**CONTRACT 86323 (Internal SUBI number for reference purposes).**

**Experimental development of approaches to early detection of late radiation effect**

Performance period: 1986-1988.

**Publications**

Report (final). Principal investigator and responsible executive – K.N.Muksinova, responsible executive – E.N. Kirillova, executive – L.D. Murzina and others, co-executives A.L. Sokhranich, A.P. Nifatov, V.S. Revina. – FIB-1, 1988. – inventory № 1805. – 121p., 24 tables, 29 figures, 76 sources. 8 papers were prepared based on contract materials, 6 of them are published.

**Background**

The information was gathered from 568 male Wistar rats that were used in SUBI’s experiment no. 86323. The collection and collation of the information was done as part of Task 2.3 (“Evaluation of the SUBI tissue archive and database as a potential part of the European archive”) of the previous EURATOM-funded project STORE (Sustaining access to Tissues and data frOm Radiobiological Experiments; contract number 23228), which was coordinated by BfS, Germany (see <http://cordis.europa.eu/project/rcn/89386_en.html>) .

The information given here is based upon SUBI’s 18 months and 42 months reports.

It has been shown in the STORE project, that the biological material from the experiments conducted by SUBI can still be used. The respective Standard Operating Procedures are available on the STORE website (<http://www.storedb.org/store_v3/documents.jsp> ).

**The study**

The study comprised four parts:

Experiment 1.1, a life span study investigating late effects of 239Рu compounds with low transportability, including 273 male Wistar rats;

Experiment 1.2, investigating the dynamics of bone marrow and liver cells damage after exposure to 239Pu compounds with low transportability, including 295 male Wistar rats which were sacrificed after 180, 256, 365 and 512 days;

Experiment 2, investigating the modification of tritium biological effect which was based on a subsample of animals from Experiments 1.1 and 1.2, including 298 rats (there was a link to study 76323, which is included in STORE ( [http://dx.doi.org/doi:10.20348/STOREDB/1056/1137](http://dx.doi.org/doi%3A10.20348/STOREDB/1056/1137) ));

Experiment 3, investigating the dynamics of leukemia development in conditions of continuous administration of tritium oxide with potable water.

***Experiment 1.1 Late effects of 239Рu compounds with low transportability***

273 male Wistar rats were involved in the experiment, polymer 239Pu in nitric acid (HNO3) pН 1.5 solutionwas administered once intravenously to 207 of them; 66 control rats received HNO3,pН 1.5 solution once intravenously.

***Results***

All rats died naturally, i.e. this is a life-span study. Average life span of deceased rats in experiment 1.1 at different administered amounts of Pu was:

1. 367±9 (70 rats)
2. 466±15 (70 rats)
3. 586±12 (67 tats)
4. 635±19 (66 rats) Control

A significant incidence increase of malignant neoplasms in the exposed rats was found, in contrast to the predominance of benign tumors in the control animals. Bone tumors prevailed in animals exposed to Pu. Histological verification showed that 9 out of 10 cases were osteosarcomas. Among the control animals, the predominant type of malignant neoplasms was lymphoreticulosarcoma of the lung (33.3% of cases), no bone cancers were found.

The malignant tumor incidence in the different groups of rats in experiment 1.1 was:

1. 92% (65)
2. 84% (59)
3. 68% (46)
4. 39% (26) control

Paraffin-fixed biological material is available for 283 male rats. The preparation method is described in STOREDB File 10865, accessible through [http://dx.doi.org/doi:10.20348/STOREDB/1041/1070](http://dx.doi.org/doi%3A10.20348/STOREDB/1041/1070) . The quantity of biomaterial stored in the repository is shown in the respective Table CONTRACT 86323\_biomaterial.

The larger number of animals for which biomaterial is available, compared to the number of animals involved in the experiment (283 compared to 273), is explained by including rats from other similar 239Pu experiments.

***Experiment 1.2 Dynamics of bone marrow and liver cells damage after exposure to 239Pu compounds with low transportability***

295 experimental and control male rats were involved in the experiment. Two types of insoluble 239Pu compounds were once intravenously administrated. The experiment design is presented in Table 1.

1а) 239Рu polymer nitrate (studies in different experimental groups 1, 8, 16, 32, 128, 180, 256, 356 and 512 days after single intravenous administration of Pu polymer nitrate);

1б) 239Рu dioxide (studies in experimental groups 8, 32, 128, 256, 412 days after single intravenous administration of Рu dioxide).

Table 1 – Contract 86323 – Experiment 1.2: Number of male Wistar rats exposed to Pu compounds with low transportability, with available cytogenetic investigations of bone marrow and liver cells

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  Contract 86323 | **Experiment 1.2а**239Pu-nitrate-polymer,IV, рН 1.5,intravenous administration  | 18.5 kBq/kg | 120 rats80 rats | myelocariocytes  |
| 55.5 kBq/kg |
| 166.5 kBq/kg | hepatocytes |
| **Control** nitric acid solution, рН 1.5 | 0.35 – 0.45 ml single |
| **Experiment 1.2b**239Pu-oxideintravenous administration | 23.2 kBq/kg | 95 rats | myelocariocytes |
| 46.3 kBq/kg |
| 92. kBq/kg |
| **Control** solution 0.1% twin,intravenous administration | 0.2 – 0.4 ml single |

***Results***

It was ascertained that:

* Stable aberrations of myelocariocytes chromosomes inheritable in a lineage of cells were found in rats exposed to internal radiation;
* A clear dose dependency of chromosomal rearrangement frequency on dose load value to skeleton and bone marrow was found;
* A similar dependency on the absorbed dose value was found for hereditary hepatocyte structures;
* The reduction of antibody-forming cells content was observed after intravenous administration of insoluble Pu compounds of high dose load.
* The suppression of natural killers functional activity was observed during the first 2-4 months after radionuclide administration.
* Dose dependency of immunity indices change was more clearly expressed after the exposure to incorporated Pu polymer nitrate.

The biological material in paraffin-fixed form (samples) was taken from 84 male rats (sacrificed after 180, 256, 365 and 512 days) to which polymeric 239Рu (74 rats) or HNO3 solution, рН 1.5 (10 rats) were administrated intravenously. These animals’ organs and tissues of main deposition (skeleton and liver) as well as bone marrow, spleen, thymus and lymph nodes were morphologically examined. The biological material from 84 rats was presented as paraffin-fixed samples in amount of 509 units.

***Experiment 2:*** ***Modification of tritium biological effect***

The experiments were conducted using Wistar male rats. The animals from the first two groups (experimental and control; 142 rats) were included into the experiment within the contract #76323 ( [http://dx.doi.org/doi:10.20348/STOREDB/1056/1137](http://dx.doi.org/doi%3A10.20348/STOREDB/1056/1137) ), preserved material was used within the contract #86323 for analysis and comparison of effects with data from two other groups (experimental and control; 156 rats). The animals from these two groups were administrated subcutaneously with pyrogenal (lipopolysaccharide, LPS), 9 monthly, single doses 1.5-2.5 MPD/100 g body weight, summarily – 13.5-22.5 MPD/100 g.

***Results***

Rate of tritium-induced leukemias at separate and pyrogenal-combined tritium administration:

1. 15.6% tritium;
2. 2.1% intact control;
3. 36% tritium + pyrogenal;
4. 0% pyrogenal.

*Types of leukemias:*

myeloleukemia 42 cases (25 – acute);

acute lymphatic leukemia – 17 cases;

erythroleukemia – 4 cases;

monoblast leukemia – 1 cases.

In 20% of cases during the period before hemoblastoses development hypoplastic states characterized by decrease of number of cells in bone marrow, lymphoid organs, pancytopenia of different intensity. It should be noted that for hemoblastosis development the following sequence of haemopoiesis disorder: hypoplastic stage – stage of erythropoietic cells hyperdysplasia – stage of granulocytopoietic cells hyperdysplasia. The two latter stages as a rule develop into leukemia, which type is mainly determined by a stage where pathology process was manifested. Tritium-induced hemoablastoses were formed against polypotent progenitors pool reduced by a factor of 2.5. Characteristics of committed progenitors of granulocyte-macrophage were also presented in the project report (method of cloning in CFC diffusion chamber).

Administration of lipopolysaccharide LPS (pyrogenal) modified nature of tritium-associated changes of intramedullary hematosis due to proliferating granulocytes by 20-40%, slower erythropoietic cells repair was noted, as well as neutrophils increase in blood. LPS administration was shown to create additional load on hematopoietic system. But if in healthy animals adaptation was quick, at prolonged tritium effect rats did not reveal adequate LPS reaction. It was specified by residual radiation damage in stem cell region, and enhanced proliferation of hemogenesis cells-progenitors hampered genome check and correction, most efficient at rest period. Prolonged tritium oxide administration was stated to lead to development of chromosomal rearrangement typical of hemablastoses long before (119-209 days) clinical manifestation of disease, before the first leukemia detection (239 days); latent period of leukemias developed at separate (449±59) and combined with LSP NTO effect (323±16 days) had the same time difference as non-random chromosome damage.

The biomaterial amounts to 7873 samples from 359 rats. More animals with the preserved biomaterial in these groups can be associated with involvement of animals with the similar LPS and 3НОН effect pattern from the other experiments. Description of four experimental groups and the biological material is presented in the respective Table CONTRACT 86323\_biomaterial.

***Experiment 3:*** ***Dynamics of leukemia development in conditions of continuous administration of tritium oxide with potable water***

The experiment involved 615 CBA mice of both genders: some of them (150 ♂ and 165 ♀, altogether 315 mice) got tritium oxide (3НОН) per os by 370 kBq/g body weight/day for 6 months. Accumulated dose for the whole period of tritium intake and clearance was 8.7 Gy. The control group of mice included 152 ♂ and 148 ♀. The majority (563 mice) was sacrificed 250, 350, 450 days after beginning of tritium administration; 52 mice died a natural death without reaching a fixed time.

***Results***

The studies performed under the previous project 76323 proved that expected lifespan of mice exposed to tritium oxide with body intake equal to experiment 3 rate and amount (with drinking water by 370 kBq/g body weight/day for 6 months, and accumulated dose for the whole period of tritium body intake and clearance 8.7 Gy) was reduced by 34%.

Analysis of histological study of male mice organs and tissues (experiment 3) sacrificed 250 days after beginning of exposure to tritium showed no difference in tumor frequency against the control. At the same time malignant tumors made 4.8 % of cases in female mice. A statistically significant increase of malignant tumors after tritium administration was found in 350 and 450 days (Table 2).

In the range of malignant neoplasms, in particular in 450-day analysis, a considerable fraction belongs to hematopoietic tissue tumors (leukemia: 13.8% (94 mice), control – 1.0% (101 mice). Liver neoplasms were presented mainly by benign hepatomas, and 1 mouse sacrificed after 450 days was diagnosed with hepatocellular cancer. Single lung adenocarcinomas were revealed only after tritium administration, control mice revealed only lung adenomas.

Table 2 – Contract № 86323, Experiment 3: Malignant tumor incidence, %

|  |  |  |
| --- | --- | --- |
| Sacrified at day 250 | Sacrified at day 350 | Sacrified at day 450 |
| control | 8.7 Gy | control | 8.7 Gy | control | 8.7 Gy |
| ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 0 | 0 | 0 | 4.8 | 0 | 2.1 | 9.6 | 8.7 | 0 | 1.9 | 14.5 | 21.7 |

***Relation of immune defects and development of tumor effects in mice***

Tritium late tumor effects are contributed by radionuclide-associated immune deficiency due to defects in all links of immunopoesis (cells deficiency in lymphoid organs, deficiency of antibody-forming cells, and reduction of natural killers’ antineoplastic activity). The most immune deficiency in exposed animals was observed during 150-270 days after the first impact. Direct relation of malignant neoplasms yield from value of decrease of humoral and cell immune factors, of value of lymphoid and hemopoietic organs, revealed before malignant neoplasms ascertainment (90-240 days after the first exposure). Similar correlation was not found at comparison of immunodeficiency factors with tumor frequency during the whole lifetime of a mouse, or after end of exposure (after 180 days).

Immunodeficiency state at tritium prolonged exposure is followed by period of malignant neoplasms histological manifestation.

To determine coefficient of biological efficiency for two types of radioactive effect in equal doses of internal tritium β-irradiation and external γ-irradiation from 137Cs source, the following factors were calculated:

1. Radiosensitive tissues damage and repair factors – 1-5
2. Residual radiation damage – 1-2.5
3. Immunoreaction suppression – 1.2-1.7
4. Late effects – 1.2-2.2 (control 140 rats, 3HOH 45 rats, γ-radiation 39 rats)

The quantity of animals with biomaterial is described in the respective Table CONTRACT 86323\_biomaterial.

***Link to other Contracts***

The study on biological effects was started within contract #86323 (this experiment) and continued within contract #89323 (see [http://dx.doi.org/doi:10.20348/STOREDB/1056/1163](http://dx.doi.org/doi%3A10.20348/STOREDB/1056/1163) ):

* Consistent doses of tritium oxide and external γ-radiation effect on immune condition in mice and rats; rats got tritium through potable water (4.1 МBq/ml, dose rate 8.5 сGy/day, 3-month accumulated dose 8.1 Gy);
* Comparison of effect in mice at tritium administration through potable water during 3 months with 1.2-5 МBq/ml at 3 exposure doses (absorbed doses from 3.1 to 9.1 Gy, dose rate from 3.3 to 9.2 cGy/day).

**Contact information**

If you want to use the material or have any detailed questions regarding the study you should contact SUBI through inter\_dep@subi.su. If you have any questions regarding this short summary report, please contact Dr. Bernd Grosche (bgrosche@t-online.de).