High Dose of h-R3, an Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Labeled With $^{188}$Rhenium Following Intravenous Injection into Rats

Bárbara O. González-Navarro, MVD, MSc*
Angel Casaco, MD, PhD †
Mariela León, DVM ‡
René Leyva, MSc ‡
Avelina León, DVM *
Edilis Santana, BSc *
Ana Margarita Bada, MSc *
Nelvis Subirós, MSc *
Normando Iznaga-Escobar, PhD †
Rolando Pérez, MD, PhD †

*El Centro Nacional para la Producción de Animales de Laboratorio (CENPALAB) [National Center for Breeding of Laboratory Animals], Havana, Cuba
†Center of Molecular Immunology, Havana, Cuba
‡Isotope Center, Havana, Cuba

KEY WORDS: toxicity, monoclonal antibody, cancer treatment, rhenium 188, h-R3

ABSTRACT
Radioimmunotherapy (RAIT) is a promising new therapeutic modality for the treatment of a variety of malignancies. Humanized monoclonal antibody (MAb) h-R3 is a product, developed at the Center of Molecular Immunology in Havana, Cuba, that was labeled with rhenium 188 ($^{188}$Re) (Center of Molecular Immunology in Havana, Cuba). It can be used in the treatment of neoplasia of transformed cerebral cells that overexpress epidermal growth factor receptor (EGF-R).

The acute local and systemic toxicity effects of single intravenous dose of $^{188}$Re labeled or unlabeled MAb h-R3 in 100 Sprague-Dawley rats were studied. The rats were distributed into 5 groups of 20 animals (10 male, 10 female) from a mean dose of 14.20 mCi/m² on 20 µg/100 µL/animal to a mean dose of 41.86 mCi/m² on 60 µg/300 µL/animal. Standard endpoints were collected at various times after administration (ie, days 0, 14, 28, 42, 56, and 70). Endpoints included clinical observations, an abbreviated neurological assessment, body weight, hematology and clinical chemistry parameters, and, at the study termination, necropsy examination, organ weight, and histological evaluations.

RAIT did not show toxic effects in the bone marrow or in peripheral blood,
only an increase of alkaline phosphatase and a decrease of liver weight when compared with the control group.

It was concluded that the proposed phase I clinical trial can be planned under the hypothesis of locoregional administration of \(^{188}\text{Re}\)-h-R3 with intensive medical management. This phase I trial can be used to enhance antitumor effects with minimum toxicity to normal tissue in patients who have grade III/IV astrocytomas who were treated previously with conventional therapies.

**INTRODUCTION**

Radioimmunotherapy (RAIT) has shown promise in the treatment of small solid tumor masses, both in experimental tumor-bearing animals and in patients. Considerable advances have been made in the field of experimental RAIT. Animal models have been useful for evaluating radiolabeled monoclonal antibody (MAb) targeting of tumors, including pharmacokinetics and dosimetry, and for the evaluation of normal tissue toxicity. A variety of animal model ligands and radionuclides have been used in these studies.\(^1,2\)

The most common brain tumor in adults is glioblastoma multiforme (GBM), which is also the most malignant form of primary central nervous system (CNS) neoplasm. Over the last 2 decades, surgery, radiotherapy, and chemotherapy have improved the survival of some patients with anaplastic gliomas; however, little progress has been made in improving the survival of patients with GBM. The median survival rate of patients treated for newly diagnosed GBM is 40 to 60 weeks\(^3\); survival is 16 to 24 weeks for those with recurrent disease.\(^4\) For patients with high grade gliomas of the brain, epidermal growth factor receptor (EGF-R) expression can be of greater magnitude than the surrounding normal tissue.\(^5\) In contrast, increased levels of EGF-R are associated with malignant transformation of squamous cells,\(^6\) and it is overexpressed in a wide variety of human tumors, including non-small cell lung cancer.\(^7\)

EGF-R signal transduction pathways have been correlated with various processes that contribute to the development of malignancies, such as cell-cycle progression, inhibition of apoptosis, angiogenesis, tumor cell motility, and metastasis.\(^8\) EGF-R overexpression has also been associated with chemoresistance and radioresistance.\(^9\)

The humanized anti-EGF-R monoclonal antibody (MAb h-R3), recently registered in Cuba for the treatment of head and neck tumors, is an \(\text{IgG}_1\) isotype, and it was obtained by cloning the variable regions of the ior egf/r3, a murine \(\text{IgG}_{2a}\) antibody that recognizes the EGF-R and inhibits the binding of EGF.\(^10,11\) A reshaped antibody was constructed using the light and heavy chains of REI and Eu, respectively, as human immunoglobulin frameworks for complementary determining region grafting.\(^12\)

Even the selection of radionuclides available for RAIT has expanded in recent years; several useful candidates are available and have shown clinical promise. Rhenium 188 (\(^{188}\text{Re}\)) constitutes an ideal radionuclide for patient imaging and RAIT because it has a 2.1-MeV maximum energy with a 0.97-cm path length similar to \(^{90}\text{Y}\), but with a shorter half-life of approximately 17 hours. The attractiveness of this radionuclide is that it can be produced efficiently from an in-house \(^{188}\text{W}\) generator. \(^{188}\text{Re}\) is produced by double neutron capture on tungsten 186 target to produce tungsten 188 (\(^{188}\text{W}\)). The production and decay scheme for the \(^{188}\text{Re}/^{188}\text{W}\) pair has been published.\(^13\) Iznaga-Escobar showed the effects of the \(^{188}\text{Re}\) direct-labeled MAb h-R3 (\(^{188}\text{Re-h-R3}\)) in RAIT of solid tumors, normal organ
biodistribution, absorbed radiation doses on normal organs and tumors, as well as the toxicity to bone marrow and normal tissues.\textsuperscript{13}

It has been shown that \textsuperscript{188}Re-h-R3 has low systemic and local toxic effects when administered intracerebrally to rats at a dose up to 50 mg of h-R3 labeled with 579 \pm 23.7 mCi.\textsuperscript{14} Systemically administered radiolabeled MAb does not accumulate in primary CNS neoplasms in amounts sufficient to deliver therapeutic levels of radiation without significant systemic toxicity.\textsuperscript{15} However, if the blood-brain barrier is interrupted by the presence of a tumor, as well as surgical treatment, then it would be necessary to know the systemic toxicity effects, assuming that the whole labeled antibody could pass into the blood system. This study was designed to determine the acute toxicity effect of single intravenous administration of humanized anti-EGF-R antibody h-R3 labeled with different doses of \textsuperscript{188}Re in Sprague-Dawley rats.

**MATERIALS AND METHODS**

**Antibody Radiolabeling**

The MAb h-R3, developed at the Center of Molecular Immunology in Havana, Cuba, at a concentration of 5 mg/mL, was reduced with 2-mercaptoethanol (2-ME) at a molar ratio of 2000:1 (2-ME:MAb) at room temperature for 30 minutes. The reduced antibody was purified to eliminate the excess of 2-ME through a Sephadex G-50 minicolumn (Pharmacia Biotech, Uppsala, Sweden) as previously described by Iznaga-Escobar et al.\textsuperscript{16,17} Following reduction of intrinsic disulfide bonds, aliquots containing 3 mg of h-R3 were dispensed into 10-mL vials. To reduce the MAb solution, 500 \mu{}L of the glucoheptonate solution (containing 50 mg of glucoheptonate, 5 mg of ascorbic acid, and 1 mg of stannous chloride/mg of MAb) were added and labeled with 4 to 42 mCi of perrhenate (ReO\textsubscript{4}{-}), eluted from a (\textsuperscript{188}W/\textsuperscript{188}Re) generator (MAP Medical Technologies Oy, Jyvaskyla, Finland). Activity was measured in a radioisotope dose calibrator (CRC-15R, CAPINTEC Inc., Ramsey, NJ).

For quality control of the radiolabeled product, ascending paper chromatography was run on Whatman 3 MM paper as the stationary phase and 0.6 % saline and acetone as the mobile phase to separate free perrhenate and \textsuperscript{188}Re-glucoheptonate, which moved with the solvent.\textsuperscript{16,17} Human serum albumin (HSA, 1 %)–impregnated ITLC-SG (Gelman Science, Ann Arbor, MI) strips were used as the stationary phase and ethanol:ammonium hydroxide:water (2:1.5 v/v) was used as the mobile phase to separate radiocolloids that remain at the base while the radiolabeled MAb and free perrhenate moved away (colloid, R\textsubscript{f} =0.0, MAb-\textsuperscript{188}Re and glucoheptonate-\textsuperscript{188}Re, R\textsubscript{f}=1.0).\textsuperscript{18}

**Immunoreactivity Assay**

Reactivity of \textsuperscript{188}Re-h-R3 and anti-human leukocyte antigen (HLA) antibody to H-125 human lung adenocarcinoma cell line was determined by flow cytometry analysis. Cells were harvested and adjusted to $10^6$ cells/mL in RPMI 1640, supplemented with 10.0 % fetal bovine sera (Gibco Laboratories, Gaithersburg, MD) and penicillin-streptomycin-neomycin antibiotics. Two hundred microliters of cell suspension were added to LP3 tubes, and centrifuged at 3000 rpm for 5 minutes. Cells were washed twice with phosphate buffered saline (PBS) solution pH 7.4, resuspended in 100 \mu{}L of saturating amounts of antibody concentration, ranging from 0.36 to 10.4 \mu{}g/mL, and incubated at 4°C for 30 minutes. The cells were then washed twice again with PBS solution. They were resuspended in 100 \mu{}L of 1:20 dilution of goat antihuman IgG, conjugated with fluorescein isothio-
cyanate (FITC, Dako, Denmark), and incubated at 4°C for 30 minutes. After washing, cells were resuspended in 500 µL of PBS solution and analyzed for immunofluorescence with a fluorescence-activated cell scan (FACS, Ortho Diagnostic Systems, Raritan, NJ). For each point of the standard curve, the immunoreactivity of the $^{188}$Re-h-R3 was expressed as the percentage of positive cells obtained after subtraction of the value given by incubating the cells with isotypic matching nonspecific anti-HLA antibody and with the conjugate alone.

**Animals**

One hundred young adult Sprague-Dawley rats of both sexes (179.4 ± 15.26 g for female and 208.6 ± 16.6 g for male animals) were obtained from the National Center for Breeding of Laboratory Animals (CENPALAB, Havana, Cuba) and adapted for 7 days to experimental conditions. Five animals were housed per cage, and free access to water and food was allowed during the study. Environmental conditions were controlled during the whole experiment: temperature was 25°C ± 2°C; humidity was 55% to 60%; and 12-hour light/dark cycles were used. The study was carried out with approval from CENPALAB’s Institutional Animal Care and Use Committee.

**Administration and Dosage**

One hundred rats were randomly distributed into 5 experimental groups of 10 animals of each sex in each group for 70 days. The test articles were administered as a single injection through the tail vein. Group I was treated with 300 µL of saline and was considered to be the negative control group. Group II received 300 µL of a solution containing the MAb h-R3 labeled with $^{188}$Re at a concentration of 2.6 mCi/200 µg/mL. Group IV received 200 µL of a solution containing the MAb h-R3 labeled with $^{188}$Re at a concentration of 2.4 mCi/200 µg/mL. Group V received 300 µL of a solution containing the MAb h-R3 labeled with $^{188}$Re at a concentration of 2.9 mCi/200 µg/mL.

**Clinical Observations, Behavioral and Laboratory Parameters**

Animals were observed twice daily to evaluate their general state of health through appearance and behavior. Body weight and food and water consumption were measured weekly during the study. Corneal, pineal, righting, and myotactic reflexes were explored on days 0, 23, and 70. Blood sampling for analysis of the following hematological and clinical chemistry parameters were taken from the right retro-orbital plexus on days 0, 14, 28, 42, 56, and 70 using a Pasteur’s pipette. Hematological parameters measured were hemoglobin, hematocrit concentration, red blood cell count, total and differential counts of leukocytes, platelet count, and reticulocyte count. The following blood biochemical parameters were analyzed: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, total protein, albumin, glucose, and creatinine. Animals were fasted for a period of 12 to 14 hours prior to blood samplings. Sedation of rats for blood sampling procedure was carried out using diethyl ether.

**Anatomical-Pathological Study**

At study termination, all animals were killed by exsanguination through a
femoral vein, and a cervical dislocation maneuver was applied under depression of central nervous function obtained with an ether atmosphere. Afterward, they were necropsied for pathological examination.

Gross examination of the external body surface, orifices, as well as cranial, thoracic, and abdominal cavities, including detailed evaluation of the application site and all organs, was conducted. Prior to fixation, the adrenals, thymus, spleen, kidneys, liver, lungs, heart, and brain were weighed in a Sartorius scale, and organ weight/body weight ratio x 100 was calculated for statistical analysis.

Samples from the brain, pituitary gland, skin, eyes, extraorbital lacrimal glands, hardierian glands, trachea, esophagus, tongue, salivary glands, thyroid, parathyroid, thymus, heart, lungs, spleen, liver, stomach, pancreas, duodenum, jejunum, ileum, cecum, colon, rectus, adrenals, aorta, kidneys, cervical and mesenteric lymph nodes, sternum/bone marrow, bladder, skeletal muscle, sciatic nerve, prostate, seminal vesicles, epididymis, testes, penis, prostate, uterus, cervix, ovaries, application site, and bone were taken. For light microscopic examination, tissues were fixed in 10% neutral formaldehyde embedded in paraffin, then cut and stained with hematoxylin and eosin (H&E). All above-mentioned tissues from the animals in the control, MAb h-R3, and high-exposure 188Re-h-R3 groups were microscopically examined in addition to the livers of the animals that were exposed to lower doses of 188Re-h-R3. Bone marrow samples from the femurs of 30 animals (3 of each sex per group) that were randomly selected were also studied. The samples were stained by the May-Grunwald–Giemsa method.

Statistical Analysis
The mean and standard deviation (SD) were calculated for all parameters.

Statistical evaluation was performed by a randomized complete analysis of variance (ANOVA) design with significance assessed at $P<0.05$. For body weight, food consumption, drinking water, hematology, and clinical chemistry, data were compared by a 2-factor ANOVA. For organ weight data, treatment comparisons were made on rank-transformed data using 1-factor ANOVA. All these tests were performed independently by sex. For bone marrow data, treatment comparisons were performed on the raw data in 1-factor (combined sexes) ANOVA. This analysis was done by a commercially available statistical software program, STATISTICA Version 6 (StatSoft Inc, Tulsa, OK). The normal range per sex for hematological and clinical chemistry parameters were preestablished, taking into account the $X \pm 2 SD$ of the values at time t=0 (pretreatment values).

RESULTS
Antibody Radiolabeling and Immunoreactivity Assay
All radiolabeled procedures were performed under aseptic conditions in a shielded hood with laminar flow. All glassware, plastics, and solutions were sterile and pyrogen-free. The purified humanized MAb h-R3 was labeled with a specific activity range from 1.21 to 14.17 mCi/mg protein. A mean of 94.91 ± 0.19% of 188Re was bound to IgG1 and 2.68 ± 0.62% to glucoheptonate as determined by paper chromatography. Instant paper chromatography of labeled MAb in acetone showed that about 2.41 ± 0.70% or less free perrhenate ran at the $R_f =1.0$. This indicates that perrhenate was reduced almost quantitatively. The radiocolloid determination was 1.50 ± 0.27%. The immunoreactive fraction (IRF) of 188Re-h-R3 measured by flow cytometry analysis and by Lidmo method on the H-125 human lung adenocarcinoma cell line was 0.78,
Clinical Observations, Body Weight, and Mortality

No clinical symptoms of toxicity were observed. Figures 1 and 2 show the rats' body weight gain during the study. The animals showed a gradual body weight increase through the entire period, and no significant differences were found between groups. Overall, there were no effects on water and food consumption when measured periodically throughout the study. No significant differences on corneal, pineal, righting, and myotactic

Figure 1. Body weight gain of female rats treated intravenously with MAb $^{188}$Re-h-R3 for 70 days.

Figure 2. Body weight gain of male rats treated intravenously with MAb $^{188}$Re-h-R3 for 70 days.
reflexes were observed when control and treated groups were compared. In contrast, 1 male rat from group III that received 20 µg of MAb h-R3 containing \(^{188}\text{Re}\) at 13 mCi/m\(^2\) manifested petechial hemorrhages in the ears on days 15, 17, 18, and 19 post-treatment, and its body weight decreased until it died.

**Hematology and Biochemistry Parameters**

Hemoglobin, hematocrit, differential leukocyte count, and platelets count were similar between intervals and sex. No significant differences between groups were found. The percentage of lymphocytes prevailed over the rest of the white cells. In the bone marrow, it was observed that the number of megakaryocytes is directly proportional to the number of platelets in peripheral blood, and no statistically significant differences between groups in reticulocyte counts were found, as they increased considerably after the second sampling \((P=0.0001)\).

No significant differences in bone marrow data were obtained when the control, MAb h-R3 only, and RAIT groups were compared. The mitosis phenomenon was observed in both systems (red and white blood cells), and it is interpreted as cellular proliferation. In the studied subjects in this report, depression of the megakaryoblastic system was not observed in either erythroid system. The cell amount was superior to 60% with integrality for both systems. The myeloid/erythroid relationship was favorable to the red in 100% of the animals. The treatment used in this study did not induce toxicity in the bone marrow (Figure 3).

Alkaline phosphatase levels were high at third sampling, surpassing the established limits of normal in the female rats (33.90 to 238.46 U/L) of the groups treated with \(^{188}\text{Re}-\text{h-R3}\), as well as in the high-dose group of male rats, rising slightly above the range of 64.30 to 390.94 U/L (Figure 4).

**Anatomical-Pathological Study**

Liver weight decrease was observed in both sexes in the saline control group compared with the other groups. Analysis of liver weight showed significant differences between the treated and control groups for both sexes, females \((P=0.0001)\), males \((P=0.0182)\) (Table 1). Other differences in the statistical study of the organ weights among groups were not found.

Based on microscopic evaluation of the liver in animals of either sex that were treated with MAb h-R3 and all doses of RAIT appeared morphologically altered. The distance between the efferent and afferent vasculature was reduced as a result of the loss of hepatic cell accumulation (data not shown). Occasional apoptotic hepatocytes were scattered throughout the parenchyma. Virtually all of these affected cells were concentrated around the central vein, although a cuff of hepatocytes in contact with the central vein remained intact. Accumulations of macrophages lined the centrilobular sinusoids, and focal collections of mononuclear inflammatory...
cells were occasionally present around the afferent vasculature (Figure 5). This apparent increase in the number of macrophages probably reflects the collapse of the hepatic lobule. Throughout this response, the hepatic parenchyma bordering the portal tract showed no detectable morphological or tinctorial changes compared with that of the control animals.

Different changes in hepatocyte morphology were indicative of apoptosis (apoptotic hepatocytes displayed the emargination and segregation of chromatin against the nuclear membrane, the cytoplasm was condensed, increasingly eosinophilic, and in occasions, vacuolated with the cytoplasmic membrane crenelated). The fragmentation of the affected cells formed membrane-bound apoptotic bodies, many of which either lacked or had vestiges of a nucleus. These bodies were subjected to phagocytosis by local macrophages lining the sinusoids and surrounding hepatocytes.

![Figure 4](image-url)  
**Figure 4.** Alkaline phosphatase levels of male and female rats treated intravenously with a single dose of MAb 188Re-h-R3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Organ Weight/Body Weight x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td>Control</td>
<td>3.69 ± 0.25</td>
</tr>
<tr>
<td>MAb h-R3</td>
<td>3.07 ± 0.25*</td>
</tr>
<tr>
<td>Low-dose 188Re-h-R3</td>
<td>3.17 ± 0.25*</td>
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<tr>
<td>Middle-dose 188Re-h-R3</td>
<td>2.90 ± 0.31*</td>
</tr>
<tr>
<td>High-dose 188Re-h-R3</td>
<td>2.93 ± 0.22</td>
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</tbody>
</table>

*Significantly different from control group, P < 0.05  
MAb h-R3 = humanized anti epidermal growth factor receptor monoclonal antibody  
188Re-h-R3 = humanized anti epidermal growth factor receptor monoclonal antibody labeled with Rhenium 188
The male rat that died also manifested misalignment of the dentition, resulting in abnormal growth of incisor teeth, as well as impoverishment of the general state, serous atrophy of the coronary fat, scarce deposit of perirenal fat, slight fatty change of the liver, and intense congestion in all organs, with emphasis in kidney and liver.

DISCUSSION

Hybridoma technology and the resulting production of MAb in large quantities have offered new perspectives in the treatment against cancer. MAb h-R3 induces very little immune response of human anti-mouse immunoglobulins, and it has been applied in the diagnosis of tumors by means of immunohistochemistry and radioimmunodetection.

A previous work related to the intracerebral treatment in Sprague-Dawley rats with relatively large doses of \(^{188}\text{Re-h-R3}\) (up to 328 \(\mu\) Ci/g of brain) was performed by the authors of this paper; this elicited no toxicity effect on the blood and a slight increase in alanine aminotransferase values in females and in creatinine blood values in both sexes. Neurological toxicity was not seen and was not dose limiting in the application site at rats’ brains.

These rats received approximately 14 times the protein dose of MAb and 4.9 times that of the radioisotope proposed by in the proposed locoregional RAIT phase I clinical trial in patients who have neuroepithelial cerebral tumors. This protocol in patients will be conducted with the radioconjugate \(^{188}\text{Re}\) labeled with MAb h-R3 in a dose-escalation toxicity study, in a range from 10 to 30 mCi, including 3 patient groups. The dose of protein of the MAb h-R3 is only 3 mg in those patients who will receive the radiolabeled antibody after surgical resection of primary brain cancer in concert with external beam therapy. To achieve this RAIT, these tumors will overexpress EGF-R, which will be shown by immunohistochemical study. According to Blumenthal et al, this pre-screening of cancer biopsies could be used to optimize treatment planning. Thus, intracavity treatment with radiolabeled antibodies is an approach that can be administered safely, alone or in combination with other treatment modalities, but it appears to be most promising in minimal residual disease.

In this study, the maximum dose of MAb h-R3 (60 mg) and \(^{188}\text{Re}\) (44 mCi/m\(^2\)) is up to 7 times the dose of MAb h-R3 and 2.5 times that of the radionuclide proposed for the clinical trial. In rats, it confirmed the absence of radiation effects on the progenitor cells of the hematopoietic system; bone marrow cellularity was above 60%, and mitotic activity in the megakaryopoietic and erythroid system was proven. In the peripheral blood, the collateral effects of dose limiting were not reported for the bone marrow. For RAIT, the hematopoietic stem cells are the most important
and most radiosensitive normal cells. Other elements of the normal hematopoietic system space are the microenvironment, and the blood-marrow barrier that suffers less radiation damage. Compared with chemotherapeutic agents, RAIT reagents have the advantage of making preclinical research more meaningful, and they produce higher tumor response rates and less toxicity in initial clinical studies.21

In dose escalation studies with only RAIT in rats, dog, and human patients, bone marrow toxicity was always the dose-limiting normal tissue; the second-highest dose-limiting normal tissue was the intestinal tract in rats, the liver in dogs, and the lung in humans. The dose-limiting side effects of RAIT on normal tissues are hematological: granulocytopenia and thrombocytopenia. Lymphopenia was observed in animals and humans within 24 hours after RAIT administration and is thought to be a result of the apoptosis of irradiated peripheral blood lymphocytes. Side effects in other organ systems are rare and occur only after serious dose escalation or combination therapies.22,23

Another effect of RAIT in the peripheral blood is anemia, but it was not evidenced in this study. Hemoglobin and hematocrit were within the normal range. In the control group, the male rats presented the highest values compared with the females, as described in the literature.24 The leukocyte differential counts showed a higher percentage of lymphocytes than neutrophils, which is characteristic of rats.24

Rats are less affected by hematological side effects after radiation, confirming the greater resistance of smaller species, because of their hematopoietic stem cell concentration is higher than larger animals.25 In the bone marrow, the number of megakaryocytes in rats was 3 times higher than it was reported in humans.24 The values registered in bone marrow agree with data reported previously.24

The effects of radiation in the normal tissue of the vascular system is reported. It manifested fundamentally at the endothelial level, and as a consequence, it also manifested as microhemorrhages and interstitial edema. The vascular bed integrity is one factor that limits the radionuclides that can be administered to a tissue; irreparable changes to other tissues due to their radioresistance should not be produced.26

In particular, the rat that died showed impoverishment of the general state, decreased body weight, malocclusion of the incisive teeth, and petechial hemorrhages in the ears during clinical inspection that could have been the result of an inadequate nutrition and oxygenation of the tissues, probably because of the weakening of the vascular wall. Through the results of the hematologic and biochemical investigation, it was determined that the death of the rat was not associated to hematological dyscrasia or dysfunction of any organ; the anatomical-pathological examination proved that the rat’s death was not due to RAIT’s effects.

Biodistribution studies in rats showed the main binding sites of the MAb h-R3, the blood activity profile, and the MAb elimination routes. The MAb h-R3 accumulation was seen in liver, lungs, kidneys, intestine, and spleen.27 Furthermore, other studies confirmed that the MAb h-R3 maintained the original in vivo recognition pattern of normal organs and tissues.27,28 The high liver uptake was related to the larger number of excretion sites and metabolism of radiolabeled MAb’s and their degradation products. The liver is one of the organs that presents higher expression of EGF-R,27,28 and the CENPALAB Sprague-Dawley rats express receptors against MAb h-R3.29 These results are of a great importance in the justification of
the findings of this study. This assumption is confirmed by the effects observed in the study groups: evidence of atrophy in the liver and apoptotic hepatocytes. An increase in alkaline phosphatase and diminishing liver weight were also detected.

The intravenous treatment effect of the immunoconjugate on critical healthy tissue (particularly the liver) could be explained by the presence of EGF-R in liver cells, which contributes to a rapid removal of labeled MAb from blood, thereby resulting in a radiation dose to the liver cells in addition to the cytotoxic effects of the MAb itself. The cytotoxic effect of the MAb plays a significant role in inducing cell death. In addition, the different morphological aspects of liver apoptosis were noted in the study rats. It evoked loss of liver mass due to the atrophy and the apoptotic hepatocytes were scattered occasionally throughout the parenchyma.

CONCLUSION

The treatment of gliomas with systemic administration of RAIT, although feasible, is fraught with difficulties, as described by Hopkins and colleagues.29 Radioimmunoconjugates penetrate the blood-brain barrier poorly, and even in situations where the blood-brain barrier is disrupted, accumulation of radioisotopes is low. The nature and location of tumors led to the development of intrathecal administration with direct tumor injection or injection into the surgical bed. This approach has proved to be possible and has shown promising results, especially for patients with lower grade tumors and small volume disease. Bone marrow toxicity is not dose limiting. Cerebral edema is frequently seen and is usually transient.31

These results indicate that, under the conditions of this study and according to the evaluations performed, the test articles, MAb h-R3 labeled with moderate levels of $^{188}$Re (up to 44 mCi/m² as total dose) resulted in low toxicity. Consequently, the Sprague-Dawley rats tolerated higher radionuclide doses of $^{188}$Re-h-R3. It was concluded that the proposed phase I clinical trial can be planned under the hypothesis of locoregional administration of $^{188}$Re-h-R3, with intensive medical management, to enhance antitumor effects with minimum toxicity to normal tissue in patients who have grade III/IV astrocytomas who were treated previously with conventional therapies.

REFERENCES


