Indian J Physiol Pharmacol 1999; 44 (1) : 229–232

STIMULATION OF IRON UPTAKE AND Hb SYNTHESIS IN IRON DEFICIENT RETICULOCYTES

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(Received on March 15, 1999)

Abstract : The effect of inhibitors and intermediates of heme synthesis, inhibitor of globin synthesis, and some iron proteins on *in vitro* iron uptake and haemoglobin synthesis by reticulocytes of iron deficient subjects was investigated in this study.

Lead, INH, ALA, mesoprophyrin, ferritin and albumin substantially increased iron uptake by iron deficient reticulocytes, while cycloheximide and glycine depressed it. The results showed that it is possible to stimulate iron uptake and Hb synthesis in iron deficiency by substances other than iron; the most effective and remarkable of them was ferritin.

Key words: iron uptake, hemoglobin synthesis, iron deficiency, anaemia, heme intermediates, heme inhibitors, globin inhibitor, reticulocytes, iron proteins

INTRODUCTION

Anemia is a condition in which the quality or quantity of circulating hemoglobin is reduced and iron deficiency anemia is one in which the rate of hemoglobin synthesis is arrested by limiting amounts of available iron (1). We have recently reported the effect of iron supplement on hemoglobin synthesis by iron deficient reticulocytes in an *in vitro* system (2). The purpose of present investigation was to see if iron uptake and Hb synthesis can be stimulated in iron deficiency by supplementing substances other than iron.

METHODS

Non-pregnant anemic (Hb < 12 g%) females attending outdoor of Mahila Chikitsalaya, Jaipur, for gynaecological problems were the subject of this study. An effort was made to select women with Hb as low as possible. Blood was collected for determination of hematologic values (3) and iron values (4). The women with serum iron less than 50 μg% or percent saturation

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below 16% were considered iron deficient and included in this study.

Iron uptake by reticulocytes was studied by incubating 1.0 ml of freshly drawn whole blood with 0.6 ml of appropriately diluted radioiron in normal saline at 37°C for 30 minutes, precipitating proteins by trichloracetic acid and measuring gamma activity of Fe^{59} in a Geiger Mueller Counter. The details of radioactive experiments have been published previously (2).

The effect of addition of additive (0.1 ml) was compared with iron uptake without additive. The additives used were lead acetate (May & Baker), isonicotinic acid hydrazide or INH (Parke Davis), cycloheximide (SD Fine), glycine (Ranbaxy), 5-aminolevulinic acid hydrochloride or ALA (Merck), mesoporphyrin (Sigma), ferritin (Boehringer Mannheim) and albumin (Reckon).

The effect of each additive was seen in five different IDA subjects. Thus there were a total of 55 iron deficient women. Their age ranged from 19 to 30 years (mean – 25.4 years).

RESULTS AND DISCUSSION

Table I shows the hematologic value of our subjects. This clearly shows the existence of severe anemia in them. This table also depicts iron values of the anemic women which confirms that iron deficiency was the cause of this anaemia. The effect of different additives on iron uptake by reticulocytes is shown in Table II. There were five different IDA patients in each additive group. The effect of each additive was consistent in all the five subjects. Since this number was too small for statistical analysis by Student's 't' test, results were evaluated by percentage change.

TABLE I : Haematologic and iron values.

Parameter	*Mean ± S.D.*
Hb (g/dl)	7.8 ± 0.9
TRBC (10^6/mm^3)	3.1 ± 0.4
PCV (%)	28.7 ± 4.8
MCV (fl)	90.6 ± 11.3
MCH (pg)	25.1 ± 4.5
MCHC (%)	27.7 ± 5.1
Reticulocyte count (%)	3.2 ± 0.8
SI (μg/dl)	41.3 ± 6.7
TIBC (μg/dl)	386.8 ± 27.7
PS (%)	10.7 ± 2.3

The values represent mean ± S.D. of 55 subjects.

The additives included in this study were inhibitors of heme synthesis (lead, INH), inhibitor of protein synthesis (cycloheximide), intermediates of heme synthesis (glycine, ALA and mesoporphyrin and intracellular iron transport proteins (ferritin and albumin).

Effect of inhibitors of heme synthesis

Lead and INH are well known inhibitors of heme synthesis (5). In our study lead in a concentration of $4x10^{-4}$M in the reaction mixture increased iron uptake by 21.4%. Similarly, INH in concentrations of $1x10^{-3}$M and $5x10^{-3}$M enhanced iron uptake by 19% and 38% respectively. The stimulation of iron uptake by inhibitors of heme synthesis was explained by Ponka & Neuwirt (6) by existence of a feedback inhibitory effect of heme on iron entry into reticulocytes. Since TCA precipitates proteins, the increased iron must have been present in protein bound from, e.g., ferritin, transferrin or some other protein, but not in hemoglobin.

Effect of inhibitor of protein synthesis

Cycloheximide is a well known inhibitor of protein synthesis which also inhibits synthesis of globin. Cycloheximide in a concentration of $2x10^{-4}$M decreased iron uptake in all the five subjects, the average fall being 11.5%. Similar observations were also reported by Ponka and Neuwirt (6) as well as Grayzel et al (7). Thus there seems to be a coordination between iron uptake by reticulocytes and globin synthesis by them.

disagreement to earlier reports of Ponka et al. (10) and Ponka and Schulman (11). Similarly, mesoporphyrin in $1x10^{-4}$M concentration increased Hb synthesis by about 30% and by about 50% in twice the concentration. No other worker has seen the effect of mesoporphyrin, however, Ponka et al (10) using protoporphyrin found increased incorporation of iron into heme in the erythrocytes.

Contrary to expectations, glycine in $5x10^{-3}$M concentration depressed Hb synthesis by 15%. It could be due to

TABLE II : Effect of additives on iron uptake and hemolobin synthesis.

Additive	*Concentration*	*Hb Synthesis & Fe Uptake (Cpm)*		*Percent change (%)*
		Wihtout Additive	*With Additive*	
Lead (Lead Acetate)	$4x10^{-4}$M	1378 (1017–2131)	1 673 (1181–2491)	+21.7 (16.1–33.3)
INH	$1x10^{-3}$M	1025 (888–1199)	1217 (1095–1376)	+19.0 (13.3–28.3)
"	$5x10^{-3}$M	1000 (910–1135)	1381 (1250–1584)	+38.0 (36.9–39.5)
Cycloheximide	$2x10^{-4}$M	1055 (1003–1121)	931 (905–968)	–11.5 (7.3–15.7)
Glycine	$5x10^{-3}$M	1560 (1488–1643)	1324 (1247–1367)	–15.0 (11.0–18.0)
ALA	$1x10^{-3}$M	1316 (1269–1395)	1586 (1485–1673)	+20.5 (17.0–24.4)
Mesoporphyrin	$1x10^{-4}$M	1301 (1006–1670)	1714 (1371–2225)	+31.7 (24.2–36.2
" "	$2x10^{-4}$M	1300 (1147–1336)	1847 (1749–2041)	+50.3 (42.4–54.9)
Ferritin	$2x10^{-13}$M	1016 (946–1071)	1258 (1195–1352)	+23.9 (16.9–34.6)
" "	$4x10^{-13}$M	1035 (969–1170)	1445 (1359–1611)	+39.9 (30.8–52.0)
Albumin	$4x10^{-4}$M	976 (932–1016)	1370 (1289–1464)	+40.2 (35.9–44.0)

No. of cases in each group is 5; Figures in parenthesis represent range of five cases.

Effect of intermediates of heme synthesis

The effect on iron uptake of addition of three intermediates of heme synthesis, viz., glycine, ALA and mesoporphyrin, is also presented in Table II. As expected, stimulation of iron uptake and Hb synthesis occurred by adding ALA and mesoporphyrin, but not by glycine. ALA addition in $1x10^{-3}$M concentration increased Hb synthesis by about 20.5%. These results are in conformity to report to of Levere and Granick (8) and more recently Kawasaki et al. (9) but in

chelation of iron by added glycine making it unavailable to heme synthesiszing site.

The other possibility is that decreased ALA synthase activity occurring in iron deficiency (12) could limit the synthesis of heme and hence hemoglobin, despite added glycine.

Effect of iron proteins

Ferritin (13), transferrin and iron binding protein (IBP), similar to albumin,

have been identified as cytosolic intermediates in iron transport in reticulocytes (14). It was, therefore, considered worthwhile to examine the effect of addition of these iron proteins on hemoglobin synthesis in iron deficient reticulocytes. Such an effect has not been examined in any other laboratory.

The effect of transferrin has already been reported by us. The addition of transferrin (containing about 0.1 µg iron) to the reaction mixture, resulted into an increased in Hb synthesis by more than 60% (2).

The effect of ferritin was examined in this study at two different concentrations. The addition of 0.18 and 0.36 ng of ferritin to the reaction mixture increased Hb synthesis by 23.9% and 39.9% respectively. This means ferritin even in sub-normal concentration triggered stimulatory response comparable to transferrin in less than one millionth concentration of transferrin on the molar basis.

The stimulatory effect of ferritin and transferrin can be explained on the basis of their role in iron transport from the cytosol to the mitochondria (14).

As we were not having iron binding protein (IBP) as referred by Nunez et al. (14), we have used a similar protein, albumin. Surprisingly, there was 40.2% rise in the ratre of Hb synthesis by adding 40 mg albumin (sub-normal concentrratrion) to the reaction mixture. This may indicate that albumin is not only structurally similar to IBP but functionally as well also.

The present work indicates that it is possibe to stumulate hemoglobin synthesis by supplementing compounds other than iron. Further work might examine possible applicatikon to humans of this *in vitro* study.

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