To the editor:

Calcium-channel blockers do not affect iron transport mediated by divalent metal-ion transporter-1

Ludwiczek et al. reported that nifedipine and other dihydropyridine-class calcium-channel blockers can reverse iron overload by stimulating the activity of divalent metal-ion transporter-1 (DMT1) and consequently mobilizing tissue iron. Since their paper disagreed with our published data regarding the activities of the DMT1 isoforms (described previously), we undertook to resolve that issue and examine the effect of nifedipine on DMT1 in vitro.

We found that nifedipine did not affect $^{59}$Fe$^{2+}$ transport (Figure 1A) nor $^{54}$Mn$^{2+}$ transport (supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article) in HEK293 cells transiently transfected with human DMT1 isoform 1A/IRE. Doxycycline-induced expression of rodent 1A+/IRE and 2/-IRE isoforms stably transfected in HEK293 cells resulted in similar protein levels on immunoblot (Figure 1B) and, as before, no differences in their ability to transport Fe$^{2+}$ ($P = .67$; Figure 1C) or Mn$^{2+}$ ($P = .32$; supplemental Figure 2). Nifedipine had no effect on metal-ion transport in cells expressing these isoforms. Other investigators observed that photodegraded nifedipine (PDN) stimulated iron uptake in erythrocytes or epidermal keratinocytes. We found that PDN stimulated uptake of Fe$^{2+}$, but not Mn$^{2+}$, in cells with minimal DMT1 expression (TetR, EV, or uninduced cells), but not in cells in which DMT1 expression was induced.

Fe$^{2+}$-evoked currents in Xenopus oocytes expressing human 1A+/IRE DMT1 were unaffected by nifedipine and only slightly inhibited by PDN (Figure 1D). Nifedipine did not affect transport of $^{59}$Fe$^{2+}$ in these cells at pH 6.5 or 5.5 (Figure 1E-F). A second dihydropyridine-class blocker, nicardipine, as well as diltiazem and verapamil also were without effect (supplemental Figure 3). PDN abolished that fraction of iron-transport activity attributable to DMT1 (Figure 1F) but stimulated iron transport in control (noninjected) oocytes, up to 50-fold more than that in untreated oocytes after 60 minutes' preincubation (Figure 1G). Treatment with either nifedipine or PDN had no effect on $^{54}$Mn$^{2+}$ transport in control oocytes or oocytes expressing DMT1 (supplemental Figure 3B). We observed higher transport activity in oocytes expressing the 1A+/IRE isoform compared with 2/-IRE (supplemental Figure 4), consistent with a previous study in which we had demonstrated that the activities of the 4 isoforms correlated with the levels of their expression at the oocyte plasma membrane. After normalization for expression levels, the 2 isoforms did not differ in their pH dependence over the pH range 5.5 to 8.5 (supplemental Figure 4A) nor in their relative abilities to transport a range of divalent metal ions (supplemental Figure 4B).

Therefore, nifedipine does not increase metal-ion transport in 3 heterologous expression systems with multiple DMT1 isoforms. Instead, our data support earlier proposals that PDN is an iron-specific ionophore. The ionophore effect of PDN was independent of DMT1 because (1) it was less apparent when cells overexpressed DMT1 than when DMT1 levels were lower or minimal; and (2) PDN stimulated the uptake of Fe, but not of Mn, while both are DMT1 substrates. Whereas the study by Ludwiczek et al. presents the interesting possibility of using nifedipine to increase iron excretion in iron-overload conditions, the mechanism by which nifedipine (or PDN) leads to such an increase remains unclear and does not involve DMT1.

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Contribution: B.M. designed research, performed research, analyzed data, performed statistical analysis, and wrote the paper; A.S. designed research, performed research, analyzed data, and performed statistical analysis; A.J.G. and J.D.S. performed research; L.Z. designed research, performed research, and analyzed data; S.G. designed research, performed research, analyzed data, and...
Figure 1. Effects of nifedipine and photodegraded nifedipine on metal-ion transport in vitro. Data presented are mean and SD for n observations. We performed statistical analyses (1- or 2-way analysis of variance [ANOVA or 2ANOVA] and 2-tailed post-hoc all-pairwise comparisons using the Holm–Sidak test) using SigmaStat version 3.5 (Systat Software) with critical significance level α = 0.05. (A) Effects of freshly prepared nifedipine (Nifd) and photodegraded nifedipine (PDN) on uptake of 45Fe2+/H9262P30 minutes (unadjusted and 2/H11002IRE isoforms of rodent DMT1. Expression was induced by treatment with 25nM doxycycline (Doxy). Immunoblot in panel B was stained by an antibody that recognizes /H11001cells. Nontransfected cells contained only the tetracycline-responsive element (TetR); HEK293 cells were stably transfected with the empty vector (EV) or with rodent 1A/IRE and 2/IRE isoforms of rodent DMT1. Expression was induced by treatment with 25nM doxycycline (Dox). Immunoblot in panel B was stained by an antibody that recognizes all DMT1 isoforms. Uptake of 2μM 55Fe2+ (n = 3) in HEK293 cells transiently transfected with human DMT1 1A/IRE. 2ANOVA and post-hoc pairwise comparisons revealed no interaction (P = .65) and that neither Nifd (unadjusted P = .27) nor PDN (unadjusted P = .062) differed from untreated. (B-C) Effects of Nifd and PDN on iron transport in a regulated-expression system in HEK293 cells. Nontransfected cells contained only the tetracycline-responsive element (TetR); HEK293 cells were stably transfected with the empty vector (EV) or with rodent 1A/IRE and 2/IRE isoforms of rodent DMT1. Insertion was induced by treatment with 25nM doxycycline (Dox). Immunoblot in panel B was stained by an antibody that recognizes all DMT1 isoforms. Uptake of 2μM 55Fe2+ (n = 3). 2ANOVA revealed an effect of PDN (P < .001), but not Nifd (P = .55), when [Dox] = 0. When [Dox] = 25nM, we found no treatment effects (P > .36). (D) Effect of Nifd and PDN on DMT1-mediated Fe2+ -evoked currents. A typical continuous current record from an oocyte expressing DMT1 and voltage-clamped at −70 mV is displayed (corrected for baseline drift). The oocyte was superfused with pH 7.5 medium for the periods shown by the empty portions of top bar or pH 5.5 medium (cross-hatched boxes). We presented 50μM Fe2+ (B) alone or with 100μM Nifd (hatched box) or PDN (gray box). (E) Effect of Nifd on 55Fe2+ transport at pH 6.5 (n = 94-97). 2ANOVA revealed no interaction (P = .89). (F) Effects of Nifd and PDN on 55Fe2+ transport at pH 5.5 (n = 22-26). 2ANOVA revealed an interaction (P < .001); within DMT1, a indicates not significant (unadjusted P = .93) and b indicates unadjusted P < .001 compared with no treatment; within PDN, c indicates not significant (unadjusted P = .99) compared with control. (G) Effect of varying PDN-treatment time on 55Fe2+ transport at pH 5.5 in control (noninjected) oocytes. 55Fe2+ uptake was measured in untreated noninjected oocytes (None) and in noninjected oocytes that were coincubated with 100μM PDN after a preincubation period with 100μM PDN ranging from 0 to 60 minutes (n = 16-19). ANOVA (P < .001) and post-hoc pairwise comparisons indicated that all groups differed from one another (unadjusted P < .01) except 0 and 30 minutes (unadjusted P = .09). Materials and methods are described in supplemental information.

performed statistical analysis; L.M.G. designed research, performed research, analyzed data, and wrote the paper; and M.D.G. designed research, performed research, analyzed data, performed statistical analysis, and wrote the paper.

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References

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