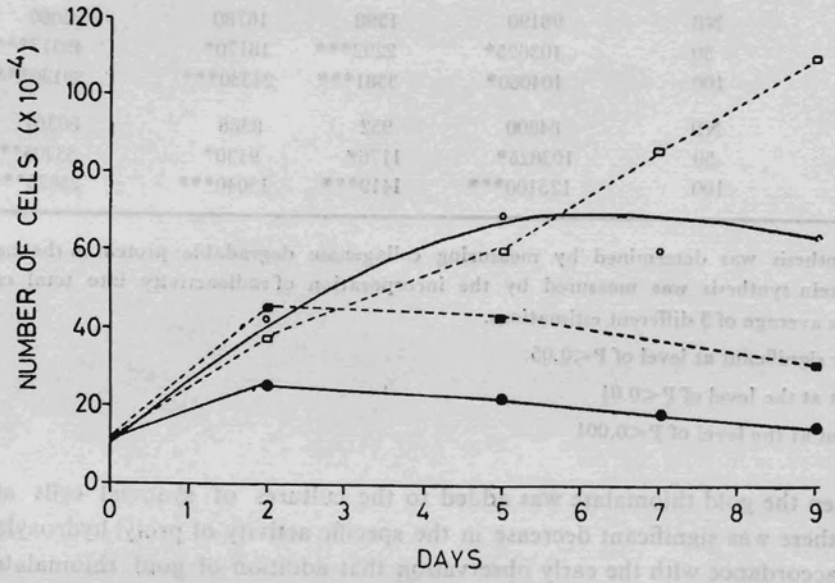

Fig. 1 : Effect of gold thiomalate (50 $\mu g/ml$ on cultured synovial cell at early-log phase. O—O RA cells (untreated) ●—●, RA Cells (treated). □—□ OA cells (untreated); ■—■, OA cells (treated). Values are the average of three different estimations. All the values after day 2 were statistically significant at $P<0.001$.

Effect of Gold Thiomalate on Prolyl hydroxylase and Collagen Synthesis : Prolyl hydroxylase which synthesize peptidyl hydroxyproline from peptidyl-proline has been shown to a marker of collagen synthesis. Effect of gold treatment on prolyl hydroxylase and collagen synthesis in confluent cultures of synovial cells is shown in Table II.

As shown in the table statistically significant increase of prolyl hydroxylase and collagen synthesis in the treated cells indicating a stiumulant effect on collagen synthesis. However, the increase was not found to be specific to collagenous proteins because there was also an increased incorporation of radioactive proline into non-collagenous proteins.

TABLE II : Effect of Gold thiomalate on prolyl hydroxylase and collagen synthesis in confluent and early-log phase synovial cell cultures.

Cell line	Concentration	Confluent cell culture			Early-log phase culture
		Prolyl hydroxylase	Collogen synthesis	Non-collagen synthesis	Prolyl hydroxylase
	µg/ml	(CPM/mg protein)	(CPM/mg protein)		
RA Cells	Nil	98190	1398	16780	96080
	50	103625*	2292***	18170*	49517***
	100	104060*	3381***	24330***	39136***
OA Cells	Nil	84800	952	8388	86364
	50	103825*	1176*	9130*	33305***
	100	125100***	1419***	13040***	25825***

Collagen synthesis was determined by measuring collagenase degradable protein in the medium and non-collagen protein synthesis was measured by the incorporation of radioactivity into total cellular proteins. Each result is average of 3 different estimations.

*Statistically significant at level of $P<0.05$

**Significant at the level of $P<0.01$

***Significant at the level of $P<0.001$

When the gold thiomalate was added to the cultures of synovial cells at their early log-phase there was significant decrease in the specific activity of prolyl hydroxylase (Table II). This is in accordance with the early observation that addition of gold thiomalate inhibit cell growth at early log phase.

DISCUSSION

The machanism of the action of gold salts in rheumatoid arthritis is not completely understood. One good explanation is that gold salts inhibit lysosomal enzyme activity in synovial membrane during disease (10). Administered gold salts have been located in synovial membrane (9) and hence gold salts directly bind with collagen fibrils in synovium making it more insoluble and less susceptible to lysosomal degradation (1). Both *in vivo* and *in vitro* studies show collagen gold interaction.

The results presented in this paper indicate that depending upon the stage of the cell growth in culture the gold thiomalate has different action.

The biochemical basis of stimulation of cell growth mediated by gold salts is not understood at present. Gold salts have been shown to inhibit lysosomal activity; this may lead to inhibition of proteolytic enzymes thus stimulating the matrix formation in cultured cells. However this does not explain the inhibition of cellular growth at early-log phase. Micronutrients such as zinc has been shown to stimulate the cell growth through increased replication of DNA. Since the gold salt increases the cell number in confluent primary cell lines it is possible that gold salts have a direct stimulatory action on cultured cells.

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