Effects of Grisorixin on the Distribution of Thallium-201 and on the Oxidative Metabolism in Cultured Myocardial Cells

Jean C. Maublant, Nicole Moins, Pierre Gachon, Maria R. Ross, Warren D. Davidson, and Ismaël Mena

Divisions of Nuclear Medicine and Nephrology, Harbor-UCLA Medical Center, Torrance, California; Department of Pharmacology, U195 INSERM, University of Clermont 1, Clermont-Ferrand, France; and Allan Hancock Foundation, University of Southern California, Los Angeles, California

Previous experiments in the dog and guinea-pig have shown that grisorixin, a monocarboxylic ionophore, can significantly increase the coronary blood flow and the myocardial uptake of ^{201}Tl, as well as have a stimulant effect on the heart. In this study, cultures of myocardial cells were used in order to isolate the cells from the vascular and extracardiac influences so that any ionophorous effect on ^{201}Tl could be evidenced. The effects of grisorixin on the oxidative metabolism were simultaneously studied. The technique described by Harary was used to prepare the cultures. The activity of the $^{14}CCO_2$ produced by oxidation of [^{14}C]glucose and [^{14}C]octanoate added to the medium of culture and the intra/extracellular ratio of ^{201}Tl concentrations (Tl i/e) were measured. In the controls ($n = 8$), the Tl i/e was 40 ± 10 while it was 17 ± 6 ($p < 0.05$) in the cells that received ^{201}Tl and grisorixin at the same time ($n = 4$), and 19 ± 5 ($p < 0.05$) in the flasks where ^{201}Tl was injected after grisorixin ($n = 7$). A significant decrease of the [^{14}C]octanoate oxidation was found in the flasks treated with grisorixin ($n = 4$, $-50 \pm 16\%$, $p < 0.01$) while the [^{14}C]glucose oxidation was not significantly lowered ($n = 3$; $-11 \pm 12\%$). The conclusion is that grisorixin decreases both the intracellular concentration of ^{201}Tl and the fatty-acids oxidation in cultured myocardial cells. The beneficial effects previously observed in vivo were probably the consequence of the strong coronary dilatation and of an indirect stimulation.

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Grisorixin is a monocarboxylic ionophore that complexes thallium and potassium (*1*) and transports them across biological membranes (*2*). Accordingly, it has been suggested that grisorixin could present some interesting properties for thallium-201 (^{201}Tl) imaging. Experiments in dogs treated with grisorixin have shown a very significant increase of the myocardium-to-background count density ratio (*3*). Measurements in the guinea-pig treated with grisorixin have demonstrated an increase of the concentration of ^{201}Tl in the heart (*4*). It has also been shown in the dog that grisorixin is a cardiac stimulant and induces a strong coronary dilatation which could explain its scintigraphic effect (*5*). However, no ionophorous effect could be demonstrated.

In order to show whether such an ionophorous and stimulant effect could be demonstrated independently of any vascular, humoral, or neuronal influence in beating cells, we utilized cultures of newborn rat myocardial cells. The oxidative metabolism was followed by measuring the $^{14}CCO_2$ liberated by the oxidation of ^{14}C-labeled substrates. The intra/extracellular ratio of the ^{201}Tl concentrations (Tl i/e) was then measured simultaneously with the rates of oxidation in the beta-oxidative and glycolytic pathways.

MATERIALS AND METHODS

The technique of cultured cardiac cells initially described by Harary (*6*) was used with small modifications.

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For reprints contact: J. C. Maublant, MD, Nuclear Medicine, Centre Jean Perrin, 63011 Clermont-Ferrand, France.

Two- to four-day-old newborn Sprague-Dawley rats were killed by spinal chord section and the heart was carefully and aseptically removed. Cells were released from the minced hearts by 0.1% trypsin solution maintained at 37°C and agitated. The supernatant containing free heart cells was decanted and replaced with the same trypsinizing medium every 6 min for a period of 1 hr. The pellet from each supernatant fraction was resuspended in 5 ml of nutrient medium which was 1 mM in $CaCl_2$ with antibiotics and 10% horse serum plus 10% fetal bovine serum. The final cell suspension was made to a concentration of 1 ml medium per heart and divided into equal volumes in sterile flasks. The flasks were incubated in a water-saturated atmosphere at a temperature of 37°C with 5% CO_2 and 95% air. The attachment of the cells could be observed 60 min after plating. They began to contract at ~16 hr after plating. They were dividing with a cycle duration of ~36 hr. They would continue to beat for several weeks if maintained in the incubator and fed every 48 hr with a new medium.

At least 2 days after preparation the flasks were partly immersed in a 37°C water bath. They were then inserted into the closed air circulation of an ionization chamber apparatus connected to a vibrating reed electrometer allowing on-line instantaneous and continuous measurements of the $^{14}CCO_2$ activity present in the system (*7*). The lowest circulating activity that could be detected was 0.27 nCi. The production of even smaller quantities could be detected by measuring the rate of increase of activity in the circulating air. At least 30 min after immersion of the flasks in the bath 5 μCi of [^{14}C]glucose, i.e., 50 μl of a 0.154 nmol/nCi solution, or of [^{14}C]octanoate, i.e., 20 μl of a 0.045 nmol/nCi solution were injected into the flasks. Thirty minutes later 20 μg of grisorixin (1.5×10^{-5} M) were added to the 2 ml of medium of the treated flasks. After stabilization of the production of $^{14}CCO_2$ and at least 30 min after injection, 1 μCi of ^{201}Tl, i.e., 100 μl of a 10 μCi/ml solution were added to the flasks. In four flasks, ^{201}Tl was injected at the same time as grisorixin.

After 5 min of incubation with ^{201}Tl the flasks were rapidly washed three times with 2 ml of cold saline. Overall, about 90 min had usually elapsed between the immersion of the flasks in the bath and the washing of the cells. The flasks were then counted for their ^{201}Tl activity. The counts were corrected for the extracellular activity not removed by the washing (*8*). This activity had been estimated during preliminary experiments by counting flasks that had been washed immediately after the injection of ^{201}Tl. The ratio of intra- to extracellular concentration of ^{201}Tl was then calculated by r x intracellular activity/(total injected activity − intracellular activity). The term r represented the ratio of the extracellular volume i.e., 2 ml, to the intracellular volume which was assumed to be proportional to the amount of proteins. It was derived from the data of Simpson et al. (*9*) who showed that there are about 500 mg of proteins per ml of cells at any time between the first and the 12th day of culture. Therefore, the value of r was set to 1,000/(amount of proteins in mg). The amount of protein present in each flask was measured by the method of Lowry (*10*). The slope of the time-activity curves generated by the electrometer was measured and converted into consumption of nano- or pmole of substrate per min and per milligram of protein.

TABLE 1
Value (mean ± s.d.) of Intra-Over Extracellular ^{201}Tl Concentration (Tl i/e) in Controls and Treated Cells

Item	n	Tl i/e
Controls	8	40 ± 10
Grisorixin and ^{201}Tl injected at same time	4	17 ± 6*
Grisorixin injected before ^{201}Tl	7	19 ± 5*

* $p < 0.001$ compared with controls.

RESULTS

The values presented in Table 1 show that the ^{201}Tl intra/extracellular gradient was significantly decreased when grisorixin was present in the medium. This effect was observed with grisorixin injected either at the same time as ^{201}Tl (58% decrease) or before (53% decrease). There was no significant difference between these two situations.

Results in Table 2 show that in these conditions of ^{201}Tl gradient there was a very significant decrease in the [^{14}C]octanoate oxidation while the [^{14}C]glucose oxidation was only moderately inhibited. Because of the small number of flasks studied with glucose and of the large standard deviation, the reaction of the glucose oxidation to grisorixin seems rather unpredictable. But, when present, these effects could be observed almost immediately in the flasks containing [^{14}C]glucose. With [^{14}C]octanoate there was usually an initial moderate increase of oxidation lasting for 10 to 20 min and then

TABLE 2
Changes (Δ) in % (mean ± s.d.) Induced by Grisorixin on Oxidation of Octanoate and Glucose. Corresponding Values of Intra- Over Extracellular ^{201}Tl Concentration (Tl i/e) are Also Shown (mean ± s.d.)

Item	n	Δ(%)	Tl i/e
Octanoate	4	−50 ± 16*	20 ± 5†
Glucose	3	−11 ± 12	17 ± 3†

† $p < 0.05$ compared with controls.
* $p < 0.01$ compared with controls.

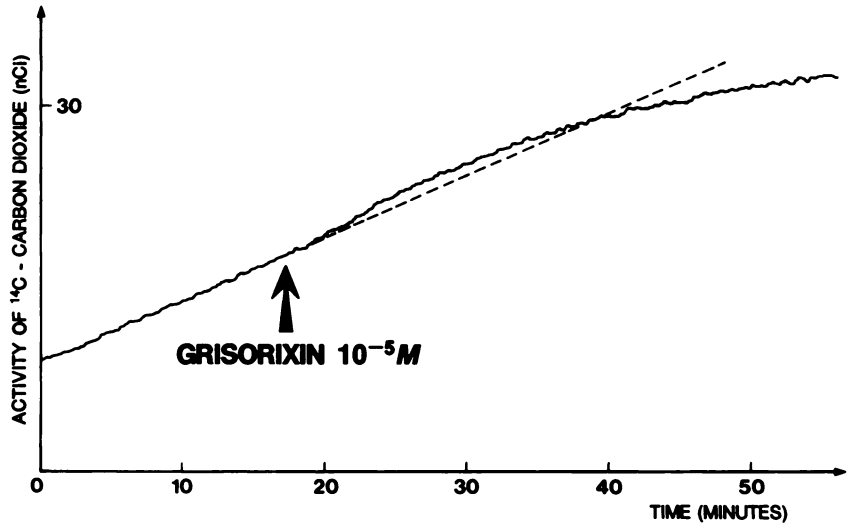

FIGURE 1
Time evolution of production of $^{14}CO_2$ from oxidation of [^{14}C]octanoate. Administration of grisorixin is followed by increased oxidation during first 20 min and then by decreased oxidation

followed by a decrease later on (Fig. 1). Globally the rates of oxidation in the absence of grisorixin were 842 ± 220 pmole/mg prot/min for glucose (n = 7) and 123 ± 79 nmole/mg prot/min for octanoate (n = 8).

DISCUSSION

These results demonstrate a clear decrease of the intracellular concentration of ^{201}Tl as well as the fatty acids oxidation with grisorixin.

It could not be determined whether the decreased intracellular concentration of ^{201}Tl was secondary to a decreased uptake or to an accelerated turnover. Experiments focusing on the washout phase would be required to answer this question. However, the absence of a significant difference in the Tl i/e when grisorixin was injected before ^{201}Tl or at the same time suggests that the effect of the drug is almost immediate after its administration and remains stable for at least 30 min.

In vivo, grisorixin presents interesting imaging properties when injected 5 min before ^{201}Tl (*11*). In the present experiments there was clearly low activity of ^{201}Tl in the cells. That difference between the in vivo and in vitro effects can be explained by the strong coronary dilatation induced by grisorixin in vivo. The tissular uptake of ^{201}Tl is roughly proportional to the local coronary blood flow (*12*). Thus, the corresponding increased uptake is probably stronger than the decrease due to the ionophorous effect of grisorixin. This suggests that grisorixin could not improve the quality of ^{201}Tl imaging any more than an equally potent pure coronary dilator.

The effects of grisorixin on the oxidative metabolism show that, despite its hemodynamic properties observed in vivo (*5*), this drug is toxic for the myocardial cell at that concentration. The biphasic effect observed on the octanoate oxidation suggests that there might be initially an increased consumption of oxygen suggesting an uncoupler-like effect on the mitochondrial oxidative phosphorylation (*13*). Similar properties have been described for other monocarboxylic ionophores on mitochondria (*14*). Therefore, although we do not have any data concerning the mechanical effect of grisorixin on these cells, our data suggest that grisorixin initially has an uncoupling, then followed by a partial inhibitory effect.

The greater impairment of the octanoate than of the glucose oxidation shows that the glycolytic pathway is better protected than the fatty-acids oxidation in presence of grisorixin. Thus the glycolytic pathway could possibly supply the cell with the ATP involved in the maintenance of membrane integrity (*15,16*).

In conclusion, we have found that the in vitro ionophorous effect of grisorixin was in contradiction with its in vivo scintigraphic properties. Moreover, grisorixin presented toxic effects on the metabolism of the cultured myocardial cells. These results suggest that grisorixin does not present any theoretical advantage over the usual coronary dilators as an adjuvant for ^{201}Tl imaging.

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