RESEARCH

Preclinical safety demonstration of the human recombinant erythropoietin HEBERITRO®

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ABSTRACT

Erythropoietin (EPO) is a glycoprotein that appears in blood as a response to hypoxia, acting on bone marrow to increase the production of erythrocytes. This glycoprotein is produced mainly by the kidneys; and its biosynthesis and release are stimulated by the reduction of tissue oxygenation and/or the reduction of the mass of erythrocytes. Here we report the results of the preclinical evaluation of the safety of HEBERITRO®, commercial name for the recombinant human erythropoietin (rhEPO) produced by the Center for Genetic Engineering and Biotechnology (CIGB), Havana, Cuba, through a comparative study of acute toxicity with EPREX®, a commercial homologue, and a study of local tolerance. The product was administered subcutaneously into Sprague-Dawley rats, at doses of 10, 30 and 60 times the therapeutic dose (TD) for the comparative acute toxicity assay, and at the TD, 10 and 20 times this value, and placebo for the local tolerance assay. The dosage and inoculation schemes were designed for measuring the short-term toxicity of HEBERITRO® after a single administration at high dosages, and the irritating potential for the subcutaneous tissue after seven daily inoculations. No signs of toxicity or morphological alterations were detected in the animals during the assays, and there were no histological changes in the organs under study. Based on these results, we conclude that this product does not induce signs of toxicity or local reactions at the administration site in the established dosage and under our experimental conditions.

Key words: erythropoietin, preclinical toxicology, EPREX®, safety

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RESUMEN

Seguridad preclínica de la eritropoyetina humana recombinante HEBERITRO®. La eritropoyetina (EPO) es una glicoproteína que aparece en la sangre como respuesta a la hipoxia, y actúa sobre la médula ósea aumentando la producción de eritrocitos. Esta glicoproteína se produce principalmente en el riñón. Su biosíntesis y secreción se estimula por la disminución de la oxigenación del tejido y/o por la disminución de la masa de hematíes. En este trabajo se evalúa la seguridad preclínica de HEBERITRO®, EPO humana recombinante (EPOhr) producida por el Centro de Ingeniería Genética y Biotecnología (CIGB), Ciudad de La Habana, Cuba, mediante estudios preclínicos. Entre ellos, el estudio comparativo de toxicidad aguda (con EPREX®, como similar comercial) y el de tolerancia local. Este producto se administró por vía subcutánea a ratas Sprague-Dawley, en tres niveles de dosis 10, 30 y 60 veces la dosis terapéutica (DT) del producto en el primer estudio y DT, 10 y 20 veces esta, y un placebo de esta. El objetivo de estos esquemas fue la determinación de la posible toxicidad a corto plazo de HEBERITRO® tras la administración única de varias dosis lo suficientemente altas y del potencial irritante para el tejido subcutáneo luego de siete aplicaciones diarias. Durante estos ensayos, no se reportaron signos de toxicidad, ni alteraciones morfológicas en los animales. Desde el punto de vista histológico no se observaron alteraciones macroscópicas ni morfológicas que evidenciaran toxicidad en los órganos estudiados. De acuerdo con los resultados, este producto no provoca signos de toxicidad ni reacciones locales en el sitio de administración, en el rango de dosis establecido y bajo nuestras condiciones experimentales.

Palabras clave: eritropoyetina, toxicología preclínica, EPREX®, seguridad

*I*ntroduction

Erythropoietin (EPO) is an essential growth factor for erythroid progenitors at the bone marrow that regulates the production of erythrocytes in response to the physiological oxygen demand. It was originally isolated from the urine of aplastic anemia patients [1] and characterized as a 34 000 Da protein. Carbohydrates comprise 40% of the structure of the molecule, and are distributed at 3 N-linked and 1 O-linked glycosylation sites with sialic acid as main component. These carbohydrates are essential for the biological activity of EPO [2]. The terminal sialic acid residues protect the molecule from rapid clearance at the liver, thus maintaining its level of activity at the bone marrow, and the enzymatic elimination of sialic acid by neuraminidases leads to loss of the biological activity of EPO *in vivo*.

Since the publication of the seminal study by Jacobson on anephric rats [3], it has been assumed that the kidneys are the physiological site for EPO synthesis, and that its production is increased in res-

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ponse to generalized anemia or a selective decrease of the oxygen supply to the kidneys. EPO interacts with its target cells through specific receptors on the cellular surface. These receptors are primarily expressed at erythroid progenitor cells, but can also be found at embryonic stem cells, multipotent hematopoietic progenitor cells, endothelial cells and neural cells [4-6].

The manufacture of human EPO (rhEPO) through recombinant DNA technology has made possible its widespread therapeutic use at clinical settings. When used at doses from 50 to 100 U/Kg this molecule, according to clinical data, can be used to treat the anemia associated to entities like chronic renal insufficiencies, zidovudine treatment in AIDS patients, rheumatoid arthritis, chemotherapy, premature birth, antologous transfusions and oncohematological diseases, among others [7-12]. No allergies have been observed upon administration of EPO in clinical trials [10], although local cutaneous reactions and arthralgias have been noted for a few patients [13]. These side effects are more likely to be related to the use of human albumin in the formulation of EPO than to its direct biological effects [13, 14]. A worsening of arterial hypertension, convulsions and thrombolytic episodes have been reported during use of EPO in patients with renal insufficiencies [15], but these side effects have not constituted a significant problem for the use of rhEPO in other clinical situations.

Although the endogenous EPO and rhEPO are similar in their biological and chemical properties, some microheterogeneities at the four carbohydrate moieties are not well tolerated [2], which makes preclinical evaluation of the safety of EPO formulations an essential need. Toxicity studies in animals can have a predictive value for humans and are indeed used for the testing of new products [16]. A study designed for the preclinical assessment of the safety of HEBERITRO® is presented here that includes the evaluation of single dose toxicity and local tolerance. With this goal, the product was compared to a commercial homologue (EPREX®) for the evaluation of single doses, and the potential for irritation at the site of delivery was assessed by repeated inoculations.

Materials and methods

The studies were performed in compliance with the ethical guidelines for the use of animals in experimentation [17], Good Laboratory Practices [18-19] and approved Standard Operating Procedures for the implementation of toxicological studies [20].

Assay animals

The Cenp: SPRD ALY® (Sprague-Dawley) rat substrain was used in the assays. The animals were provided by the Gnotobiotic Rodents Division of the National Center for the Production of Laboratory Animals (CENPALAB, Havana, Cuba). Upon arrival, the rats underwent a clinical examination, were weighed, and individually housed in Makrolon® boxes with sterile sugarcane wood-shavings. They were kept under observation for 7 days before starting the study, under controlled environmental conditions (temperature from 19 to 21 ºC, average relative humidity of 68% and light-darkness cycles of 12 hours) that were also maintained throughout the assays. Food (ALY co., CENPALAB, Cuba) was delivered daily at a proportion of 25 g per animal, and water was supplied *ad libitum.*

Formulation

The EPO formulations used in the study were HEBERITRO®, manufactured at the Center for Genetic Engineering and Biotechnology (Havana, Cuba), and EPREX®, marketed by Johnson & Johnson (United Kingdom) for the treatment of anemia. In total, four batches of HEBERITRO® were used for both assays, all with a purity higher than 99% (HPLC-RP), a biological activity of 70.4 x 106 U/mL, and a total protein concentration (Lowry) of 0.39 mg/mL. Additional tests performed on the batches included sterility, apirogenicity, pH, volume, and stability. EPREX®, manufactured by Johnson & Johnson, was obtained from a commercial batch (B10060), and the placebo was composed of the same components used on the formulation of HEBERITRO® minus the active principle.

Experimental design

The assays were designed following the regulations described by the ICH/EMEA (International Conference on Harmonization/European Agency for the Evaluation of Medicinal Products) [21-24]. The design also took into account factors described at guideline 425 from the OECD [25]. The animals were randomly assigned to the acute toxicity or local tolerance studies, using a list generated by the Marsman FR software [26], and inoculated subcutaneously.

Experimental groups and dosage

A total of 70 rats, both male and female, were used for the acute toxicity study. Males had an average weight of 234.5 g, females had an average weight of 182.6 g, and the age ranged from 5 to 6 weeks. The local tolerance study included a total of 40 male rats, randomly selected. The variations in body weight for both experiments did not exceed 20% of the average (199.3 g for an age of 6 to 7 weeks). The animals were distributed as shown in table 1.

Inoculation frequency

A single inoculation at day one of the study was performed for the acute toxicity study, whereas 7 consecutive inoculations, 1 per day, were administered for the local tolerance assay. The subcutaneous route was used in both cases (this is the route to be used for clinical trials in humans), in the interscapular space.

Assay length and observations

The acute toxicity study lasted for 14 days, after which the animals were euthanized. The environmental conditions described above were kept throughout the study, and daily clinical observations were conducted with the aim of registering any behavioral variations or signs of toxicity. These clinical evaluations followed the assessment method described by DiPascuale and Hayes [15], and included changes in skin, fur, pigmentation and appearance of mucous membranes, eyes, respiratory system, central and autonomous nervous system, and somatomotor activity. Body weight was measured at days 1, 7 and 14, and food

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intake was quantified daily. Hematological tests were done for all the animals per treatment group at day 1 of the assay (before inoculation) and before euthanasia, using a *Celltac* digital hematological analyzer. The parameters evaluated were: red cell and leukocyte counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte counts, platelets, hemoglobin and hematocrit.

The local tolerance study lasted for 8 days. The parameters examined during observations were similar to those used for the acute toxicity assay, in addition to the evaluation of local signs such as irritation, edema, erythema and induration. Five animals from each group were sacrificed 24 hours after the first administration, and the remaining individuals were sacrificed upon conclusion of the study.

Histopathological study

On the day appointed for euthanasia, a cervical dislocation procedure was practiced for the animals after anesthetizing with ether, and blood was drawn from a cut on the femoral artery. The body surfaces and cavities were scrutinized, and a necropsy was performed for macroscopic inspection of every organ. Samples were taken from the spleen, liver, bone marrow, mesenteric lymph nodes and the inoculation site, to be stained with eosin-hematoxylin and examined under a Carl Zeiss (Germany) simple optical microscope at magnifications of 40 and 100X [27].

Data processing

The variables used for statistical processing were body weight (BW), average weekly food intake (FI), the hematological parameters and the microscopy findings (FM). In all cases, central tendency and dispersion statistics were calculated (mean, standard deviation, maximum and minimum values). For the treatment of the hematological parameters, the variable FD was created (difference between the final and initial value).

The assumptions of normal distribution and homogeneity of variance were verified by the Kolmogorov Smirnov and Shapiro-Wilk tests [28] and by the Levene test, respectively, before the analysis of the BW and FI variables for each evaluation time point or gender. Depending on whether the data fit a normal distribution or not, a parametric analysis of variance (ANOVA) or a non-parametric alternative (Kruskall-Wallis test) [28] was used. Paired comparisons were performed on consecutive intervals, using either the paired t test or the Wilcoxon test [28], depending on whether the data fit a normal distribution. The results from the histopathological studies were analyzed by making cross-tabulated classification tables, using the test for associated independence (Fisher's exact test) [28]. The data were processed with the SSPS 8.0 statistical application software, running on the Windows operating system [29].

Results and discussion

Clinical observations

No side effects or signs or toxicity were observed after daily observations of the animals inoculated with HEBERITRO® or EPREX® in the comparative acute toxicity assay. There were no changes in fur or pigmentation and the eyes and mucosal surfaces appeared normal, as did the somatomotor activity and behavior. Proper responses to stimulation were obtained, and no deaths were reported during the study. Upon evaluation of the inoculation site, no signs of damage attributable to the administration of rhEPO were evidenced. The only abnormalities detected were clinical signs (hemorrhagic areas) that appeared on the local tolerance study, but these were related to the method and the reiterative nature of the inoculation rather than to the use of rhEPO itself, since they also appeared with the same intensity in the placebo group. The results of the hematological tests were normal when compared to their values before the start of the study.

Table 1. Distribution by groups of the animals for the toxicity studies of HEBERITRO®.

Group	# animals	Treatment	Product/Dose (UI/kg)	Volume (µL)
Acute toxicity				
I	10	-	Placebo/0	940
II	10	10 times the therapeutic dose	HEBERITRO®/1500	160
III	10	30 times the therapeutic dose	HEBERITRO®/4500	470
IV	20	60 times the therapeutic dose	HEBERITRO®/9000	940
V	20	60 times the therapeutic dose	EPREX/9000	940
Local tolerance				
I	10	-	Placebo/0	300
II	10	therapeutic dose	HEBERITRO®/150	20
III	10	10 times the therapeutic dose	HEBERITRO®/1500	150
IV	10	20 times the therapeutic dose	HEBERITRO®/3000	300

Body weight and food intake

Body weight increas ed steadily and significantly during the acute toxicity study, as shown in figure 1.

This result held true if the same data were analyzed independently per gender or evaluation time point (days 0, 7 and 14) ($p = 0.01$) (Tables 2 and 3). There were no significant differences between the treatment groups ($p > 0.05$), including the group treated with the commercial homologue, EP REX®. The Food Intake variable oscillated between the groups for each evaluated time point. Significant differences were detected between the groups at day 1: for males, I-III, $p = 0.008$; I-IV, $p = 0.001$ and for females, II-VI, $p = 0.001$; III-V, $p = 0.008$ at the same point. Despite

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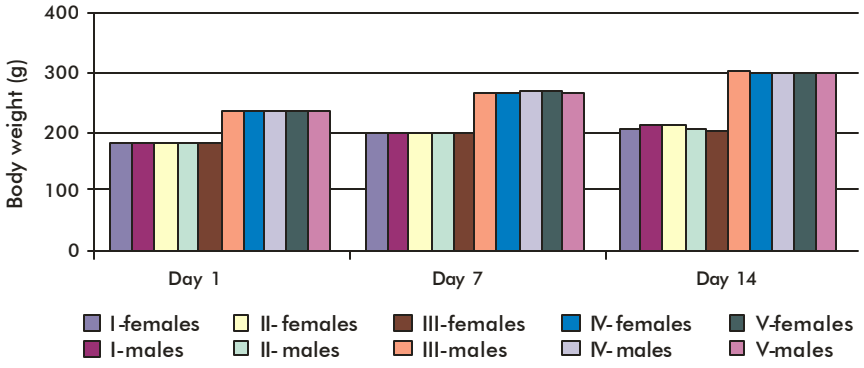

Figure 1. Behavior of body weight, in grams (g). Comparative acute toxicity assay.

these differences, it is possible to detect a significant increase of this parameter for all the groups during the study, translated into a normal evolution of body weight for both genders which constitutes an indicator of health for the animals and further substantiates the non-toxicity of the cytokine under study.

An increase in body weight was also observed during the local tolerance assay, although the differences between the evaluation at days 0 and 8 were not statistically significant for any experimental group.

A steady increase in body weight was observed for the animals belonging to both the acute toxicity (single inoculation with 10, 30 and 30 times the TD) and local tolerance (daily administrations for 7 days at the TD and 10 and 20 times the TD) studies during the extent of the experiments. The absence of negative effects on body weight gain is favorable for the evaluation of the substance under study, since one of the primary clinical symptoms of stress or illness on this rat strain is precisely the decrease of body weight [15, 30]. Therefore, an increase in body weight is an indirect evidence of non-toxicity for the substance under analysis. The behavior of this parameter on the animals inoculated with HEBERITRO® or EPREX®, even at high doses, fits the reported growth curves for body weight in healthy animals from IFFA-CREDO [31].

Macroscopic findings

The results of the necropsies for both studies yielded no evidence of anatomical changes indicative of any alterations, since the morphology of all the organs was normal from a macroscopic point of view. Hemorrhagic areas were observed when examining the inoculation site for 4 animals belonging to group I (placebo) and 3 animals from group IV (20 times the TD) after two doses. This sign is presumably related to the trauma caused by repeated subcutaneous injection at the same site. The macroscopic findings thus corroborate the clinical observations of no alterations attributable to erythropoietin, with no evidences of local irritation or damage. The absence of macroscopic damage in organs and tissues of the experimentation animals is by itself an important point for the assessment of the safety of the product under assay in both studies, and it is therefore imperative to underscore the fact that there were no reports, independently from the dose or volume of administration.

Hematological parameters

When analyzing the results of the hematological tests, differences were detected for the FD variable in the following parameters:

MCH males (I-III p = 0.042)
(II-III p = 0.02)
(II-IV p = 0.008)
(III-IV p = 0.005)
(IV-V p = 0.002)

MCHC males (I-II p = 0.028),
(I-V p = 0.032),
(II-IV p = 0.048),
(IV-V p = 0.041)

females (I-II p = 0.026)
(I-V p=0.005)
(IV-V p=0.015)

Table 2. Acute toxicity study: variations in average body weight (g) (mean ± standard deviation).

Group	Day 1		Day 7		Day 14	
	Females	Males	Females	Males	Females	Males
I	182.2±8.44	234.6±9.76	194.80±13.70	264.6±6.43	204.2±15.93	303.2±7.40
II	182.8±15.35	234.8±10.99	194.80±17.67	265.6±11.72	208.4±17.39	296.8±13.29
III	182.4±12.3	234.8±7.43	195.60±15.82	270.80±8.35	209.6±17.81	298.0±8.66
IV	182.4±8.80	234.5±10.93	196.80±11.60	268.5±7.03	202.8±19.77	300.6±9.81
V	182.2±5.51	234.1±7.40	197.30±12.54	265.90±5.95	201.8±13.31	295.8±9.01

$p < 0.05$

Table 3. Local tolerance study: variations in average body weight (g) (mean ± standard deviation).

Group	Day 1	Day 8
I	201.00 ± 21.31	236.00 ± 20.43*
II	196.20 ± 20.43	241.20 ± 21.39*
III	200.40 ± 8.62	240.60 ± 9.24*
IV	199.40 ± 15.09	241.80 ± 7.05*

*p< 0.01

The differences were found in the groups inoculated with HEBERITRO®, EPREX®, or with the placebo. The values stayed within the normal range for the species used in the assay [32-34].

The high counts of hemoglobin and the high hematocrit (Table 4), together with the decrease in leukocytes, confirm the therapeutic effect of EPO. These results are related to the mobilization of hematopoietic progenitor cells to the peripheral blood, given the mechanism of action of the product under assay [35].

Histopathological study

Acute toxicity

No morphological signs of toxicity were observed in spleen, liver, mesenteric lymph nodes or the bone marrow (Table 5). Focal cellular reactions on the hepatic histology were observed for all groups, including the placebo, with an incidence of 3-5 cases per group. These reactions are characterized by mononuclear cell clusters composed of no more than 5 to 6 cells at the periphery of the central lobular venule (Figure 2), without signs of degeneration or cellular necrosis, and as mentioned above they affect all treatment groups including group V, which received EPREX®. These reactions have been described in the literature as a consequence of the intense metabolic activity of this organ, and their spontaneous appearance has been reported for this animal species [36, 37]. The fact that they are present in the control groups indicates that their appearance is not dependent on the effect of the different doses under study for the rhEPO in any of the assayed formulations. It is also important to remark that even in this aspect the histopathological results are similar between HEBERITRO® and EPREX®.

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Table 4. Acute toxicity study: hematological parameters (mean ± standard deviation).

Group	Leukocytes (%)		Erythrocytes (x10^{12}/L)		Hemoglobin (g/dL)		Hematocrit (%)		VCM (μm^3)		HCM (pg)		CCMH (%)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
							Males							
I	6.5±1.8	3.78±1.1	5.21±0.2	5.52±0.3	11.9±0.5	13.2±0.9	33.9±1.5	39.5±3.4	64.8±2.2	71.6±3.2	22.9±0.6	24.0±0.4	35.1±0.4	33.6±1.1
II	5.84±1.1	2.6±0.5	5.34±0. 3	6.1±0.4	11.9±0.3	13.1±0.5	33.9±1.5	41.3±2.8	64.0±1.7	67.6±2.1	22.4±.9	21.5±0.9	35.2±0.7	31.8±1.3
III	7.48±2.5	3.6±0.9	5.21±0 .3	5.66±0.2	11.8±0.3	13.0±0.4	33.9±1.2	39.3±2.4	65.4±2.6	69.4±4.1	22.7±1.0	22.9±0.8	34.8±0.6	33.2±1.3
IV	7.27±1.2	4.63±2.2	5.22± 0.2	5.74±0.2	11.9±0.4	12.8±0.5	32.6±4.5	37.7±2.4	64.9±2.1	67.6±8.1	22.8±0.6	22.3±07	35.2±0.5	34.0±1.8
V	8.15±2.2	4.41±1.7	5.04±0.6	5.91±0.5	11.9±0.7	13.0±0.7	31.7±2.8	37.8±2.8	62.8±2.3	64.2±3.9	23.9±1.3	22.1±1.1	37.9±1.5	34.5±1.4
							Females							
I	6.64±2.3	2.04±1.1	5.56±0.52	5.69±0.2	12.2±0.8	12.8±0.2	34.5±2.7	36.7±0.9	62.0±1.6	64.6±2.1	21.9±0.8	22.6±0.7	35.3±0.7	34.9±0.6
II	5.74±1.8	2.96±0.9	5.09±0.4	4.89±1.5	11.7±0.7	12.3±1.8	32.2±1.6	34.4±5.5	63.6±2.6	66.4±3.4	23.2±1.4	27.2±8.0	36.5±0.9	40.6±10.7
III	4.87±0.8	3.48±1.2	5.50±0.5	5.41±0.5	12.4±0.5	12.7±1.4	34.8±2.2	36.1±4.1	63.3±2.6	68.2±2.6	22.7±0.9	24.2±0.9	22.7±0.9	24.4±0.9
IV	6.44±1.4	3.84±1.3	5.50±0.3	5.63±0.2	12.3±0.6	13.2±0.4	34.4±1.6	36.2±1.2	62.4±1.8	64.4±2.3	22.3±0.8	22.4±0.7	22.3±0.8	22.4±0.7
V	6.40±1.8	3.89±0.8	5.22±0.3	5.85±0.3	12.6±0.5	13.7±0.5	32.0±1.3	37.6±1.5	61.3±1.3	64.3±2.5	24.1±0.9	23.4±0.4	24.1±0.9	23.4±0.4

Table 5. Acute toxicity study: frequency of appearance of macroscopic observations at the inoculation site, with a single dose and the animals euthanized after 24 hours.

Group	Without alterations in subcutaneous tissue	Discrete reaction
I*	4/5	-
II	4/5	1/5
III*	1/5	3/5
IV	1/5	4/5

Legend:
Discrete reaction: Focal reaction, with low numbers of cells, without additional alterations.
*Groups having an animal catalogued as non-evaluable.

Local tolerance

The histopathological findings at the inoculation site were analyzed at 2 time points: 24 hours and 8 days. These results are shown in table 6.

At 24 hours, group I (placebo) and II (DT) behaved identically, and 80% of the treated animals showed no alterations of the subcutaneous cellular tissue. However, 60 and 80% of the animals in groups III (intermediate dosing) and IV (high dosing), respectively, had a discrete focal reaction characterized by its very focalized nature, low numbers of cells, and the absence of additional alterations, as shown in figure 3.

The signs reported among the animals that were inoculated daily for 7 days were mainly inflammatory reactions of varying intensities, classified as discrete, moderate or severe according to their extension, the number of cells present, and the absence or presence of hemorrhages. Most of the animals did not have any alterations of the subcutaneous tissue at this point in the assay. The group inoculated with the therapeutic dose showed the best results, with no apparent alterations in 7 out of the 10 animals; followed, in descending order, by groups I (placebo) and III (20 times the TD), with 4 and 5 animals without alteration out of 10, respectively. The lowest number corresponded to group IV (30 times the TD). Regarding the number of animals with inflammatory reactions ranked as dis-

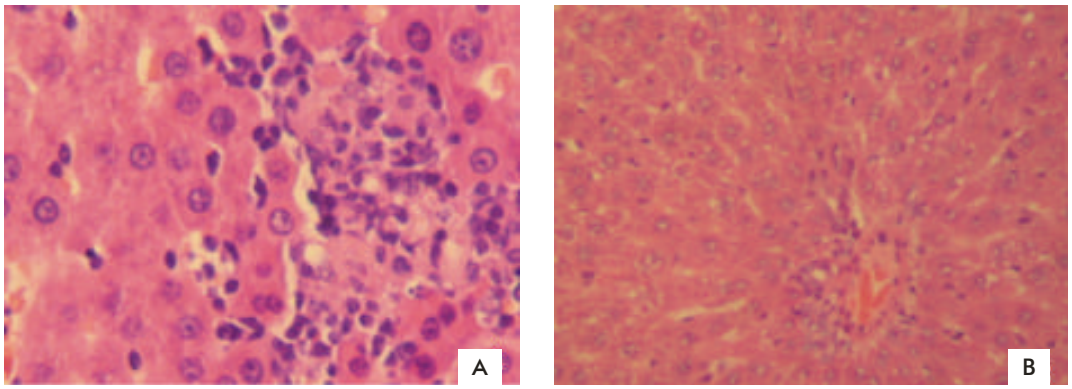

Figure 2. Acute toxicity study. A. One of the observed histopathological findings: Clusters of mononuclear cells in the liver of an animal from group V (60 times the therapeutic dose). B. Appearance of a normal liver.

crete, the highest percentage is found in the placebo group, with 4 animals out of 10. The incidence of these reactions was similar for the re-maining groups, so it was possible to prove that it does not depend on the administration of rhEPO. The incidence of moderate inflammatory reactions (Figure 4) was 2, 1, 1, and 3 animals out of 10 for the groups I, II, III and IV, respectively; and only 1 animal, belonging to group III, showed a reaction classified as severe. No alterations of the subcutaneous cellular tissue were observed at the inoculation site for most animals from groups II and III, although some animals from groups I and IV did present alterations with similar characteristics. These cases coincide with the biggest inoculation volumes, suggesting that the cause for this reaction is the presence of human albumin in the formulation.

The results suggest an adequate level of tolerance to administration by the subcutaneous route, even at the highest dose and inoculation volumes. It should be underlined that no morphological signs of irritation or necrosis of the muscular tissue were observed in any of the groups under study. Given the absence of toxicity in both assays and the similarity in the response profile to that reported for EPREX® (a registered and internationally recognized commercial homologue), the results of the use of HEBERITRO® support the safety of our product.

Table 6. Local tolerance study: Frequency of appearance of macroscopic findings at the inoculation site, with 7 inoculations and euthanized at day 8.

Group	Without alterations in subcutaneous tissue	Discrete reaction	Moderate reaction	Severe reaction
I	4/10	4/10	2/10	-
II	7/10	2/10	1/10	-
III*	5/10	2/10	1/10	1/10
IV*	3/10	3/10	3/10	-

Legend:
Discrete reaction: Focal reaction, with low numbers of cells, without additional alterations.
Moderate reaction: Extended focal or diffuse reaction, with a higher number of cells.
Severe reaction: Reaction usually diffuse, with extended hemorrhaging.
*Groups having an animal catalogued as non-evaluable.

Conclusions

The results of the preclinical studies of HEBERITRO® indicate that it is not a toxic agent and does not induce local reactions at the site of administration even when used in amounts higher than the therapeutic dose, having therefore an adequate safety margin for its use in clinical trials in humans.

It can be concluded, on the basis of the results of the experimental phase and the histopathological study, that the biological response to the products HEBERITRO® and EPREX® is essentially equivalent.

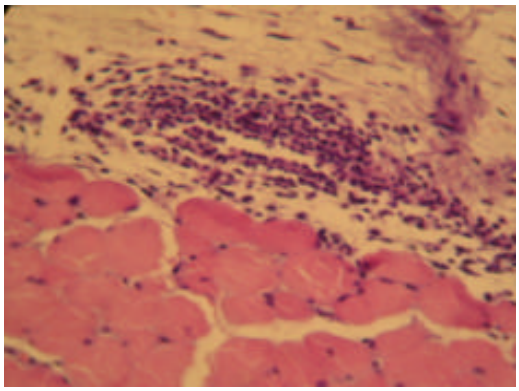

Figure 3. Discrete focal reaction at 24 hours, group V (60 times the therapeutic dose).

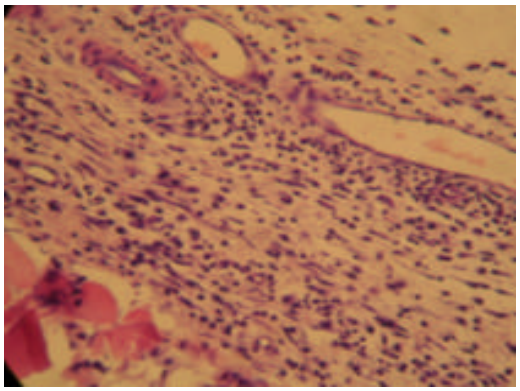

Figure 4. Moderate focal reaction at day 7, group I (placebo).

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