## Studiul influenței tratamentului cu ciclofosfamidă asupra hematiilor din sângele embrionului de găină

## A study upon the influence of cyclophosphamide treatment on the red blood cells of the chicken embryo

Delia Anca HAŞ-LĂZĂU

School 08, Mihai-Bravu, delia\_lazau@yahoo.com

**Abstract.** The aim of this study is to show the effect of cyclophosphamide on the developing red blood cells of the 3-4 days old chicken embryo, when the hematopoiesis is at its peack, located at the vitelline sack level.

I have chosen to work with the chicken embryo red blood cells because they have an intense mitotic activity as well as a tumoural cell-like behaviour.

It is vital to know the particularities of the cell cycle of the healthy and tumoural cells, keeping in mind that most of the cytostatics act upon the cell which are developing their cell cycle (Menkes B., Prelipceanu O., Checiu I., Căpălnăşan I. 1979).

The cyclophosphamide is not stage-dependent, as it acts in all the stages of the cell cycle, its mutagen effect being accompanied also by a cell cycle stopping (Paşca C., Crăciun C., Ardelean A. 2000).

Cyclophosphamide supply determines retrenchment of the cell division, transforming the normal cells into multinucleated cells, with normal ploydia. The cyclophosphamide is a cytostatic using for cancer therapy (Schiavoni G., Mattei F., Di Pucchio T., Santini S. M., Bracci L., Berardelli F., Proietti F. 2000).

Reshearches have done lots of studies along the years both on mice and rats, concerning the effects of cyclophosphamide on: thymus and burse fabricio (Giurgea R., Toma V., 1977), stromal cells of bone marrow (Anton E. 1997), pulmonary thrombocytopoiesis (Sulkowki S., Sulkowska M., Musiatowikz B. 1997).

## Cuvinte cheie: ciclofosfamidă, citostatice, hematii, hematopoieză, sac vitelin.

Keywords: cyclophosphamide, cytostatics, red blood cells, hematopoiesis, vitelline sack level.

We have incubated hen eggs – hybrids between different races (mixed race) – of maximum 5 days.

The second day, the eggs were opened, as follows:

The administration of the cyclophosphamide took place in the 4th day of incubation.

The cyclophosphamide solution was made with bidistilled water.

We have administrated 0.5 ml cyclophosphamide / chicken embryo.

Before that, we have sterilized this solution in a  $0.2\,\mu$  diameter porus filter.

From the surviving embryos we have sampled blood coming from the corio-alanthoid vessels, in the 5th, 6th and the 7th day of incubation, smears were made.

The smears were coloured using the May-Grundwald-Giemsa method, as follows:

- May Grundwald 3 min;
- tap water 1 min;
- Giemsa 30 min. The Giemsa solution was dilluted: 4 drops colour substance/1 ml tap water;

As a summary, we can say that by studying the embryos to which we added 50 µm cyclophosphamide and which we have examide after 3 days, we can observe the following characteristic changes:

- the gathering of the metaphase cells exhibiting unusual morphological properties (red blood cells more wide in diameter (double) and others with a single nucleus but a large volume; the red blood cells exhibit a heavy polychromatophilia; there are oval red blood cells, acidophilic and with a normal aspect, as well as a

large number of non-mature, round, basophil).

- the suppression of the cell multiplication and the overturning of these mitotic cells back into cells with more than one nucleus and with abnormal ploydia;
- the disappearance after 24 hours of those cells which are not viable, as well as the beginning of a great regeneration of the lost red blood cells, when young, hemocitoblastic, basophile cells appear, probably belonging to a final series, as the activity in the vitelline sack begins.

This study enabled us to show a clear effect of the cyclophosphamide upon the red blood cells of the chicken embryo as well as the minute type of action, very important for the optimum concentration / time balance in obtaining a therapeutic effect

Coordonatorștiințific:Ioana-MihaelaTOMULESCU-UniversitateadinOradea;FacultateadeȘtiințe,e-mail:imtomulescu@yahoo.com;Iacob CHECIU, Universitatea de Vest dinTimișoara,Catedra de Biologie,e-mail:icheciu@cby.uvt.ro

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