INVESTIGATIONS OF TERATOGENIC EFFECTS
INDUCED BY COPPER UPON EARLY
POSTIMPLANTATIONAL MOUSE EMBRYOS

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INTRODUCTION (online version)

Previous investigations (Checiu et all. 2001) performed on preimplantational mouse embryos showed that copper “in excess” administered to pregnant mouse females in the first 4 days of gestational period, followed by recovery of embryos at 12 hours after last administration has no teratogenic effect.

In these experiments, the embryos were examined before implantation, meaning that the evaluation was made at a short time after the last administration of CuCl$_2$ (12 hours). It is possible that by indirect offer (via mother) the appearance of developmental abnormalities asks for a longer time period.

For this reason we considered that new investigationes regarding the teratogenic potential of copper upon early postimplantational period (day 9 of gestation), are period with a high sensitivity to the teratogenic action of exogenous factors.

In our experimental model the control of teratogenic effect of copper is possible to be made during the entire prenatal developmental period, at different time intervals.

MATERIAL AND METHODS

Copper (CuCl$_2$ solution) was administered in a single dose to pregnant mice females on day 9 of pregnancy, when embryos were in 12-15 somites stage. We choose this developmental stage taking in account ours previous results on chicken embryos (Checiu et all. 2000) where we proved that 12-18 somites stage is the period with the higher sensitivity at copper action.

Experiments were performed on albino mice (NMRI strain) with 20-30 g body weight from the colony of the Department of Biology. Virgin
females and males were kept under a 12 hours day and 12 hours night regimen with food and water “ad libitum”.

Females were mated overnight with males (5 females:1 male). The morning after the mating period, the females with vaginal plug were considered on day 1 of gestation.

By preliminary investigationes we established the level of DL 50 dose. We worked with 3 experimental groups, each of them with 5 females on day 9 of gestation:

- group a had received by intraperitoneal injection (i.p.) 0.1 ml/10g body weight from a solution 35 mg% CuCl$_2$.
- group b had received by i.p. 0.1 ml/10g body weight from a solution 70 mg% CuCl$_2$.
- group c had received by i.p. 0.1ml/10g body weight from a solution 100 mg%CuCl$_2$.

After these administrations we established that the concentration of 100mg% is lethal and we used it no more, and only the concentration of 35mg% and 70mg% were used further more in our experiment.

The experimental groups, received by i.p. administration, 35mg% and 70mg% CuCl$_2$ as following :

- group I : 10 pregnant females on day 9 of gestation received by i.p. injection 0.1 ml/10g body weight from a solution 35mg% CuCl$_2$.
- group II: 15 pregnant females on day 9 of gestation received by i.p. injection 0.1ml/10g body weight from a solution 70mg% CuCl$_2$.
- group III: 10 pregnant, untreated females on day 9 of gestation.

The control of the treatment effects was made in lot I on day 11 of gestation (48 hours after CuCl$_2$ administration), on day 12 of gestation (72 hours after administration) and on day 16 of gestation.

In group II, the control of the treatment effects was performed on day 11, 12,16 and 19 of gestation.

In the untreated group III, the control was made in the same days of gestation (see above) as in the experimental groups.

RESULTS AND DISCUSSIONS

PRELIMINARY INVESTIGATIONS

After 1-2 hours after CuCl$_2$ administration the females from group b and c (70mg% and 100mg%) were sleepy, had difficulty in breathing and slow, abnormal movements (especially in the posterior members).
At 24 hours after CuCl₂ administration the animals from group c (100mg%) were dying. Maternal necropsy shown a marked alteration of the organs from abdominal cavity (suggesting the contact with a fixative liquid). The aspects cited above were diminished or even absent in group b (70mg% solution) and totally absent in group a.

RESULTS IN EXPERIMENTAL GROUPS

**Group I.** Females from group I were killed by cervical dislocation on day 11 of gestation (3 females), on day 12 (5 females), and on day 16 (2 females). After opening the abdomen, the uterus was removed and placed in a Petri dish with physiological saline at 37°C. The uterine wall was sectioned with 2 pairs of forceps so, that from each deciduum one embryo was obtained. All embryos were examined under a dissection microscope. The number of resorbtions, dead and living embryos was registred. Also, the presence of external abnormalities was noted.

*Control on day 11 of gestation (3 females) showed:*
- 1 gestant female had 10 embryos: 1 embryo with an atipic bud tail; 9 embryos were normal (28-30 somites, beating heart, anterior and posterior limb buds present, anterior neuropore closed, posterior neuropore opened, eye primordium present);
- 2 gestant females with 9 respectivelly 11 embryos. All those embryos were morfolgically normal.

*Control on day 12 of gestation (5 females) showed:*
all 45 embryos recovered (10,8,7,9,11 embryos/litte) did not show external abnormalities (the anterior and posterior neuropore closed, cerebral vesicles with normal appearance, eye primordium present, limb buds present, beating heart).

*Control on day 16 of gestation (2 females) showed:*
the fetuses (7 and 9 respectivelly) showed no external abnormalities, their developmental stage was similar with fetuses from control, untreated group. Data obtained showed that the concentration of 35mg%, copper has no teratogenic effect.
Group II.
In this group 15 pregnant females received 0.1 ml/10g body weight from a 70% CuCl₂ solution on day 9 of gestation.

On day 11 of gestation (4 females) showed:
- 24 embryos (70%) from the total number of embryos (35) were normal developed.
- 11 embryos (30%) presented external abnormalities under dissecting microscope such as:
  - neural tube open in the diencephal-rhombencephal region
  - neural tube open only in rhombencephal region
  - stasis in posterior limb buds
  - a marked retardation of general development given by a low number of somites (20-22).

On day 12 of gestation (4 females) showed:
- From 38 embryos (10, 9, 11, 8 embryos/litter), 28 embryos (75%) were normal developed but 10 embryos (25%) showed different malformationes. Those malformationes involved the neural tube (anterior neuropore opened) and telencephalon (deformed cerebral vesicles). Also, a marked retardation of developmental rate was noted. Fig.1a,b

On day 16 of gestation (3 females) showed:
- 15 fetuses were recovered (7, 2, 6 embryos/litter). From these 15 embryos, 13 (87%) showed a normal developmental rate but 2 (13%) showed an important brain malformation: exencephaly. It is to be noted that in this group were 11 early resobtions (1, 8, 2/female), which probably initially were malformed embryos which then arrested in their development.

On day 19 of gestation (4 females) showed:
- 30 fetuses (7, 8, 8, 7 embryos/litter) were recovered and examined. 22 fetuses were normal (73%) and 8 (27%) showed a marked exencephaly 5 early resorbtions (3, 1, 0, 1 resorbtions/litter) were noted. (Fig.2a,b)

UNTREATED GROUP
Control of pregnant females from this group showed:
On day 11 of gestation (4 females):
-36 embryos (10,8,9,9 embryos/litter) presented:
- 28-30 somites
- beating heart,
- anterior and posterior limb buds present,
- anterior neuropore closed,
- posterior neuropore opened,
- eye primordium present

**on day 12 of gestation (2 females):**
- 19 embryos (10,9 embryos/litter) were recovered. They showed normal external aspect:
  - cerebral vesicles of normal aspect
  - anterior and posterior neuropore closed
  - eye primordium present
  - limb buds present, beating heart.

**on day 16 of gestation (2 females):**
- 10 and 8 embryos normally developed were recovered.

**on day 19 of gestation (2 females):**
- 11 and 9 embryos normally developed were recovered.

In all these experiments we used 50 gestant mice females (for this a number of 90 females and 10 males was necessary).

In literature, exencephaly was reported in mouse embryos after cadmium treatment. Intraperitoneal injection of CdCl₂ at 6 mg/kg at gestational day 7.5, in mouse, induced exencephaly in about half the littermates (Takagi et al. 2000). Exencephaly and axial skeletal abnormalities induced by maternal exposure to cadmium in the mouse also reported Padmanabhan and Hameed 1986, Sato 1994, Webster and Messerle 1980.

The embryonic susceptibility to selenium teratogenicity was examined in rats, using postimplantation embryo culture. Rat embryos at day 9.5 of gestation were cultured by the roller bottle method for 48 hr in the presence of Se compounds. This compounds caused abnormalities such as deformed optic vesicle and swollen rhombencephalon in the viable embryos (Usami and Ohno 1996).

**CONCLUSIONS**

The administration of an aqueous solution of 35mg% CuCl₂, 0.1ml/10g body weight to pregnant mice females (on day 9 of gestation)
show no teratogenic effect upon embryogenesis (day 11 and 12 of development) and fetal period (day 16).

The higher concentration (70% CuCl₂ solution) has a strong teratogenic effect. The control at 48 and 72 hours after treatment show abnormalitis of CNS, bud limb, and a general developmental retardation. In the late control we noticed that the main organ affected was the brain, which presented an important malformation: exencephaly.

Taking in account our experimental results we are able to affirm that highy doses of copper consumed accidentally especially in early period of gestation are very dangerous.

REFERENCES

• Checiu I., Maria Checiu, Ioana Tuduce, Delia Checiu: The ultrastructural modifications at chick embryos induced by cooper ion (Cu ²⁺). Annals of West University of Timisoara, Series of Biology, vol III, 2000, pg. 32-46.


FIG. 1a. Mouse embryo, 12 days of gestation. Control

FIG. 1b. Mouse embryo 12 days of gestation, mother received 0.1 ml / 10 g body weight, mg% CuCl₂, at 9 days of gestation, anterior neuropore opened, arrow
FIG. 2a. Mouse foetus, 19 days of gestation. Control

FIG. 2b. Mouse foetus, 19 days of gestation, mother received 0.1 ml/10 g body weight, mg%CuCl₂, at 9 days of gestation, exencephaly