INFORMATIVENESS OF CLINICAL HEMATOLOGIC CRITERIA FOR THE EARLY DIAGNOSIS OF ACUTE RADIATION SICKNESS IN PIG-TAILED MONKEYS

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KEY WORDS: radiation sickness in monkeys; primary response; blood cell composition; adhesion of leukocytes; fluorescence of uranin.

The choice of adequate criteria for the early diagnosis of acute radiation sickness is an essential prerequisite for the extrapolation of experimental data from animals to man. The inclusion of monkeys in the extrapolation series, however, increases the reliability of the criteria developed and enhances their prognostic value. Differences in the course of radiation sickness in different species of monkeys must be borne in mind, since most radiobiological experiments in the USSR and elsewhere have been undertaken on Macaca rhesus.

The aim of this investigation was to study the diagnostic value and informativeness of the basic clinical manifestations of acute radiation sickness and certain hematologic parameters in the early stage after exposure to ionizing radiation in experiments on monkeys of a different species, the pig-tailed monkey (Macaca nemestrina), which is used comparatively less often in experimental radiobiology.

EXPERIMENTAL METHOD

Experiments were carried out on 10 male monkeys aged 1.5-3 years and weighing 4.7 ± 0.4 kg. The source of radiation consisted of gamma-quanta from ^{60}Co in a dose of 6-6.5 Gy (dose rate 1.2 Gy/min), corresponding to $CD_{100/20}$. The experimental results were subjected to

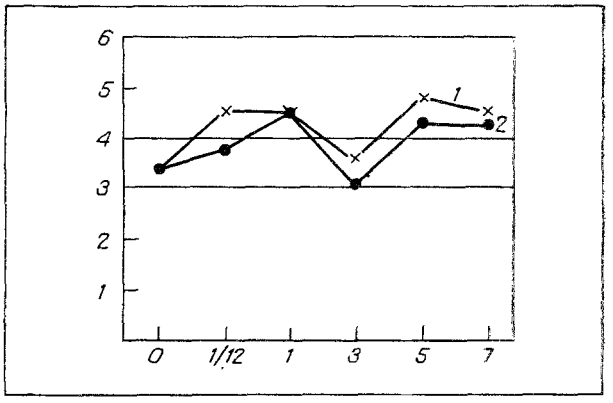

Fig. 1. Dynamics of spontaneous and evoked motor activity in irradiated monkeys. Abscissa, time after irradiation (in days); ordinate, activity (in points). 1) Evoked motor activity, 2) spontaneous.

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TABLE 1. Dynamics of Peripheral Blood Leukocytes in Irradiated Monkeys (6.0-6.5 Gy)

Parameter	Before irradiation	Time of investigation after irradiation					
		6 h	1 day	3 days	5 days	7 days	14 days
Leukocytes, $\times 10^9$/liter	16,0±2,5	8,4±1,4	6,6±1,8	2,9±0,7	2,4±0,5	1,4±0,2	0,78±0,1
Lymphocytes, $\times 10^9$/liter	8,3±0,3	1,1±0,4	1,98±0,34	0,86±0,14	0,9±0,12	0,2±0,12	0,6±0,08
Neutrophils, $\times 10^9$/liter	7,2±0,2	7,2±0,5	4,3±0,36	1,94±0,22	1,46±0,13	1,19±0,04	0,18±0,04

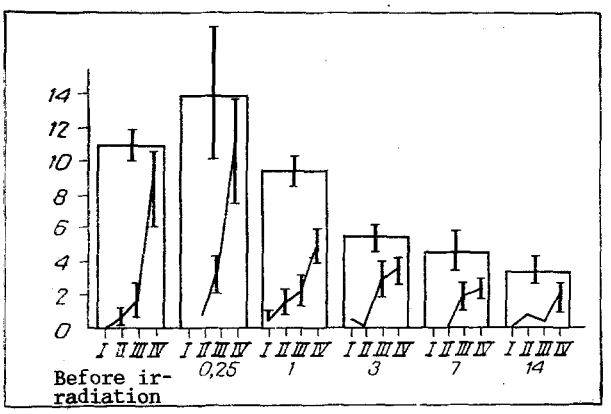

Fig. 2. Dynamics of changes in reticulocytes and differential reticulocyte count in irradiated monkeys. Abscissa, type of reticulocytes (I-IV) and time after irradiation (in days); ordinate, number of reticulocytes (in ‰).

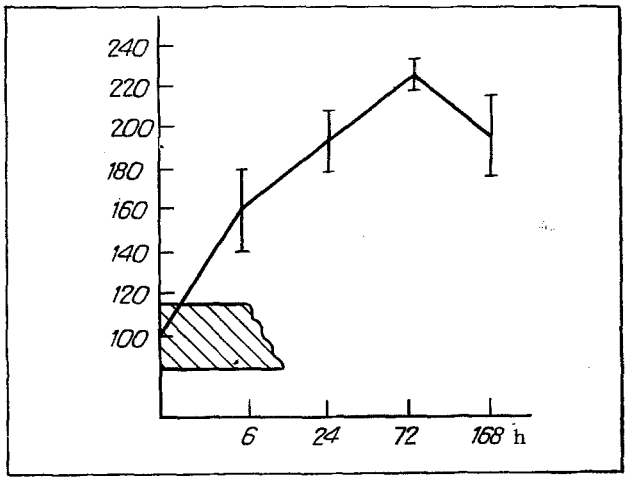

Fig. 3. Changes in adhesiveness of peripheral blood leukocytes of monkeys. Abscissa, time after irradiation (in h); ordinate, adhesiveness of leukocytes (in % of background).

statistical analysis with determination of arithmetic mean values by Student's t test (differences considered significant at the $p \leq 0.05$ level).

Clinical observation and investigation of spontaneous and evoked motor activity, and also of the food excitability of the animals were carried out in accordance with a 6-point scheme. Voluntary and evoked motor activity were characterized by a scale of states ranging from motor restlessness, assessed at 6 points, to complete immobility (0 point). A full peripheral blood count was carried out 6 h and 1, 3, 7, and 14 days after irradiation, and thereafter every 5 days until the end of the observations. The number of reticulocytes was counted at the same times and included a differential reticulocyte count, distinguishing four types of cells by degree of maturity, in the manner of Engel [5]. Meanwhile, starting with results obtained on animals of other species [4, 7], the adhesive ability of the leukocytes was

investigated by McGregor's method [9] and the intensity of fluorescence of uranin (fluorescein sodium) in the blood plasma of the irradiated animals was measured by microspectrofluorometry (λ of excitation 436 nm, λ of fluorescence 530 nm). The intensity of fluorescence was expressed in conventional units.

EXPERIMENTAL RESULTS

Observation of the animals revealed a mild primary response, as shown mainly by the absence of vomiting in the majority of monkeys (80%). Spontaneous and evoked motor activity differed only a little from the background level until 7 days (Fig. 1); food excitation of the animals also was preserved.

In the latent period (1-8 days) the state of the animals was satisfactory. Diarrhea was rare (two cases). A hypothermic reaction (lowering of the rectal temperature by 0.8-1.4°C between 4 and 7 days) was observed in 30% of cases.

Signs of illness, evidence of the beginning of the climax of the disease, occurred on the 12th-15th day. At these times petechial hemorrhages began to appear on the skin and visible mucous membranes. At the height of the disease diarrhea was present in 50% of cases. However, motor activity declined only during 1-3 days before death. Bleeding developed from the nose and gums, followed by dystrophic changes (edema). The mean length of survival of animals before death was 17 days.

The leukocyte count fell by 50-60% as early as 6 h after irradiation, and by the 30th day it had fallen by 7-8 times (Table 1). The early (6 h) and profound [down to (0.5-0.8) $\times 10^9$/liter] development of lymphocytopenia was observed. Neutrophils, however, began to appear appreciably from the peripheral blood at an earlier stage – after the 3rd day. The decrease in the number of erythrocytes and hemoglobin concentration was not significant at any time of the investigation.

The initial (6 h after irradiation) reticulocytosis was subsequently (after 1-3 days) replaced by reticulocytopenia, with marked changes in the differential reticulocyte count toward an increase in the number of immature forms (Fig. 2).

The intensity of fluorescence of uranin in the monkeys' plasma fell significantly (by 22%; $p < 0.05$) only on the 3rd day after irradiation. Later no changes were observed in this parameter.

In the experimental monkeys, just as in rats and dogs [4], the adhesiveness of the leukocytes was increased during the first days of irradiation, to reach a maximum by the 3rd day, and the normal state had not been restored even after 7 days (Fig. 3).

A radiation load of 6-6.5 Gy was thus high enough for the experimental monkeys: acute radiation sickness of the III degree developed and they all died between the 16th and 20th days. The clinical manifestations of the disease were clearly visible on the 12th-15th days after irradiation. Meanwhile the lesions in the pig-tailed monkeys showed certain specific features: mildness of the primary response (absence of vomiting, preservation of motor activity and of food excitability) and of the dyspeptic manifestations in the latent period. Meanwhile in rhesus monkeys the primary response is a constant feature and occurs very demonstratively [1, 2, 6, 8]. This particular feature of pig-tailed monkeys is in agreement with evidence of the lower lability of their CNS during exposure to extremal factors [3]. The mildness of the primary response in pig-tailed monkeys makes it impossible to predict the severity and possible outcome of the sickness soon (during the first few hours) after irradiation. Data on fluorescence of uranin in the blod plasma also are insufficiently informative: they point to the severity of the lesion only on the 3rd day. This test thus proved to be unsuitable for the early diagnosis of radiation sickness. Changes in several blood parameters were more reliable from this point of view. Significant disturbances in the dynamics and severity of the lymphocytopenia occurred soon after irradiation. It will be recalled that considerable changes in the blood lymphocyte count were found as early as 6 h after irradiation. The parameters characterizing adhesiveness of the leukocytes and the reticulocyte count, especially if including analysis of the differential reticulocyte count, are informative for early diagnosis.

The results of this investigation confirm the view that the further search for informative criteria for the early diagnosis and prognosis of acute radiation sickness must take into account differences in the responses of animals of different species to irrradiation.

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REGULATION OF DNA SYNTHESIS BY FIBRONECTIN AND ITS PROTEOLYSIS PRODUCTS IN SKIN FIBROBLASTS OF HEALTHY DONORS AND PATIENTS WITH SYSTEMIC SCLERODERMA AND RHEUMATOID ARTHRITIS

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The fibronectins are a family of high-molecular weight glycoproteins which are involved in cellular adhesion and migration, organization of the cytoskeleton, and processes of embryonic development, hemostasis, thrombosis, wound healing and malignant transformation [11]. In addition it is becoming increasingly evident that fibronectin plays a role in the pathogenesis of various diseases [3, 6]. Investigations have demonstrated the role of fibronectin and its fragments as growth factors and regulators of cell growth [4, 7, 9, 10]. It was shown previously that removal of gelatin-binding fragments (gel-fragments) from tryptic digests of the fibronectin molecule leads to marked stimulation of DNA synthesis in fibroblasts, and gel-fragments themselves inhibit DNA synthesis by 50-75% [2].

In the investigation described below a comparative analysis was undertaken of the effect of fibronectin and its tryptic hydrolysis products on DNA synthesis in cultures of skin fibroblasts from patients with rheumatoid arthritis (RA) and systemic scleroderma (SSD), and also of healthy blood donors (HD).

EXPERIMENTAL METHOD

Cell cultures and technique of culture were described previously [1]. The cultures were tested at the 3rd-10th passages in the stationary phase of growth. DNA analysis was carried out, using medium 199 with 0.5% solution of embryonic calf serum. Cells were synchronized in this same medium, cultured for 24 h. ^{14}C-Thymidine (USSR) was used as labeled precursor

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