

Protective effect and the therapeutic index of indralin in juvenile rhesus monkeys

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(Received 15 January 2014; revised 9 May 2014; accepted 13 May 2014)

The radioprotective effect of indralin in rhesus monkeys was examined over 60 d following gamma irradiation. Male and female rhesus macaques (*Macaca mulatta*) 2–3-years-old and weighing 2.1–3.5 kg were used. Animals were exposed to total-body gamma irradiation from ⁶⁰Co at a dose of 6.8 Gy (lethal dose, 100% lethality over 30 days). Indralin (40–120 mg kg⁻¹) was administered intramuscularly 5 min prior to radiation exposure. Indralin taken at a dose of 120 mg kg⁻¹ protected five out of six monkeys (compared with the radiation control group, in which all 10 animals died). The average effective dose of indralin in the monkeys exposed to gamma irradiation for 30 min was equal to 77.3 (63.3–94.3) mg kg⁻¹, and the maximum tolerated dose of indralin administered to monkeys was 800 mg kg⁻¹. Indralin reduced radiation-induced injuries in macaques, thus resulting in a less severe course of acute radiation syndrome. Delayed and less pronounced manifestation of the haemorrhagic syndrome of the disease, and milder forms of both leukopenia and anaemia were also noted. The therapeutic index for indralin, expressed as the ratio of the maximum tolerated dose to the average effective dose, was equal to 10. Therefore, indralin has a significant radioprotective effect against radiation and has a high therapeutic index in rhesus monkeys.

Keywords: monkeys; radioprotectors; indralin; acute radiation syndrome; haemorrhagic syndrome

INTRODUCTION

The radioprotector indralin (B-190) is used for medical protection of personnel during emergencies at nuclear power plants [1]. It has been demonstrated to have a high radioprotective effect in experiments with seven animal species, including dogs subjected to the condition of gamma-, gamma-neutron and high-energy proton irradiation [1, 2]. The study of drug effectiveness in large animals is of crucial importance in the final stage of pre-clinical testing of new medical preparations; this includes protective agents against radiation. To date, the radiation protective effects of cysteamine [3, 4], 2-aminoethylisothiuronium (AET) [5, 6], 2-(1 decylamine) ethane thiosulfonic acid [7], cysteamine-S-phosphate (cystafos) [8, 9], amifostine [4, 10–13],

5-methoxytryptamine (mexamine) [14–17], 5-androstenediol [18] and CBLB502, an agonist of toll-like receptor 5 [19], have not exceeded 50% in experiments conducted on dogs and monkeys. This value is significantly lower than those reported from studies using small laboratory animals (mice or rats), with the exception of the radioprotectors amifostine [12, 20] and indralin [21]. These agents provided 100% protection in large animals >45 d after exposure to lethal doses of gamma irradiation. The radioprotective activity of indralin is associated with a partial neutralization of the radiobiological ‘oxygen effect’ phenomenon (further radiation-induced damage as a result of the increase in cellular oxygen tension). Indralin as a direct alpha1-adrenomimetic [22, 23], has a vasoconstrictive effect and is able to increase tissue oxygen consumption. These outcomes promote the

development of acute circulatory and tissue hypoxia, e.g. in the haematopoietic tissues of large animal such as dogs [24, 25]. The radioprotective efficacy of indralin on haematopoiesis is first of all implemented by the protection of haematopoietic stem cells in the bone marrow and spleen. This enables rapid post-radiation recovery of the blood [26]. Indralin also reduces radiation injury to the salivary glands [27]. Furthermore, in conditions of non-uniform irradiation, indralin has demonstrated a mitigating effect when administered following exposures to lethal doses of whole-body radiation [28]. When administered after carboplatin, indralin attenuates carboplatin haemotoxicity [29, 30]. The present study describes our findings of both radioprotective and toxic effects of indralin in experiments on rhesus monkeys.

MATERIALS AND METHODS

Animal experiments

Experiments were conducted on juvenile male and female rhesus monkeys (*Macaca mulatta*) aged from 2–3 years with body weights of 2.1–3.5 kg. The average weight of the animals, with lower and upper quartiles, was 2.8 (2.2–3.0) kg in the control radiation group and 2.7 (2.2–3.1) kg in the experimental group of monkeys (those receiving the radioprotector). All animals were fed a standard forage diet and drinking water. The diet consisted of fruit (apples) and vegetables (carrots, red beets, cabbages, potatoes), as well as protein from chicken eggs. Euthanasia was not used in this study.

This study was performed taking into consideration the requirements according to regulatory legal acts on the procedure for experimental work on animals, including national (Order of Ministry of Health Care of Russian Federation No. 267 from 19 June 2003) and international ethical guidelines for medical and biomedical research involving animals (World Medical Association Declaration of Helsinki, 2000).

Irradiation

The monkeys were exposed to bilateral total-body gamma irradiation in the experimental ^{60}Co facility of model GUBE-2000 (2000 Ci of ^{60}Co charge) (Russia) at a lethal dose of 6.8 Gy (the radiation dose causing 100% mortality of animals by 30 d after irradiation = $\text{LD}_{100/30}$) and a dose rate of 22.8 cGy min^{-1} . The animals were irradiated without anaesthesia. The absorbed dose in the body (D_{ab}) was calculated as follows: $D_{\text{ab}} = D_{\text{ex}} \times 1.137 \times 0.877$, where D_{ex} = exposure radiation dose in the air, 0.877 = a transition coefficient for calculation of absorbed dose in air from exposure dose in air, and 1.137 = transition coefficient of the gamma ray ^{60}Co energy of 1.25 MeV for the calculation of absorbed dose in body from absorbed dose in air.

Drug administration

Indralin (CAS 156799–03–0, Farmzaschita, Federal Biomedical Agency, Moscow, Russia) was administered intramuscularly (i.m.) to monkeys in the thigh area at doses of 40, 60, 80 or 120 mg kg^{-1} as a 2.5% solution 5 min before exposure to radiation. After irradiation, the animals received antimicrobial agents according to a single treatment protocol: levomycetin (Ferein, Moscow, Russia) 200 mg kg^{-1} administered orally once daily on Days 1–10; penicillin (Biosynthes, Penza, Russia) 150 mg kg^{-1} and streptomycin (Biochimic, Saransk, Russia) 50 mg kg^{-1} i.m. once daily on Days 10–20. Four unirradiated macaques received toxic doses of indralin i.m. (800 and 1000 mg kg^{-1}).

Determination of the radioprotective effect of indralin

The radioprotective properties of indralin were estimated from the survival analysis of the animals over the 60 d following irradiation, and by the presence of clinical symptoms of acute radiation syndrome (ARS). Changes in body weight and rectal temperature were monitored. We also examined the abdominal region by palpation, assessed pulmonary function by observing respiration, and visually inspected the skin and mucous membranes, including the oral cavity and anal area. Leukocyte, platelet, erythrocyte and reticulocyte counts, haemoglobin values and the erythrocyte sedimentation rate (ESR) were determined from the peripheral blood of the animals. Clinical manifestations of acute radiation injury in the intestines included diarrhoea, and tension of the abdomen. These symptoms were evident from Days 5–10. During the prodromal period was monitored by recording of vomiting, diarrhoea and locomotor and behavioural disorders during the first day after irradiation. Toxic doses of indralin were based on clinical signs of poisoning and death of the animals within three days of the treatment.

Statistic analysis

Data are expressed as mean \pm standard error. The presence of a radioprotective effect was evaluated by analysing the survival curve using the Kaplan–Meier method (Cox's F -test). Statistical analysis was performed using the computer program 'Statistica' version 7. Statistical probability was determined using the non-parametric Mann–Whitney U-test with a minimum sample size of three, a two-tailed Fisher exact test, and factorial analysis of variance. P -values < 0.05 were considered to indicate a significant difference between the control and indralin-treated groups at a particular time-point. The dose that induced 50% of the effect (ED_{50}) for indralin was calculated according to probit analysis with the Litchfield and Wilcoxon modification method [31]. The graphs were drawn using the computer program GraphPad Prism version 5.

RESULTS

Effects of indralin on survival of irradiated monkeys

The radioprotective effects of indralin in juvenile rhesus monkeys (*Macaca mulatta*) are shown in Fig. 1. After 6.8 Gy gamma irradiation ($LD_{100/30}$; dose rate $22.8 \text{ cGy min}^{-1}$), i.m. administration of indralin at a dose of 120 mg kg^{-1} protected five out of six monkeys compared with the death of all 10 animals in the control radiation group ($P < 0.02$, Fisher's two-sided exact test). When the dose of the drug was reduced 1.5 times (to 80 mg kg^{-1}), the radiation protective effect of indralin was equal to 50% (three out of six monkeys survived). After the administration of 60 mg kg^{-1} , one out of four monkeys survived to Day 60 of the experiment. Indralin at a dose of 40 mg kg^{-1} was not effective, and the three experimental animals died from ARS. The ED_{50} of indralin in the monkeys exposed to gamma irradiation ($LD_{100/30}$) for 30 min was equal to $77.3 (63.3\text{--}94.3) \text{ mg kg}^{-1}$. Indralin reduced the severity of lethal radiation damage in monkeys up to a milder course of ARS.

Clinical manifestations of a prodromal reaction to irradiation in the monkeys

Under the conditions of gamma irradiation described above, the monkeys from the control radiation group had a clearly manifested prodromal reaction. Vomiting was observed in the majority of the exposed animals (90% of cases) mostly between 30 and 60 min after exposure to radiation; 50% of the monkeys had experienced repeated vomiting. The

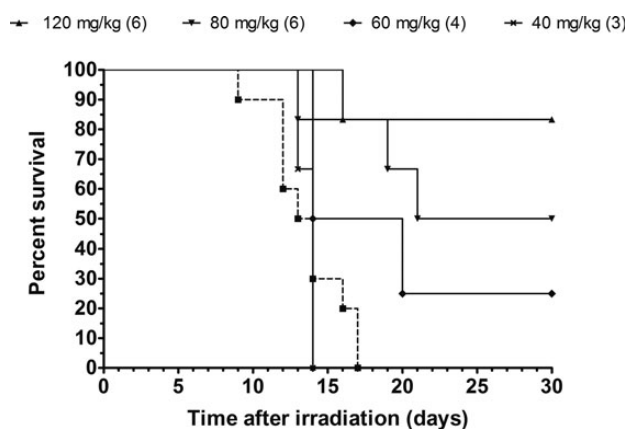


Fig. 1. Survival of monkeys (*Macaca mulatta*) following treatment with varying doses of indralin (40–120 mg/kg) after whole-body gamma irradiation (6.8 Gy; $LD_{100/30}$). Asterisk indicates $P < 0.05$, i.e. significant difference between the control and indralin-treated group by Cox's F -test through the Kaplan–Meyer survival curve method. Monkeys were injected with indralin i.m. 5 min before irradiation. The number of animals in each experimental group is shown in parentheses. All the monkeys that were alive on Day 30 survived until Day 60.

earliest periods of vomiting were observed 9 and 14 min after exposure to radiation. The development of diarrhoea in the same period was less frequent (20% of cases). The mobility of the animals decreased within 15–60 min after irradiation, and this was maintained for several hours.

Behavioural changes were also observed in the animals. They remained in a seated position with their heads greatly lowered; they also rejected food such as fruits. In some cases, animals could only lay on their sides. At that time, one monkey had a limiting seizure of the extremities. After the cessation of vomiting, locomotor activity and interest in food was gradually restored. External manifestations of the primary reaction to irradiation were not detected until 24 h after exposure.

Clinical manifestations of ARS in irradiated monkeys

By the end of the first week of ARS, 45% of the animals from the control group had diarrhoea and tension of the abdomen, and its palpation appeared to cause discomfort (Fig. 2A). Body temperature did not change significantly. The exposed monkeys had decreased motor activity and suppressed interest in food. The body weight of the animals had decreased by an average of 10% by Day 7, and this continued till the weight loss was 15–20% when death from ARS occurred ($P < 0.05$) (Supplementary Table S1, Fig. 2B).

From Day 9 post-irradiation (two days earlier than indralin-treated animals), the irradiated monkeys from the control group showed the first signs of haemorrhagic syndrome in the form of an isolated petechial rashes, usually in the abdominal area, the inner surface of the thighs, the groin area, or the eyes and chin, as well as in the oral mucosa. A few days before death, by the 11th–13th day of the disease, the majority of irradiated animals had pronounced nasal and intestinal bleeding, extensive haemorrhages of confluent character in the skin, which came away in the form of strips, and edema in the area of the chin, presenting as a bruise (Fig. 2C, 2D).

Severe leukopenia and thrombocytopenia were observed in peripheral blood samples (Fig. 3A, 3B). Haemorrhagic syndrome resulted in acute and profound decrease in erythrocyte counts and haemoglobin levels by up to 50% of initial levels (Fig. 3C, 3D). In monkeys from the control group, the leukocyte and erythrocyte count nadir in peripheral blood was $0.7 (0.4\text{--}0.9) \times 10^9 \text{ l}^{-1}$ and $2.5 (2.1\text{--}2.9) \times 10^{12} \text{ l}^{-1}$, respectively, by the 14th day of ARS (Supplementary Table S1, Fig. 3A, 3C).

During this period, animals that survived for two weeks underwent a febrile reaction, with a rise in body temperature from $38.3^\circ\text{C} (38.2\text{--}38.5)$ to $39.2\text{--}40.2^\circ\text{C}$ (Fig. 2E) and an increase in the ESR from $3 (2\text{--}4) \text{ mm h}^{-1}$ to 56 mm h^{-1} (Fig. 3E). Monkeys lay on their sides and rejected food. Irradiated monkeys from the control group died between Days 9 and 17

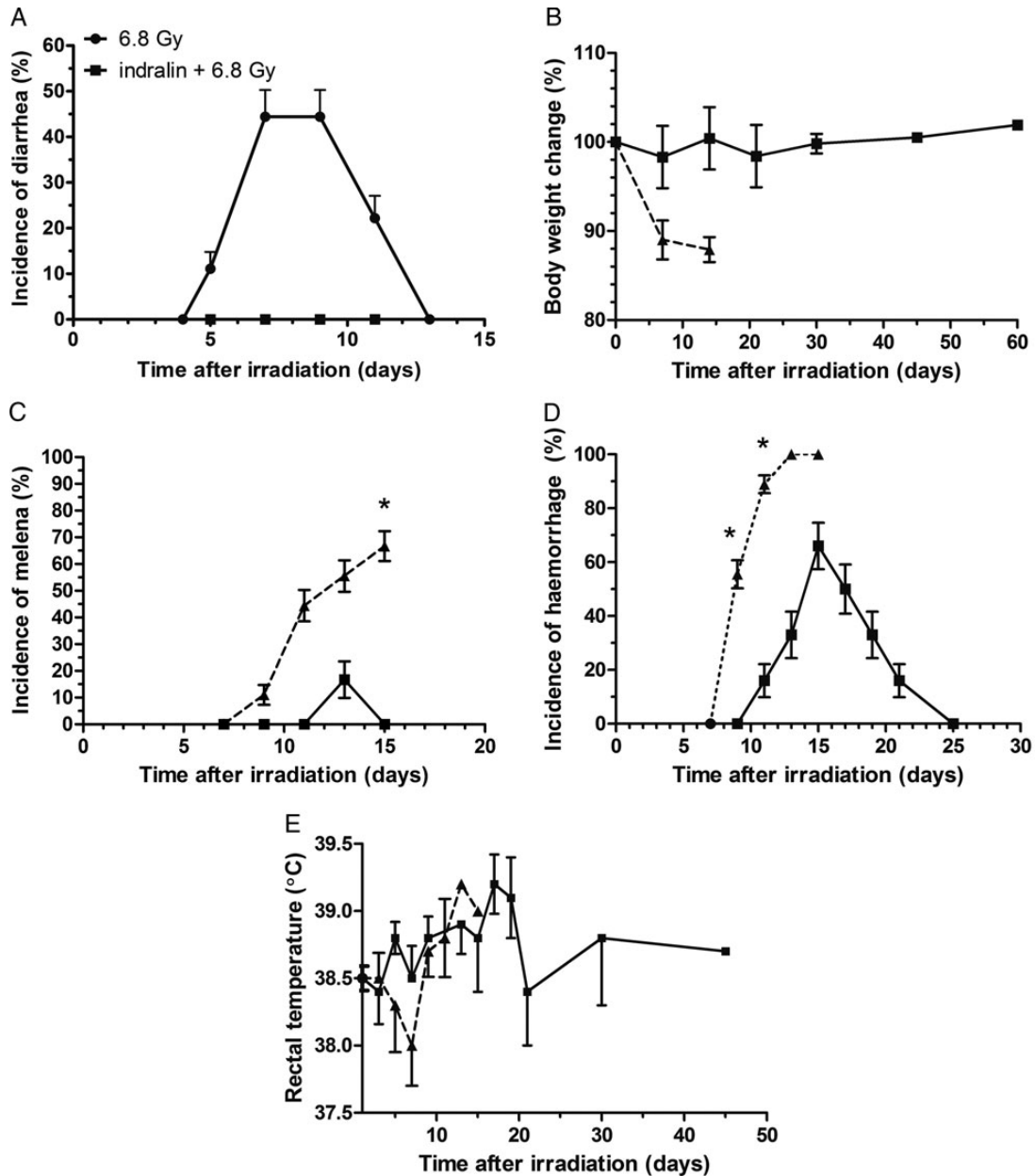


Fig. 2. Incidence of clinical manifestations of acute radiation syndrome: diarrhoea (A), body weight changes (B), melena (C), haemorrhage (D) and rectal temperature (E) in control and indralin-protected (80 mg/kg) monkeys after total-body gamma irradiation (6.8 Gy). Asterisk indicates $P < 0.05$, i.e. significant difference between the control and indralin-treated group by a non-parametric two-tailed Fisher exact test and Mann-Whitney U-test.

after exposure to radiation (mean lifespan of control irradiated monkeys 14.1 ± 0.9 d) (Fig. 1).

Sex differences in the radiosensitivity of the monkeys were not evaluated in this study. The mean lifespans of the deceased animals after irradiation were 13.4 ± 1.4 d for females ($n = 5$) and 14.8 ± 1.0 d for males ($n = 5$). On the seventh day after radiation exposure, the leukocyte counts were $1.53 \pm 0.82 \times 10^6 \text{ l}^{-1}$ for females ($n = 5$) and $1.31 \pm 0.23 \times 10^6 \text{ l}^{-1}$ for males ($n = 5$).

Anatomical examination of radiation injuries on the bodies of monkeys

Autopsy determined that the cause of death in these animals was ARS, with severe aplasia of the bone marrow, spleen, and lymph nodes, and multiple haemorrhages in the intestinal mucosa, endocardium, pericardium, heart muscle, and lung tissue; features of catarrhal gastroenteritis and haemorrhagic ulcerative colitis were also observed. There was one case of low-lobar pneumonia with a haemothorax, which

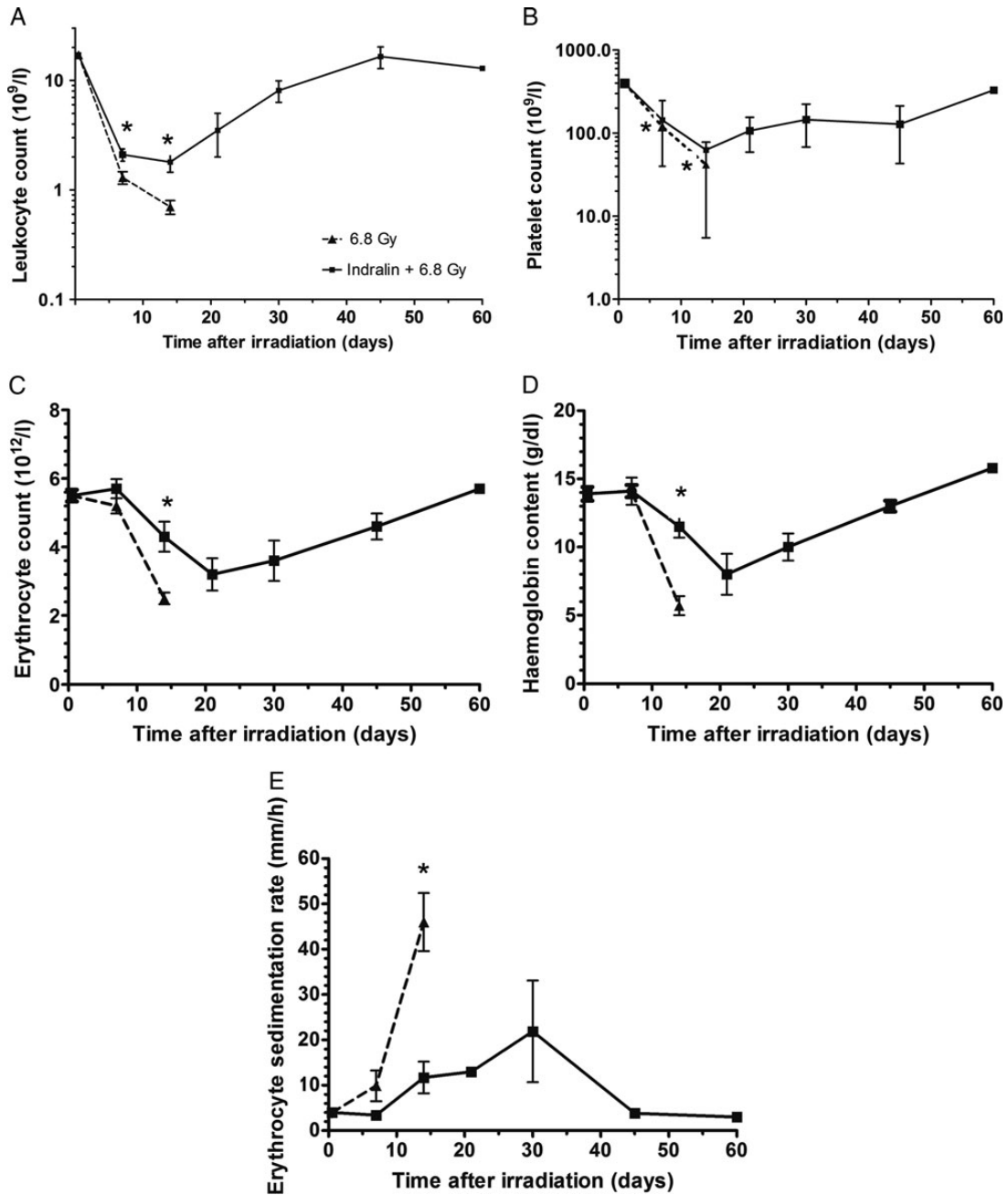


Fig. 3. Haematological patterns: leukocytes (A), platelets (B), erythrocyte count (C), haemoglobin content (D) and erythrocyte sedimentation rate (E) associated with acute radiation syndrome in control and indralin-treated (80 mg/kg) monkeys after whole-body gamma irradiation (6.8 Gy). Asterisk indicates $P < 0.05$, i.e. significant difference between the two groups that were compared by a non-parametric Mann–Whitney U-test.

was detected in the monkey that died on Day 21 of ARS after treatment with 80 mg kg⁻¹ of indralin.

Radioprotective effects of indralin on the clinical manifestations of ARS

Indralin did not reduce the severity of the prodromal reaction to irradiation; however, no obvious signs of disease

progression were observed. The radioprotective effect of indralin most clearly manifested itself during the development of the haematopoietic syndrome of ARS (Days 9–20 after exposure). Haemorrhaging of the skin and visible mucous membranes was evident in the experimental monkeys on the 12th day of the disease, which was significantly later than in the control irradiated animals who did not

receive indralin. The haemorrhaging was less intense and disappeared after two weeks (Fig. 2D), which may account for the decreased anaemia in this group of animals. The erythrocyte count on the 14th day of ARS in indralin-treated (80 mg kg⁻¹) animals was 1.7 times higher compared with the control radiation group, and the erythrocyte count nadir occurred later (on Day 21 of ARS) in the treated animal group (Fig. 3C). The body weight of the indralin-treated animals remained virtually unchanged, which was significantly different to the weight loss observed in the control group ($P < 0.05$) (Fig. 2B).

Improvement in the general health of the protected monkeys was observed within the third week after irradiation; the animals regained motor activity and appetite and were emotionally responsive to the researcher. Haemorrhaging in the skin and mucous membranes could no longer be seen. The leukocyte count nadir ($(0.9\text{--}1.2) \times 10^9 \text{ l}^{-1}$) that occurred on Day 14 of ARS was higher in indralin-treated monkeys compared with the control radiation group ($P < 0.05$) (Supplementary Table S1, Fig. 3A). Notably, the leukocyte count of surviving monkeys treated with 120 mg kg⁻¹ indralin on Day 14 of ARS was from $0.8 \times 10^9 \text{ l}^{-1}$ and higher compared with low line of $1.1 \times 10^9 \text{ l}^{-1}$ in the monkeys treated with 60–80 mg kg⁻¹ of indralin. Macaques from both groups who had lower leukocyte counts died.

At that time, a moderate increase in body temperature and ESR was recorded (Figs 2E, 3E). In general, changes in platelet and erythrocyte counts, haemoglobin content, ESR, and body temperature were correlated with the clinical manifestations of haemorrhagic syndrome associated with ARS, including melena (Figs 2C, D and E, and 3B, C, D and E). Remarkable increases in leukocyte and reticulocyte counts were observed beginning in the third week. Anaemia persisted at that time, but by the 45th day after exposure to radiation, the indralin-protected monkeys had recovered from both leukopenia and anaemia. Furthermore, the platelet count had increased to $340 \times 10^9 \text{ l}^{-1}$ by the end of the observation period (Fig. 3B).

Acute toxicity of indralin in monkeys

Indralin tolerance in rhesus monkeys was evaluated following i.m. administration of the radioprotector at toxic doses of 800 and 1000 mg kg⁻¹. All three monkeys that received a dose of 800 mg kg⁻¹ survived. One monkey died from poisoning 20 min after administration of indralin at a dose of 1000 mg kg⁻¹. This animal showed signs of significant respiratory distress.

The clinical picture of the action of indralin administered at a dose of 800 mg kg⁻¹ was as follows. Indralin, being a direct alpha1-adrenomimetic [22, 23], caused piloerection in the site of the animal withers a few minutes after administration, which persisted for 3 h. Repeated vomiting was observed every 2 min (six times) in one of the three monkeys. The depressed effect of the protectant was observed 5 min after

administration. The monkeys lay on their backs, retaining the ability to respond to external stimuli, though clearly demonstrating impaired motor coordination. The animals continued to be in a state of slight drowsiness with half-closed eyes, and were often restless. One hour later, the monkeys preferred to sit, with persistent severe ataxia.

During that period the animals refused to eat apples and sugar when ataxia is proceeding. Severe ataxia lasted up to 2 h. The monkeys sat hunched with a lowered head and closed eyes. By the fourth hour, their movements remained awkward. However, their interest in the environment increased, with a restored quick response to sound signal in the form of an aggressive posture. The condition of the animals was satisfactory after 24 h; they took food and quickly responded to the researcher without demonstrating ataxia. We observed moderate body weight decreases in the monkeys exposed to radiation and received prophylactic administration of indralin at a dose of 120 mg kg⁻¹. This decreased weight was likely due to decreased appetite, which is a side-effect of a high dose of the radioprotector.

DISCUSSION

Indralin has radioprotective effects on haemopoietic tissue, the intestines, the skin, the testis and the salivary glands [26, 27, 32–34]. The radioprotective effects of indralin have been studied in seven species of experimental animals (mice, rats, hamsters, guinea pigs, rabbits, dogs and monkeys) [21, 23, 26, 32, 35]. Indralin is a direct adrenomimetic agent that induces its protective effects through alpha1-adrenergic receptors [22]. The radioprotective actions of indralin are inhibited by tropaphen and chlorpromazine (which are non-selective adrenoblockers [21, 23]), prazosin (which is a selective alpha1-adrenoblocker [22]), and theophylline (which is an inhibitor of cyclic adenosine monophosphate (AMP) degradation [21]). The radioprotective effect of orally administered indralin lasted up to 1 h [35]. The monoamine oxidase (MAO) inhibitor iproniazid was found to double the duration of action by indralin [21]. Finally, indralin is a direct alpha1-adrenomimetic that increases arterial pressure and reduces pulse rate [35].

The protective mechanism of indralin is the result of acute hypoxic effects in radiosensitive tissues. As a direct alpha1-adrenomimetic, indralin causes changes in microcirculation due to the vasoconstriction of pre-capillaries and the simultaneous increase in oxygen consumption by cells through the stimulation of tissue respiration [21, 23–25]. The respiration of 100% oxygen during irradiation decreases the radioprotective effects of indralin [23, 24]. As a radioprotector, indralin, along with partial shielding of the abdomen, protects dogs irradiated with a triple lethal dose of radiation [35]. As a mitigator of radiation effects, indralin increases the survival of dogs by more than 50% when administered after irradiation [36]. The damage-mitigating property of indralin increases

dramatically under conditions of non-uniform irradiation [37].

Based on the data presented in this study, the therapeutic index (TI) of the radioprotective action of indralin in monkeys was established as the ratio of the maximum tolerated dose of indralin and ED₅₀ of indralin administered before exposure to the absolute lethal dose of radiation. In this case, the TI equalled 10. The TI of indralin in the experiments on dogs under the same conditions would be a little more than 10 [2]. Based on the published data on the toxicity and radiation protective properties of amifostine in experiments on dogs [12, 13, 20, 38], and using the same principle for its calculation, the TI was found to be close to three. Given the high potential for the radiation protective efficacy of amifostine and indralin in the experiments on dogs, the latter has a TI three times higher. The large TI for indralin in the experiments on dogs and monkey is due to the high specificity and potential of radioprotective alpha1-adrenergic receptor action as compared with that of radioprotectors from the family of aminothiols, which implement their protective effect via biophysical and biochemical processes in cells [2].

SUPPLEMENTARY DATA

Supplementary data is available at the *Journal of Radiation Research* online.

ACKNOWLEDGEMENTS

The research cited in this article was from publications up until December 2013. The opinions contained in this paper are the views of the authors.

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