Antianemic Activity of Water-Soluble Na,Ca,Fe-Polygalacturonate

A. B. Vyshtakaliuk[1], V. V. Zobov[1,2], S. T. Minzanova[1], A. V. Lantsova[1], V. F. Mironov[1], G. R. Petrova[1], F. H. Ziatdinova[1], O. V. Tsepaeva[1], A. I. Konovalov[1]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 7, pp. 51-53, July, 2010
Original article submitted July 14, 2009

A pronounced antianemic effect of water-soluble Na,Ca,Fe-polygalacturonate is demonstrated. The drug in solution is more effective than in the solid form; it promotes absorption of nutrient calcium and iron. During this treatment, consumed calcium does not impair iron absorption.

Key Words: *pectin polysaccharides; iron; calcium; absorption; hematological values*

The control of bioavailable iron deficit is a priority problem today [6]; this condition leads to the development of alimentary anemia and other iron deficiencies. We showed high antianemic activity [1,3] and low toxicity [2] of Na,Fe,Co,Cu-polygalacturonate (PG).

Here we studied a new synthetic preparation: water-soluble Na,Ca,Fe-PG [5], a source of bioavailable Fe^{2+} and Ca^{2+} ions.

The efficiency of simultaneous use of calcium and iron remains unclear, because calcium was shown to inhibit absorption of iron [5], though no specific effect of calcium on iron absorption was detected. A previous study [4] showed an S-shaped relationship between iron absorption and calcium content in the ration, while another study [7] showed that the use of calcium additives supporting the bone system health is not fraught with the risk of iron deficiency development.

MATERIALS AND METHODS

Water-soluble Na,Ca,Fe-PG contains 16-23 mg calcium and 10 mg iron per 1 g substance.

Antianemic activity of Na,Ca,Fe-PG and reference drugs was studied on laboratory animals. Male mice (25-30 g; 7-8 per group) received 0.5 or 0.25% solution in doses of 480 or 360 mg/kg, respectively, instead of drinking water. Female rats (160-250 g, aged 3-4 months; 3-4 per group) were subjected to blood loss of 0.5-1.2 ml before the experiment and received the substance in solid and dissolved form. Solid Na,Ca,Fe-PG was given in the form of boluses with flour once a day in a dose of 126 mg/kg, which corresponded to 1.3 mg/kg iron. Antianemic reference drugs aktiferrin and sorbifer durules were also given in boluses with flour in doses equivalent to 1.3 mg/kg iron. Dissolved Na,Ca,Fe-PG was given to animals instead of drinking water (0.2% solution), iron consumption being 1.3 mg/kg/day. The groups receiving the preparation in solution and the absorption experiment controls (receiving no preparation) received additionally "empty" flour boluses. Young rats (23-24 per group) received the preparation on days 3-30 of life through maternal milk and until the age of 70 days instead of drinking water (1% solution, 1250 mg/kg).

Blood for the analysis was collected from the caudal vein. The dynamics of hematological values was studied: hemoglobin concentration (by hemoglobin cyanide method) and erythrocyte count (counting in Goryaev's chamber, light microscopy). Calcium and iron absorption were evaluated in female rats at the

[1]A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan Research Center, Russian Academy of Sciences; [2]Kazan State University, Russia. ***Address for correspondence:*** egrad@hitv.ru. A. B. Vyshtakaliuk; zobov@iopc.knc.ru. V. V. Zobov; minzanova@iopc.knc.ru. S. T. Minzanova

0007-4888/10/1501-0045

end of the experiment as follows. Total amount of fodder and water consumed and the amount of excrements were measured for 5 days. The concentrations of calcium and iron in the fodder, water, and feces were evaluated by atomic absorption in solid specimens and by spectrophotometry in water. The levels of consumed and released metal were then calculated and the absorption was calculated from the difference.

The data were statistically processed using Student's *t* test.

RESULTS

The results of three experiments on different groups of animals indicate that consumption of Na,Ca,Fe-PG leads to elevation of hemoglobin level and increase in erythrocyte count.

Hemoglobin concentrations virtually did not differ from the initial levels during the first 10 days of the experiment in laboratory mice of all groups (Fig. 1). On days 33 and 70, hemoglobin concentrations increased significantly in both experimental groups in comparison with the initial level and virtually did not change in the control.

The need in iron and calcium is maximum in young growing animals. This fact presumably explains the reduction of hemoglobin level and erythrocyte count in 10-week-old rats in the control group (145.7±9.4 g/liter and 6.63±0.31×10^6/μl) *vs.* adult controls (174.0±10.4 g/liter and 7.52±0.67×10^6/μl, respectively). Hemoglobin concentration in 10-week-old rats daily receiving Na,Ca,Fe-PG in a dose of 1250 mg/kg was significantly higher (234.7±5.8 g/liter; $p<0.001$) compared to the control. However, erythrocyte count (6.63±0.31×10^6/μl) virtually did not differ from the control.

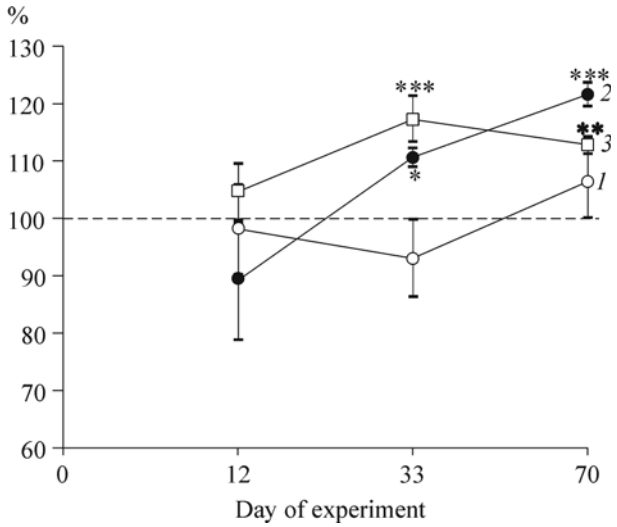

Fig. 1. Time course of hemoglobin concentration in laboratory mice. Initial level of hemoglobin (140.3±2.1 g/liter) is taken for 100%. *1*) control; *2*) Na,Ca,Fe-PG, 360 mg/kg; *3*) Na,Ca,Fe-PG, 480 mg/kg. *$p<0.05$; **$p<0.01$; ***$p<0.001$ compared to 100%.

The dynamics of hemoglobin concentration increase in female rats receiving solid Na,Ca,Fe-PG after bleeding was similar to that in the groups treated with aktiferrin and sorbifer (Table 1). On days 14 and 30 of the experiment, hemoglobin concentrations in the group receiving 0.2% solution of Na,Ca,Fe-PG (174.6±1.2 and 178.0±3.1 g/liter, respectively) were significantly ($p<0.05$) higher than the mean values in the groups treated with aktiferrin and sorbifer (164.0±2.9 and 165.7±2.7 g/liter). Erythrocyte count on day 14 of the experiment in the group treated with solid Na, Ca,Fe-PG did not differ from the initial level before blood loss (Table 1). Erythrocyte count on day 14 in the group treated with 0.2% Na,Ca,Fe-PG solution

TABLE 1. Time Course of Hematological Values in Laboratory Rats after Blood Loss (*M*±*m*; %)

Parameter	Preparation		Day of study		
			14	30	53
Hemoglobin level	Na,Ca,Fe-PG	solid	117.4±4.2	118.6±5.2	125.3±3.6
		dissolved	125.3±0.9	127.7±2.2	126.0±0.9
	Aktiferrin		121.1±3.0	119.9±2.0	125.8±0.9
	Sorbifer		115.0±2.6	117.8±3.6	130.7±4.3
Erythrocyte count	Na, Ca, Fe-PG	solid	97.1±4.3	122.1±4.0	133.0±7.4
		dissolved	150.1±5.8	128.8±5.5	126.1±2.1
	Aktiferrin		127.6±1.5	123.7±6.1	137.8±3.9
	Sorbifer		113.5±2.6	106.4±13.0	112.5±7.6

Note. Parameters after blood loss are taken for 100%: hemoglobin level 139.4±1.5 g/liter, erythrocyte count 8.15±0.18×10^6/μl.

TABLE 2. Effects of Na,Ca,Fe-PG on Absorption of Calcium and Iron from Rations in Laboratory Rats

Parameter	Control	Na,Ca,Fe-PG	Aktiferrin	Sorbifer
Ca absorption, %	28.46	44.80	49.23	36.90
Fe absorption, %	55.02	66.44	74.41	55.98

(12.23±0.47×10^6/μl) differed significantly (p<0.001) from the mean values in the groups treated with aktiferrin and sorbifer (9.64±0.24×10^6/μl). Later, the counts of erythrocytes in the groups receiving solid and dissolved Na,Ca,Fe-PG were similar to those in the group treated with aktiferrin, but were higher than in the group treated with sorbifer. Hence, solid Na,Ca,Fe-PG is not less effective than antianemic drugs aktiferrin and sorbifer and the efficiency of dissolved Na,Ca,Fe-PG is even higher.

Absorption of calcium from the ration increased greater in the groups treated with aktiferrin and Na, Ca,Fe-PG and negligibly in the group treated with sorbifer. Iron absorption did not change under the effect of sorbifer and increased under the effects of aktiferrin and Na,Ca,Fe-PG (Table 2). Calcium share added to the ration with PG is about 1.5% of summary consumption of this element by animals, which is inessential for iron absorption. On the other hand, 18-19% total iron is consumed with the drugs, which can lead to an increase in its absorption and manifestation of antianemic activity. On the other hand, calcium and iron ions are bound to a polymeric matrix when consumed with pectin biopolymer. The metal ions are cleaved gradually from the biopolymer and are not absorbed simultaneously, due to which the antagonistic interactions between calcium and iron are minimized and do not prevent the antianemic effect of iron.

The study was supported by Program No. 5 OKhNM Russia Academy Sciences.

REFERENCES

1. A. B. Vyshtakaliuk, A. N. Karaseva, V. V. Karlin, *et al.*, *Byull. Eksp. Biol. Med.*, **143**, No. 1, 46-49 (2007).
2. A. B. Vyshtakaliuk, A. N. Karaseva, V. V. Karlin, *et al.*, *Toksikol. Vestn.*, No. 6, 10-15 (2006).
3. A. B. Vyshtakaliuk, A. N. Karaseva, V. V. Karlin, *et al.*, *Khim.-Farm. Zh.*, **42**, No. 5, 78-81 (2008).
4. L. Hallberg, *Am. J. Clin. Nutr.*, **68**, No. 1, 3-4 (1998).
5. L. Hallberg, M. Brune, M. Erlandsson, *et al.*, *Ibid.*, **53**, No. 1, 112-119 (1991).
6. K. Haram, S. T. Nilsen, and R. J. Ulvik, *Acta Obstet. Gynecol.*, **80**, No. 8, 683-688 (2001).
7. S. S. Harris, *Nutr. Clin. Care*, **5**, No. 5, 231-235 (2002).