REVISTA ESPAÑOLA DE FISIOLOGIA, 45 (2), 163-170, 1989

Iron Mobilization on Three Animal Models of Inflammation

M. T. Carbonell, M. P. Sáiz, M. T. Martí, J. Queralt* and M. T. Mitjavila**

Departamento de Bioquímica y Fisiología
Facultad de Biología
Universidad de Barcelona
08028 Barcelona (Spain)

(Received on October 24, 1988)

M. T. CARBONELL, M. P. SAIZ, M. T. MARTI, J. QUERALT and M. T. MITJAVILA. *Iron Mobilization on Three Animal Models of Inflammation.* Rev. esp. Fisiol., 45 (2), 163-170, 1989.

The effect of acute, subchronic, and chronic experimental models of inflammation upon hematocrit, hemoglobin, serum iron and ferritin iron and nonheme iron concentration in the liver and spleen has been studied in the rat. In the acute model (carrageenan oedema) no iron mobilization took place, whereas in the chronic models differences in iron mobilization were observed, related to their different chronicity and to the time elapsed from induction. The carrageenan-induced granuloma (from 12 h to 8 days) (subchronic model) was accompanied by a decrease of plasma iron (12 and 24 h), a later decrease of the hematocrit values (2 and 4 days) and high ferritin and nonheme iron concentrations in the liver and spleen for 4 days, followed by a tendency to return to the control values. The anemia in the adjuvant arthritis (from 1 to 4 weeks after induction) (chronic model) was observed at 7 days and is related to increased iron stores in the liver and spleen. However, the iron store levels in liver decreased and fell later below control values. The increase of ferritin and nonheme iron concentrations may be responsible for the reduced availability of iron release from tissue.

Key words: Serum iron, Ferritin iron, Nonheme iron, Inflammation, Carrageenan oedema, Carrageenan-induced granuloma, Adjuvant arthritis.

Trace metals such as Cu, Zn and Fe have important roles in many biochemical reactions and in chronic inflammation in man (11, 21) and animals (2, 3, 8, 14, 20) which is associated with a disturbed metabolism of these metals. According to KONIJN and HERSHKO (15) the alterations of iron metabolism are due to an increased ferritin synthesis not resulting from a preceding block in iron release, but as part of a general stimulus for the synthesis of acute phase reacting proteins. This is followed by a low serum iron, a low iron binding capacity (TIBC) and impaired iron exchange by some cellular transport systems (12).

Few experiments have been carried out

* Departamento de Ciencias Fisiológicas Humanas y de la Nutrición. Facultad de Farmacia. 08028 Barcelona.

** To whom all correspondence should be addressed.

on inflamed animals to appreciate the time-course of the blockade of iron in storage organs (7, 19), which is responsible for the characteristic anemia of the inflammatory disorders. The purpose of the present study is to investigate the evolution of some hematological parameters related to iron (hematocrit, hemoglobin, and serum iron), and the liver and spleen iron stores (ferritin iron and nonheme iron) in rats submitted to three models of inflammation of different chronicity, in order to better understand the iron mobilization in inflammatory processes.

Materials and Methods

Sprague Dawley rats were used in all the experiments. They were kept in standardized conditions and fed with commercial rat chow (A-04 Panlab, S.L.) containing 85 mg Zn/kg and 305 mg Fe/kg. Three experimental inflammatory conditions (acute, subchronic and chronic) were induced in these animals.

Carrageenan oedema (acute model). — The study was performed on two groups (inflamed and control) of 10 male rats with a body weight of 140-170 g which were fasted for 18 h before the beginning of the experiment with water *ad libitum*.

The inflammation was induced in one group of rats by subplantar injection of 0.05 ml of 1 % (w/v) carrageenan (Marine Colloids Inc., Springfield, N. J.) in 0.9 % NaCl in the right-hind-paw according to the method of WINTER *et al.* (24). The paw volume was measured using a Ugo Basile mercury plethysmograf before injection (0 h) and at 1, 2, 3, 4 and 5 h after the carrageenan injection and were expressed as percentage of volume increase with respect to 0 h.

Carrageenan-induced granuloma (subchronic model). — The experiment was carried out on six groups of five male rats weighing 175-215 g. The granuloma pouches were induced on five groups by the method of FUKUHARA and TSURUFUJI (10) by the injection into a dorsum air pouch of 4 ml of 2 % (w/v) solution of carrageenan in 0.9 % NaCl. The rats fasted overnight were sacrificed and the inflammatory response was evaluated at 12 h and at 1, 2, 4, and 8 days after induction on the basis of weight of granuloma.

Adjuvant arthritis (chronic model). — The arthritis was induced in four groups of eight female rats each, with a body weight of 160-180 g at the beginning of the experiment, by a single intradermal injection (0.1 ml) in the right-hind-paw of *Mycobacterium butyricum* (Difco Laboratories) suspended in liquid vaseline (5 mg/ml). Four additional groups of control animals only received 0.1 ml of liquid vaseline under the same conditions. The right and left hind paw volumes were assessed in non fasted rats at 1, 2, 3 and 4 weeks after arthritis induction.

Treatment of samples. — At the end of each experiment, the rats were anesthetized by ether inhalation. Blood was collected by heart puncture and the liver and spleen were removed and weighed. Acid-washed glassware and deionized water were used.

The hematocrit was determined in microhematocrit capillary tubes and hemoglobin concentration with Drabkin's reagent. Plasma iron levels were assayed by the beta-phenanthroline method, recommended by the International Committee for Standardization in Haematology (13). Ferritin iron was measured according to the DRYSDALE and MUNRO method (5) and the nonheme iron by the TORRANCE and BOTHWELL technique (23).

Results were expressed as mean ± SEM. The values of the inflamed animals were compared with their respective controls by the Student's t test.

Results

Carrageenan oedema. — The percentage of the paw volume increase after the oedema induction is 22, 39, 41, 46 and 30 % after 1, 2, 3, 4 and 5 h respectively. Hematocrit, hemoglobin, plasma iron and organ iron content of inflamed animals are similar to their control group (table I).

Carrageenan-induced granuloma. — The granuloma tissue begins to develop 12 h after induction, and continues to increase with time and at 8 days reaches 17.8 g. Plasma iron shows an important drop as early as 12 h, and the hematocrit decreases later (table II). Liver and spleen weights (table II) have a tendency to increase, but at the fourth day liver weight falls to its normal value. There is a remarkable increase in iron (essentially in the form of nonheme iron) in these two organs after the induction of the inflammatory process with the highest values at the fourth day (the nonheme iron increased 94 and 91 % in regard to the control group for liver and spleen respectively).

Adjuvant arthritis. — Figure 1 shows the time-course of uninjected hind-paw-volume (systemic inflammation) in control and arthritic rats. The arthritic rats have an important increase of the left hind-paw-volume 14 days after induction when compared to control groups. The injected hind-paw (local inflammation) also shows an increase in volume and to a greater degree (data not shown).

The hematological parameters, liver and spleen weights and their iron content are summarized in table III. A significant re-

Table I. *Body weights, hematological values, liver and spleen weights and their iron content in control and in carrageenan paw edema rats.*
Mean ± SEM of 10 rats. Comparisons to the control group by the Student's t-test: * $p < 0.05$.

Parameters	Control	Inflamed
Weight, g	150.0 ± 3.0	157.0 ± 2.0
Hematocrit	40.6 ± 0.81	39.9 ± 0.90
Hemoglobin, g/100 ml	14.0 ± 0.42	13.6 ± 0.53
Plasma iron, µg/100 ml	132.0 ± 22.8	119.0 ± 11.0
Liver		
Weight, g	5.3 ± 0.09	5.6 ± 0.09*
Ferritin iron, µg/g	21.3 ± 3.42	22.1 ± 2.37
Nonheme iron, µg/g	122.0 ± 9.9	129.0 ± 17.0
Spleen		
Weight, g	0.5 ± 0.03	0.5 ± 0.03
Ferritin iron, µg/g	11.4 ± 1.70	15.0 ± 2.98
Nonheme iron, µg/g	58.0 ± 3.6	72.0 ± 6.1

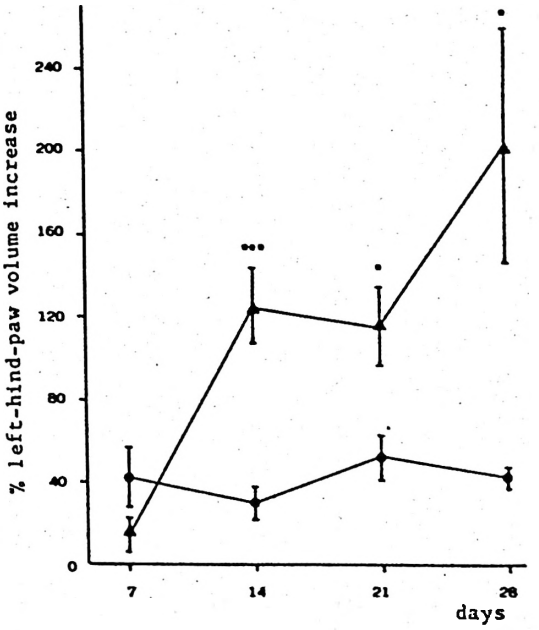

Fig. 1. *Time-course of left-hind-paw volume increase in control (•) and adjuvant arthritic rats (▲). (Mean ± SEM of 8 rats.)*

Table II. *Body weights, haematological values, liver and spleen weights and their iron content in control (0 days) and in carrageenan-induced granuloma in rats.*

Mean ± SEM of 5 rats. Comparisons to the control group by the Student's t-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Parameters	0 days	12 hours	1 day	2 days	4 days	8 days
Weight, g	188.0±10.0	210.0±3.0	173.0±4.0	179.0±4.0	185.0±9.0	216.0±8.0
Hematocrit	39.7±1.0	39.6±0.8	39.9±0.9	36.8±0.6*	36.6±0.8*	37.1±1.2
Hemoglobin, g/100 ml	14.6±0.6	14.8±0.7	14.3±0.8	13.6±0.5	13.2±0.5	14.2±0.7
Plasma iron, µg/100 ml	145.0±26.0	50.0±2.0**	50.0±5.0**	130.0±3.0	93.0±10.0	83.0±11.0
Liver						
Weight, g	7.3±0.4	7.6±0.4	8.7±0.4*	8.5±0.2*	6.4±0.2	7.7±0.2
Ferritin iron, µg/g	13.4±1.4	24.1±3.3*	29.6±2.3***	24.5±5.3	30.0±2.7***	20.4±2.0*
Nonheme iron, µg/g	68.0±11.0	104.0±10.0*	92.0±7.0	118.0±15.0*	132.0±14.0**	79.0±5.0
Spleen						
Weight, g	0.7±0.02	0.6±0.04	0.6±0.04	0.7±0.06	0.9±0.09*	0.9±0.07**
Ferritin iron, µg/g	20.0±2.5	21.5±2.8	31.1±6.0	34.0±3.6*	58.8±6.4***	22.3±2.7
Nonheme iron, µg/g	65.0±4.9	80.0±3.1*	97.0±5.0**	70.0±4.7	124.0±11.1**	60.0±7.2

Table III. *Body weights, haematological values, liver and spleen weights and their iron content in control and arthritic rats.* Mean ± SEM of 8 rats. Comparisons to the control group by the Student's t-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	7 days		14 days		21 days		28 days	
Parameters	Control	Inflamed	Control	Inflamed	Control	Inflamed	Control	Inflamed
Weight, g	198.0±5	190.0±2.0	211.0±5.0	165.0±3***	217.0±5	169.0±6***	231.0±13	186.0±4**
Hematocrit	42.0±0.5	28.0±2.8***	41.0±0.3	35.0±3.3	42.0±0.4	37.0±4.5	41.0±0.7	38.0±2.6
Hemoglobin, g/100 ml	16.3±0.3	11.8±1.4**	15.4±0.4	13.2±1.6	14.8±0.3	14.0±0.7	14.3±0.4	13.4±0.8
Plasma iron, µg/100 ml	258.0±17	263.0±19	262.0±27	98.0±4***	268.0±20	162.0±21**	271.0±11	179.0±21**
Liver								
Weight, g	6.7±0.3	6.4±0.3	7.3±0.3	6.7±0.5	7.6±0.2	6.7±0.8	7.9±0.5	6.6±0.3*
Ferritin iron, µg/g	20.0±1.8	30.0±2.9*	41.0±6.4	29.0±4.2	49.0±4.8	30.0±2.4**	60.0±4.1	40.0±4.7**
Nonheme iron, µg/g	67.0±5.6	86.0±6.1*	140.0±6.0	133.0±14.5	192.0±16.4	121.0±9.5**	169.0±9.7	144.0±7.5
Spleen								
Weight, g	0.5±0.02	0.6±0.02***	0.6±0.03	0.9±0.14*	0.6±0.04	0.7±0.02	0.5±0.03	1.0±0.16*
Ferritin iron, µg/g	30.0±2.8	40.0±3.5*	46.0±5.6	132.0±17.3***	126.0±16.5	154.0±24.7	128.0±9.5	143.0±15.9
Nonheme iron, µg/g	90.0±7	121.0±10.0*	242.0±20	405.0±46**	520.0±51	666.0±85	753.0±77	805.0±48

duction in hematocrit (28 %) and hemoglobin levels is observed on day 7, followed by low values of serum iron on day 14. The liver weights of inflamed animals remained constant for 3 weeks but the spleen weights increased significantly with the exception at 21 days. The adjuvant arthritis induced an increase in ferritin and nonheme iron concentration in the organs studied only at 1 week for the liver and for 2 weeks in the spleen. Although the iron in the liver of arthritic rats decreased significantly at the end of the study period (3 and 4 weeks), the presence of this metal in the spleen remained constant with regard to their respective controls.

Discussion

The characteristic anemia of chronic inflammatory diseases has little clinical significance. KONIJN and HERSHKO (15) explained it by an increase of ferritin synthesis in parenchymal cells of the storage organs, with a concomitant blockade of iron released from them. In turpentine abscess, the ferritin synthesis takes place in two waves (16). The first wave preceded the decrease of serum iron and TIBC which began at 12 h post-induction (15), and the second wave had peak activity at 48 h, but no increment in liver ferritin iron was detected. No other observations on ferritin iron stores in inflamed processes have been found. In our results, the absence of iron mobilization in the acute inflammatory model (carrageenan oedema) may be due to a short delay (16), while synthesis of ferritin and storage of iron could have taken place in the two other models. In the carrageenan granuloma two waves of increment of iron stores in the liver seem to take place, which are related to the oscillations of plasma iron levels. In the arthritic model, the symptoms of inflammation appear soon and persist for 4 weeks and, as a result, an increase of hepatic ferritin iron at one week and a decrease of plasma iron from the second week were observed.

According to FELDMAN and KANEKO (6) high concentrations of ferritin inhibit catalase, so that an accumulation of hydrogen peroxide occurs which leads to the conversion of ferritin into hemosiderin, but hemosiderin iron is much less mobilized from stores than is ferritin iron (17). This may explain the greater increase of nonheme iron (which includes hemosiderin), with regard to the ferritin iron, in the liver of carrageenan-induced granuloma (2 and 4 days), and in the spleen of arthritic rats (2 and 3 weeks).

The alterations on hemoglobin, hematocrit and serum iron observed in the adjuvant arthritis are related to those observed in arthritic dogs (7, 8) and in arthritic rats (18, 19). Changes in nonheme iron values probably depend on the injected dose (19) and on the time elapsed from the induction (7). However, increased iron excretion (18) may be responsible for the decrease of ferritin and nonheme iron in the liver from 21 days of arthritis induction. The iron metabolism alterations detected in arthritic rats could be related to the changes of Cu and Zn levels previously seen in arthritic animals (20).

However, loosely-bound iron has also been detected in small amounts (μM) in synovial fluid (22) and is responsible for the formation of hydroxyl radicals that lead to peroxidation processes of the polyunsaturated lipids of the cellular membranes during inflammation. The changes in the metabolism of Fe, Cu and Zn may have a protective role against free radicals, so that the increment of ferritin and transferrin synthesis may prevent the presence of the loosely-bound iron. Moreover, ceruloplasmin (a cuproprotein with ferroxidase activity) and superoxide dismutase (copper-zinc metalloenzyme) protect against formation of hydroxyl and superoxide radicals respectively (1, 4, 9).

Hence, in the liver and spleen important

functions in the regulation of trace metal metabolism take place, implying the role of acute-phase plasma proteins such as ceruloplasmin and ferritin. The increase of ferritin iron and its diversion to hemosiderin iron may be responsible for the reduced availability for the iron release from tissue, associated with a defensive mechanism against the free radical formation.

Acknowledgements

This work was supported by the grant from the CIRIT number AR 85-147 (carrageenan oedema) and by the grant from the CAICYT number PB 85-0234 (carrageenan granuloma and adjuvant arthritis). The valuable technical assistance of Miss J. Valentín is gratefully acknowledged.

Resumen

Se estudia en ratas el efecto de modelos inflamatorios experimentales agudos, subcrónicos y crónicos sobre el hematocrito, la hemoglobina, la sideremia y concentración de hierro ferritínico y no hemínico en el hígado y en bazo. En el modelo agudo (edema por carragenina) no se detecta movilización del hierro, mientras que en los modelos crónicos la movilización está relacionada con la distinta cronicidad y con el tiempo transcurrido desde la inducción. El granuloma por carragenina (de 12 h a 8 días, modelo subcrónico) provoca un descenso de la sideremia (12 y 24 h), un descenso más tardío del hematocrito (2 y 4 días) y concentraciones elevadas de hierro ferritínico y no hemínico en el hígado y bazo durante 4 días, seguido de una tendencia a recuperar los valores de los controles respectivos. En la artritis por adyuvante (de 1 a 4 semanas, modelo crónico), se observa anemia al día 7 relacionada con un aumento de las reservas de hierro. No obstante, las reservas hepáticas disminuyen posteriormente situándose, al finalizar las pruebas, por debajo del control. El incremento de las concentraciones de hierro ferritínico y no hemínico, pueden ser responsables de la baja disponibilidad para liberar hierro de los tejidos.

Palabras clave: Sideremia, Hierro ferritínico, Hierro no hemínico, Inflamación, Edema por carragenina, Granuloma por carragenina, Artritis por adyuvante.

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