REVISTA ESPAÑOLA DE FISIOLOGIA, 48 (2), 103-106, 1992

Relationship Between Erythropoietic Rate and Iron Donating Capacity of the Fe/Transferrin Complex in Mouse

J. L. Scaro*, C. Miranda, M. Carrera, I. Torrejón and B. Martín

Instituto de Biología de la Altura
Departamento ERITAL. CONICET
Universidad Nacional de Jujuy
S. Salvador de Jujuy (Argentina)

(Received on October 10, 1991)

J. L. SCARO, C. MIRANDA, M. CARRERA, I. TORREJÓN and B. MARTÍN. *Relationship Between Erythropoietic Rate and Iron Donating Capacity of the Fe/Transferrin Complex in Mouse*. Rev. esp. Fisiol., 48 (2), 103-106, 1992.

The transfer of iron from the Fe/transferrin complex to the erythroid cells was studied in *in vitro* system in mice in which a drastic and opposite change in their erythropoietic activity was produced by bleeding or actinomycin D administration. A reduction of iron donation in the serum of bled animals was found, whereas the aplastic condition induce in the donors of the serum by actinomycin D did not produce any change in the transfer process. It was also found that in spite of the normalization of the saturation in the serum of bled animals, the diminished donation remained unchanged. The possibility that conditions other than quantitative could produce this behavior is discussed.

Key words: Iron, Transferrin, Erythropoiesis, Erythropoietic rate.

Iron for erythropoiesis is delivered to the user cells by the Fe/transferrin complex (7). The existence of a competition for iron between transferrin and the membrane sites has been postulated (1, 4). It is suggested that *in vitro* donation of iron from the Fe/transferrin complex to the reticulocytes may depend, in addition to the quantitative availability of the metal for erythropoiesis, on alterations in the function of the specific iron carrier for the synthesis of hemoglobin (9). Results of studies aimed to evaluate the influence of the erythropoietic rate on the iron donation capacity of the serum in mice with acute changes in their erythropoietic rate, are herein reported.

* To whom all correspondence should be addressed.

Materials and Methods

Female FWD/C3H mice, eight to ten weeks of age were used. The animals were kept in a balanced diet containing a standardized amount of iron. The erythropoietic rate was increased by removing

one third of the blood volume from the retrorbital sinus of the eye. In another group of prospective serum donors an erythropoietic drastic reduction was induced by i.p. injection of 10 μg of actinomycin D (7). Serum was obtained from blood taken by cardiac punction into iron free syringes four days after either bleeding or AM administration. A pool of sera from normal donors with hematocrit in the range of 43-47 (SN) or from bled donors (S-B) with hematocrit between 32-37 as well as from AM treated donors (S-AM) with hematocrit in the range of 32-39 were kept lyophilized until use. The amount of iron donated from S-N was considered as normal values. The *in vitro* technique was as described elsewhere (2). Briefly, it consisted in the incubation of a volume of washed erythrocytes from normal mice with an equal volume of the serum under study. The red cells were washed twice with normal balanced saline solution (NKM) made of NaCl 0.8 g; KCl 0.075 g; $MgCl_2$, $6H_20$ 0.031 g; K_2HPO_4 0.006 g; NaH_2PO_4 $2H_2O$ 0.006 g and glucose 0.1 g/100 ml of solution.

The cells suspensions were transferred to 1/3 diameter glass tubes in which the gaseous phase was air containing 3 % of CO_2 (3). The proportion of reticulocytes in the circulation in the normal mouse, as shown in previous studies (8), is 15 to 20 fold greater than in the blood of normal human beings.

In other experiments the iron saturation of the transferrin in the S-B was brought to a similar level of that of S-N by addition, made before labelling, of an adequate amount of ^{56}Fe. After measuring the amounts of ^{59}Fe taken up by the cells, their hemic fraction was separated using the method of KASSENAAR *et al* (5).

Results

Table I shows the amounts of iron transferred from each serum used. A significant reduction of the amounts donated from S-B as compared with N-S and S-AM can be seen. It was also found that the normalization of transferring saturation of S-B before labelling, does not modify the pattern of donation of this serum. Table II shows that when RBC with a significantly greater demand of iron were incubated with both, S-B or S-M, the amounts of iron taken up by the cells could be increased two or three fold above the original low value though the difference between the amounts donated to the cells from each serum persists at all leves of demands studied.

The amounts of iron delivered from a mixture of both sera are shown in table III. When the label was in the S-N fraction of the mixture it produced a higher donation than the normal values, whereas

Table I. *Iron transfer (ng) to reticulocytes from the sera of normal, bled and actinomycin treated mice in an* in vitro *system.*

Group	Serum	N of sera	Iron transferred
1	Normal	6	15.2 ± 0.9
2	Bled donors	6	10.8 ± 1.8*
3[a]	Bled donors + Fe	5	11.0 ± 1.3 NS[b]
4	Actinomycin D treated donors	6	15.8 ± 0.98 NS[c]

a Before labelling, an adequate amount of ^{56}Fe was added to the sera to bring their iron content to the level of group 1.
b vs group 2.
c vs group 1.
* $P < 0.05$ vs group 1.

Table II. *Total iron (ng) taken up from the serum of either normal or bled donors after incubation with cell substractums with increased demand for iron.*

Ht of RBC donors	Iron transferred	
	Normal donors	Bled donors
45	14.9 ± 0.98	9.8 ± 0.65
40	18.7 ± 1.23	13.9 ± 0.87
35	24.4 ± 1.98	17.9 ± 1.43
30	30.3 ± 2.10	24.8 ± 1.44

Table III. *Iron transfer (ng) from the sera of normal and bled donors and from their mixture when labelling was in normal or in the bled fraction of the mixture.*

3) Mixture of labelled normal serum with non labelled normal serum; 4) Mixture of labelled normal serum with non labelled bled serum; 5) Mixture of non-labelled normal serum with labelled bled serum. Values are media ± S. D. ($p < 0.05$).

Group	Serum	Iron transferred
1	Normal	15.9 ± 0.88
2	Bled	10.3 ± 0.77[a]
3	Normal Fe + Normal	8.9 ± 0.99
4	Normal Fe + Bled	10.0 ± 0.65[b]
5	Normal + Bled Fe	6.8 ± 1.01[b]

a vs group 1.
b vs group 3.

the opposite occurred when the label was in the S-B fraction.

There is a close relationship between the values of ^{59}Fe taken up by the whole cells and by their hemic fraction (data not shown).

Discussion

The amounts of iron donated to the cells by S-B are significantly lower than those transferred by either S-N or S-AM. An explanation for this difference could be based on the fact that the quantity of transferrin-bound iron in S-B is lower, probably due to the intense process of erythropoietic regeneration that takes place at the time the serum is collected. However this interpretation is not substantiated by the observation that when the saturation of S-B was normalized its reduced donating capacity persists.

That the diminished donating capacity of S-B here discussed does not result from the exhaustion of the available serum iron, is also supported by the observation that S-B is capable of increasing its iron donation several fold above lower values when the demand from the cell substratum increases correspondingly though the magnitude of the difference in donating capacity of each serum remains the same at all levels of iron demand. It should also be mentioned that if the reduced iron donation capacity of S-B were caused by its lower content of iron, it should have resulted in the utilization of almost all the iron in the serum including the tracer fraction which is not the case in these studies.

The fact that the Ht values in donors of either S-B and S-AM are similar contrasts with the marked difference in their donating capacity. Since iron donation in the latter group does not differ from the normative values, it would suggest that the Ht have no influence in iron donation capacity of this serum as is the case in the S-B.

The observation, made in experiments using mixtures of both sera, that the labelling of the S-N fraction of the mixture results in higher transfer of iron 59 as compared with the normative control value and that the opposite is the case when the tracer was added to S-B, is consistent with the notion that when iron is bound to the transferrin molecule there is no exchange of the metal from one molecule to another.

These results support the possibility that the different behavior of the sera here studied is due to intrinsic changes in the molecule or could be mediated by humoral factors acting on the molecule or both.

These data can also lead to the conclusion that the diminished capacity of S-B to donate iron can be partially related to factors or conditions independent of quantitative values of the metal in the circulation. They also may help to explain results of studies made in the sera of children affected by malnutrition which proved to have a reduce iron donating capacity (2). We then have suggested that that abnormal behavior could be partially independent of the amounts of iron in the circulation.

Acknowledgements

This work has been supported by the "Consejo Nacional de Investigaciones Científicas y Técnicas" (República Argentina). The authors thank Miss Isabel Zingariello for her valuable technical assistance.

Resumen

Se estudia la transferencia de hierro desde el complejo Fe/transferrina a las células eritroideas utilizando un modelo *in vitro* en ratones a los que se ha producido un drástico cambio en los valores de la tasa eritropoyética, por sangría o por inyección de actinomicina D. Se encuentra una reducción significativa en la cantidad de hierro transferido desde el suero de dadores anemizados, pero no desde los que se ha inducido una anemia hipoplástica. La citada reducción no se modifica por la normalización de la saturación de los sueros de los animales sangrados. Se discute la posibilidad de que otras condiciones, además de las impuestas por la disponibilidad de hierro, puedan ser la causa de esta reacción.

Palabras clave: Fe, Transferrina, Eritropoyesis, Tasa eritropoyética.

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