

Parenteral administration of glipizide sodium salt, an inhibitor of adenosine triphosphate-sensitive potassium channels, prolongs short-term survival after severe controlled hemorrhage in rats*

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Objective: Recent experimental evidence suggests that activation of adenosine triphosphate (ATP)-sensitive potassium channels contributes to vascular failure and early mortality after hemorrhagic shock. The present investigation evaluated the effects of the water-soluble sodium salt of glipizide, an inhibitor of ATP-sensitive potassium channels, in anesthetized and awake rats subjected to severe controlled hemorrhage.

Design: Prospective, randomized, controlled study.

Setting: Animal research laboratory.

Subjects: Male Wistar rats.

Interventions: Anesthetized rats were subjected to bleeding to reduce mean arterial pressure to either 40 or 35 mm Hg, which was maintained constant for 60 mins. In addition, awake rats underwent blood withdrawal of 4.25 mL/100 g over 20 mins. At the end of the hemorrhage period and 30 mins later, the animals received intravenous (5 and 20 mg/kg) or intramuscular (10 and 40 mg/kg) injections of glipizide sodium salt or vehicle.

Measurements and Main Results: In anesthetized rats subjected to pressure-controlled hemorrhage, glipizide sodium salt improved mean arterial pressure in a dose-dependent manner.

Compared with the vehicle-treated animals, mean arterial pressure increased from 41.6 ± 4.6 to 63.1 ± 3.1 mm Hg in the 20 mg/kg intravenous group and from 33.2 ± 4.9 to 54.0 ± 4.7 mm Hg in the 40 mg/kg intramuscular group 60 mins after a 40-mm Hg shock. Furthermore, the drug did not affect the hemorrhage-induced changes in blood glucose concentrations. However, the higher doses of glipizide sodium salt attenuated the increments in plasma concentrations of lactate, alanine aminotransferase, creatinine, and amylase. Moreover, the higher doses markedly improved short-term survival after pressure- and volume-controlled bleeding. Overall, the intramuscular injections of the drug exerted salutary effects that were comparable to the intravenous administration.

Conclusions: In rats, parenteral administration of the water-soluble glipizide sodium salt attenuates vascular and end-organ dysfunction associated with severe hemorrhagic shock and prolongs short-term survival. The intramuscular administration provides comparable benefits as obtained by the intravenous injection. (Crit Care Med 2003; 31:2429–2436)

KEY WORDS: hemorrhage; shock; resuscitation; adenosine triphosphate-sensitive potassium channels; mean arterial pressure; survival

Prehospital management of hemorrhagic shock (HS) traditionally relies on vigorous fluid administration to gain time until definitive control of bleeding (1, 2). However, during large-scale accidents, natural or industrial disasters, terrorism

acts, or combat operations, a vast number of casualties can develop simultaneously. Such environments present numerous obstacles in providing adequate medical care to the surviving victims including a reduced availability of medical personnel and logistic constraints that limit the volume of available resuscitation fluids (1, 3). Therefore, the introduction of an effective pharmacologic therapy for HS that requires small amounts of drug to be administered may considerably increase medical capabilities and improve outcome. Ideally, a drug for emergency treatment of HS should be suitable for intramuscular (as opposed to intravenous) injections to facilitate its administration by nonmedically trained personnel.

Recent experimental evidence suggests that activation of adenosine triphos-

phate (ATP)-sensitive potassium (K_{ATP}) channels in the plasma membrane of vascular smooth muscles, immediately after the onset of HS, may contribute to the vascular failure and early mortality (4–6). Likewise, opening of K_{ATP} channels has been demonstrated during myocardial ischemia (7) as well as in systemic states of oxygen supply-dependency, such as endotoxemia and hypoxic lactic acidosis (8). These channels, which are physiologically modulated by changes in ATP, lactate, and pH of the intracellular environment, maintain adequate perfusion in regional vascular beds under metabolic stress (9–12). Activation of K_{ATP} channels results in hyperpolarization of the vascular smooth muscle, reduction of the entry of extracellular calcium via voltage-dependent calcium channels, and, ultimately, vascular smooth muscle relax-

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ation and increased blood flow to ischemic tissues (6, 7, 9–12). Since HS is associated with global acidosis, regional ischemia, cellular hypoxia, and depletion of intracellular high-energy stores, K_{ATP} channels are activated systemically. This process leads to hypotension, reductions in perfusion pressure and left ventricular preload, and a decreased contractile response to vasoconstrictor agents (6, 13).

Pharmacologic inhibition of K_{ATP} channels with sulfonylureas, such as glibenclamide, glipizide, and tolazamide, may be of therapeutic value for the acute management of HS. In a preliminary study in a rodent model of lethal pressure-controlled HS, we have demonstrated that an intravenous administration of glibenclamide and tolazamide caused an increase in mean arterial pressure (MAP) and reduced early mortality (4). In anesthetized pigs subjected to pressure-controlled hemorrhage, combined resuscitation with glibenclamide and lactated Ringer's solution also improved systemic and regional hemodynamics and metabolism (5). Furthermore, intravenously injected glibenclamide increased MAP, attenuated acute renal failure, and improved survival in sheep subjected to severe uncontrolled aortal bleeding (14).

Since all of the sulfonylureas are practically insoluble in aqueous solution (15), additional formulation studies are required to identify a salt of a clinically approved K_{ATP} channel inhibitor suitable for parenteral administration. Moreover, intramuscular administration of drugs involves additional requirements, including a low volume of injection and appropriate absorption and distribution. These factors are especially salient when the administration is expected to occur in HS, which is associated with reduced

blood flow to the skeletal muscle (6). Therefore, the purpose of the present investigation was to optimize the formulation of glipizide, one of the most potent sulfonylurea agents (16), and evaluate its effects, when administered intravenously (iv) or intramuscularly (im), to anesthetized and awake rats subjected to severe controlled HS.

MATERIALS AND METHODS

Preparation of a Water-Soluble Glipizide Salt. Glipizide (glydiazinamide) possesses a pyrazine moiety, which is basic. This property suggests that water solubility of glipizide may be improved by converting it into a pharmacologically acceptable salt, for example, a sodium salt (15). Glipizide sodium salt was prepared *in situ* at 300 mg/mL with stoichiometric sodium bicarbonate in 10% glycerol/deionized water (v/v). This solution was further diluted to 30 mg/mL with deionized water and filtered via a 0.22 μ m filter (Millipore, Bedford, MA).

Surgical Procedures and Experimental Protocols. The animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health and were performed with the approval of the Institutional Animal Care and Use Committee.

One hundred and fifteen male Wistar rats weighing 400–450 g (Charles River Laboratories, Wilmington, MA) were anesthetized with sodium pentobarbital 60 mg/kg injected intraperitoneally. Body temperature measured via a rectal probe was maintained at 37°C using a servo-controlled homeothermic blanket (Harvard Apparatus, Holliston, MA). The trachea was cannulated with a polyethylene tubing (PE240; Becton Dickinson, Sparks, MD) to facilitate respiration with room air. The right carotid artery was cannulated with a polyethylene catheter (PE50; Becton Dickinson), which was connected to a calibrated pressure transducer (Maxxim Medical, Athens, TX) for the continuous measurement of MAP and

heart rate. The latter variables were digitalized and recorded using a PowerLab 400 converter and a Chart 3.6 data acquisition program (AD Instruments, Mountain View, CA). The right jugular vein was cannulated with the PE50 catheter for the administration of drugs. In addition, the left femoral artery was cannulated with the PE50 catheter for blood withdrawal. At the end of the instrumentation, all animals received intravenous injections of heparin solution 500 units/kg.

Pressure-Controlled Hemorrhage. Following surgical preparation and a 30-min stabilization period, 100 anesthetized rats were subjected to a 5-min blood withdrawal from the femoral artery to decrease MAP to the target pressure of either 40 (n = 60) or 35 (n = 40) mm Hg. The target MAP was maintained constant for 60 mins by either additional blood withdrawals or injections of small volumes of normal saline as necessary. At the end of the hemorrhage period (T_0), glipizide sodium salt or vehicle was administered with the same dose repeated 30 mins later. The rats subjected to a 40 mm Hg HS were randomly assigned to six groups (n = 10 animals/group) to receive the following: a) glipizide 5 mg/kg iv; b) glipizide 20 mg/kg iv; c) vehicle 0.4 mL iv; d) glipizide 10 mg/kg im; e) glipizide 40 mg/kg im; and f) vehicle 0.4 mL im. Furthermore, the rats subjected to a 35 mm Hg HS were randomly assigned to two groups (n = 20 animals/group) to receive either glipizide 40 mg/kg im or vehicle 0.4 mL im (Table 1).

Volume-Controlled Hemorrhage. Another 40 male Wistar rats weighing 250–275 g (Charles River Laboratories) were instrumented with the PE50 catheter in the jugular vein 1 wk before the experiment and were studied awake. The animals underwent blood withdrawal of 4.25 mL/100 g over 20 mins. At the end of the hemorrhage period (T_0), glipizide sodium salt or vehicle was administered with the same dose repeated 30 mins later. Rats were randomly assigned to four groups (n = 10 animals/group) to receive the following: glipizide 20 mg/kg iv, vehicle 0.4 mL iv, glipizide 40 mg/kg im, and vehicle 0.4 mL im (Table 1).

Table 1. Study Groups

	Intravenous (iv) Administration	Intramuscular (im) Administration
Pressure-controlled hemorrhagic shock		
40 mm Hg shock	Glipizide 5 mg/kg iv (10) Glipizide 20 mg/kg iv (10) Vehicle iv (10)	Glipizide 10 mg/kg im (10) Glipizide 40 mg/kg im (10) Vehicle im (10)
35 mm Hg shock		Glipizide 40 mg/kg im (20) Vehicle im (20)
Volume-controlled hemorrhagic shock	Glipizide 20 mg/kg iv (10) Vehicle iv (10)	Glipizide 40 mg/kg im (10) Vehicle im (10)
Nonshocked animals		
Anesthetized	Glipizide 20 mg/kg iv (5)	Glipizide 40 mg/kg im (5)
Awake	Glipizide 20 mg/kg iv (5)	Sham-operated (5) Glipizide 40 mg/kg im (5)

Number of animals in each group is indicated in parentheses.

Experiments in Nonshocked Rats. Fifteen instrumented, anesthetized rats were randomly assigned to three groups (n = 5 animals/group) to receive a) no additional treatment (sham-operated); b) glipizide 20 mg/kg iv; and c) glipizide 40 mg/kg im, with the same dose of the drug repeated 30 mins later. Moreover, another ten chronically instrumented, awake animals were assigned to two groups (n = 5 animals/group) to receive a single injection of glipizide 20 mg/kg iv or 40 mg/kg im (Table 1).

All intramuscular injections were split between the quadriceps muscle on both lower limbs. Animals subjected to pressure-controlled HS were monitored under pentobarbital anesthesia (25 mg·kg⁻¹·hr⁻¹, intraperitoneally). At the end of the observation period, all surviving animals were killed with an intravenous injection of thiopental sodium 100 mg/kg iv.

Biochemical Analyses. Arterial blood was collected for the analysis of blood glucose (OneTouch Monitoring System; Lifescan, Milpitas, CA), plasma lactate (GM7 Analyzer; Analox Instruments, London, UK), and plasma concentrations of alanine aminotransferase, creatinine, and amylase (VetScan Chemistry Analyzer; Abaxis, Sunnyvale, CA).

Determination of Plasma Glipizide Concentrations. Plasma proteins were denatured using 1 M hydrochloric acid and diluting with water. The samples then were passed through Bond Elut C-18SPE cartridges (Varian, Harbor City, CA) and eluted with methanol/water (80/20, v/v). The samples were dried and reconstituted with methanol/water (50/50, v/v). The samples were analyzed by high-performance liquid chromatography using a YMC-Pack ODS-AQ column (4.6 × 150 mm, 5 μ; YMC, Kyoto, Japan) with an acetonitrile/water (modified with 0.02% formic acid) gradient running from 5 to 90% over 8 mins and ultraviolet detection at 235 nm. The limit of detection for this analysis was 0.5 μg/mL.

Drugs and Reagents. All drugs and reagents were obtained from Sigma-Aldrich (St. Louis, MO), unless indicated otherwise.

Data Analysis. Results are expressed as mean ± SEM. Continuous data were assessed by analysis of variance for repeated measures. If the *F* value was statistically significant, two-tailed Student's *t*-test was used to evaluate differences between groups and within groups toward the baseline (*T*₀). Survival rates were compared between groups at the individual time points using chi-square test. A *p* < .05 was considered statistically significant.

RESULTS

Effects of Glipizide Sodium Salt on Hemodynamics and Survival in Rats With Hemorrhagic Shock. In anesthetized rats subjected to the 40 mm Hg hemorrhage, intravenous and intramuscular administrations of glipizide sodium

salt increased MAP in a dose-dependent manner (Figs. 1 and 2), whereas the changes in heart rate remained nearly unaffected (Table 2). For example, MAP increased from 41.6 ± 4.6 mm Hg in the vehicle iv group to 63.1 ± 3.1 mm Hg in the glipizide 20 mg/kg iv group and from 33.2 ± 4.9 mm Hg in the vehicle im group to 54.0 ± 4.7 mm Hg in the glipizide 40 mg/kg im group at 60 mins (*p* < .05). Importantly, the higher doses of the drug (20 mg/kg iv and 40 mg/kg im) extended mean survival time more than three-fold (Figs. 1 and 2; *p* < .05). Likewise, in rats subjected to the 35 mm Hg bleeding, the intramuscular administration of glipizide sodium salt prevented MAP from further deterioration compared with the vehicle group (Fig. 3; *p* < .05) and had no effect on heart rate (not shown). In addition, mean survival time was about 50% longer in the drug-treated animals (*p* = .045). As depicted in Figure 4, both intravenous and intramuscular

injections of glipizide sodium salt caused a marked and comparable delay of death in awake animals after volume-controlled hemorrhage (*p* < .01).

Effects of Glipizide Sodium Salt on Metabolic Status and Organ Functions in Rats With Hemorrhagic Shock. As displayed in Table 2, blood glucose concentrations increased more than two-fold during the bleeding period and then declined toward preshock values in all groups of animals exposed to the 40 mm Hg HS. Although some variations were noticed between the individual groups, glipizide sodium salt had no significant effect on the overall time course of these changes. Likewise, the drug had no effect on the changes in blood glucose concentration in rats subjected to the 35 mm Hg HS (not shown). In contrast, at the higher doses given intravenously or intramuscularly, glipizide sodium salt markedly attenuated the increase in plasma lactate concentration induced by

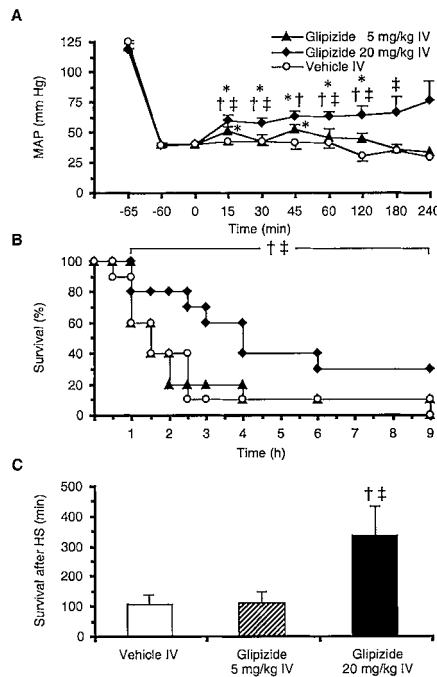


Figure 1. Effects of intravenous (IV) administration of glipizide sodium salt on (A) mean arterial pressure (MAP) and (B and C) survival of anesthetized rats subjected to a 40 mm Hg hemorrhagic shock. Rats were subjected to a 5-min blood withdrawal to reach the target MAP, which was maintained constant for 60 mins. At the end of the hemorrhage (*T*₀), glipizide sodium salt or vehicle was injected with the same dose repeated 30 mins later. Data are mean ± SEM, n = 10 animals/group. **p* < .05 vs. intragroup posthemorrhage baseline (*T*₀); †*p* < .05 vs. vehicle iv group; ‡*p* < .05 between glipizide 20 mg/kg iv and glipizide 5 mg/kg iv groups.

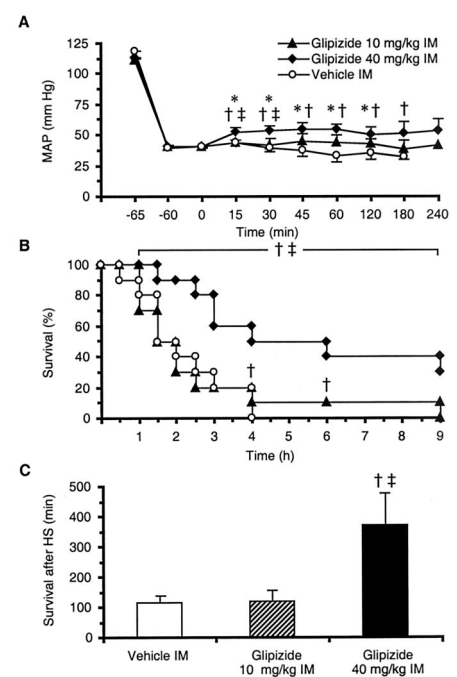


Figure 2. Effects of intramuscular (IM) administration of glipizide sodium salt on (A) mean arterial pressure (MAP) and (B and C) survival of anesthetized rats subjected to a 40 mm Hg hemorrhagic shock. Rats were subjected to a 5-min blood withdrawal to reach the target MAP, which was maintained constant for 60 mins. At the end of the hemorrhage (*T*₀), glipizide sodium salt or vehicle was injected with the same dose repeated 30 mins later. Data are mean ± SEM, n = 10 animals/group. **p* < .05 vs. intragroup posthemorrhage baseline (*T*₀); †*p* < .05 vs. vehicle im group; ‡*p* < .05 between glipizide 40 mg/kg im and glipizide 10 mg/kg im groups.

Table 2. Effects of Glipizide Sodium Salt in Anesthetized Rats Subjected to a 40 mm Hg Hemorrhagic Shock

	-65 Mins	0 Mins	30 Mins	60 Mins	120 Mins	180 Mins	240 Mins
Heart rate, beats/min							
Glipizide 5 mg/kg iv	349 ± 15	328 ± 16	316 ± 15 ^a	328 ± 25	317 ± 27	314 ± 22	
Glipizide 20 mg/kg iv	339 ± 17	317 ± 11	310 ± 11 ^a	313 ± 15	332 ± 12	332 ± 20	336 ± 27
Vehicle iv	340 ± 12	338 ± 11	356 ± 17	342 ± 24	319 ± 40		
Glipizide 10 mg/kg im	325 ± 13	310 ± 20	317 ± 17	313 ± 26	300 ± 46	295 ± 30	
Glipizide 40 mg/kg im	351 ± 13	313 ± 19	312 ± 7	327 ± 12	305 ± 8	298 ± 16	294 ± 22
Vehicle im	328 ± 18	317 ± 19	309 ± 15	304 ± 26	274 ± 44	265 ± 40	
Blood glucose, mg/dL							
Glipizide 5 mg/kg iv	68.7 ± 1.8	157.0 ± 12.9	110.0 ± 20.7	97.4 ± 18.3 ^b	118.5 ± 4.5 ^b	103.0 ± 18.0	
Glipizide 20 mg/kg iv	73.6 ± 2.4	161.5 ± 28.0	133.1 ± 30.6	118.5 ± 20.5	102.0 ± 14.0 ^b	93.8 ± 22.2 ^b	119.7 ± 6.7
Vehicle iv	71.0 ± 3.0	150.7 ± 15.9	124.8 ± 23.7	109.0 ± 11.3 ^b	101.3 ± 9.5 ^b		
Glipizide 10 mg/kg im	65.8 ± 2.1	170.8 ± 31.4	131.5 ± 34.1	139.8 ± 46.3	121.3 ± 21.2	101.0 ± 5.0 ^b	
Glipizide 40 mg/kg im	63.2 ± 1.9	166.0 ± 19.8	149.9 ± 23.0	139.5 ± 23.5	128.4 ± 27.0	127.0 ± 29.5	109.8 ± 12.4 ^b
Vehicle im	62.7 ± 3.8	162.0 ± 24.6	136.3 ± 17.4	98.5 ± 17.5 ^b	92.0 ± 13.1 ^b	65.7 ± 13.8 ^b	
Plasma lactate, mmol/L							
Glipizide 5 mg/kg iv	3.0 ± 0.2	8.2 ± 0.4	8.9 ± 0.9	8.0 ± 0.8	6.9 ± 0.8	6.8 ± 0.8	
Glipizide 20 mg/kg iv	3.1 ± 0.2	7.8 ± 0.4	6.4 ± 0.6 ^{a,b,c}	5.0 ± 0.5 ^{a,b,c}	4.7 ± 0.7 ^{a,b}	4.1 ± 0.7 ^b	4.6 ± 0.9 ^b
Vehicle iv	3.0 ± 0.2	7.8 ± 0.4	8.4 ± 0.4	8.4 ± 0.8	8.5 ± 0.8		
Glipizide 10 mg/kg im	2.8 ± 0.1	8.0 ± 0.6	7.5 ± 0.7	7.4 ± 0.6	7.6 ± 0.6	7.1 ± 0.0	
Glipizide 40 mg/kg im	2.9 ± 0.2	8.2 ± 0.5	6.5 ± 0.5 ^{a,b}	6.0 ± 0.5 ^{a,b}	6.1 ± 0.7 ^{a,b}	4.9 ± 0.6 ^{a,b,d}	4.6 ± 0.6 ^b
Vehicle im	2.8 ± 0.1	8.4 ± 0.3	8.4 ± 0.7	8.2 ± 0.4	8.7 ± 0.9	7.7 ± 0.8	

Rats were subjected to a 5-min blood withdrawal to reach the target mean arterial pressure, which was maintained constant for 60 mins. At the end of the hemorrhage (T_0), glipizide sodium salt or vehicle was injected either intravenously (iv) or intramuscularly (im), with the same dose repeated 30 mins later. Data are mean ± SEM, n = 10 animals/group.

^a $p < .05$ vs. corresponding vehicle group; ^b $p < .05$ vs. intragroup posthemorrhage baseline (T_0); ^c $p < .05$ between glipizide 20 mg/kg iv and glipizide 5 mg/kg iv groups; ^d $p < .05$ between glipizide 40 mg/kg im and glipizide 10 mg/kg im groups.

the 40 mm Hg HS (Table 2; $p < .05$). Moreover, the drug prevented the HS-induced elevation in plasma alanine aminotransferase concentration and also reduced the increments in creatinine (glipizide 20 mg/kg iv) and amylase (glipizide 40 mg/kg im) concentrations (Fig. 5; $p < .05$).

Effects of Glipizide Sodium Salt in Anesthetized, Nonshocked Rats. In anesthetized, nonshocked animals (Table 3), parenteral administration of glipizide sodium salt tended to decrease MAP gradually, although no statistically significant intragroup difference was noticed. Likewise, the effect of the drug on heart rate was negligible. However, in the glipizide-treated rats, blood glucose concentration was reduced by 20 to 30% throughout the study, whereas it increased by 25% in the sham-operated animals at the end of the experiment ($p < .05$).

Pharmacokinetics of Glipizide Sodium Salt. In nonshocked, awake rats, mean plasma glipizide concentration peaked 5 mins after a bolus injection of 20 mg/kg iv ($T_{1/2} = 2.30 \pm 0.17$ hrs) and 60 mins after a bolus injection of 40 mg/kg im ($T_{1/2} = 4.71 \pm 1.20$ hrs), followed by a gradual decline (Table 4). Hemorrhagic shock resulted in slower changes in plasma concentrations of the drug. The maximal increments in plasma concentrations of glipizide sodium salt

occurred at 60 and 120 mins following intravenous injections of 5 and 20 mg/kg, respectively. Furthermore, after intramuscular injections, plasma concentrations of the drug were the highest at the last time points studied.

DISCUSSION

The present study demonstrates that parenteral administration of glipizide sodium salt, an inhibitor of K_{ATP} channels, delays death of rats subjected to severe pressure- and volume-controlled hemorrhage. In addition, in anesthetized animals subjected to pressure-controlled HS, glipizide sodium salt improves MAP in a dose-dependent manner. Apparently, the higher doses of the drug also delay the onset of end-organ dysfunction, as judged by reductions of the HS-induced increments in plasma concentrations of lactate, alanine aminotransferase, creatinine, and amylase.

Recent investigations indicate that in trauma involving major vascular injury, resuscitation with large volumes of crystalloid fluids, when administered before full control of bleeding, may be associated with increased morbidity and mortality rates (17–19). Furthermore, under some situations, such as mass casualties and combat operations, the availability of intravenous resuscitation fluids is limited

and urgent intravenous access is not always possible (1, 3). These situations require a rapidly administrable drug that can be injected by nonmedical personnel with the ultimate goal to extend the time until definitive medical intervention (“golden hour”) (1). Although the present study evaluated parenteral therapy with glipizide sodium salt only under conditions of controlled hemorrhage without determining long-term survival, the results are consistent with the notion that inhibition of K_{ATP} channels might be a potentially effective pharmacologic intervention in the previously mentioned situations (4, 5, 14). All vehicle-treated rats died, whereas 30% of the animals that received higher doses of the drug were alive as long as 9 hrs after the 40 mm Hg hemorrhage. Importantly, the marked differences in the survival rates were noticed throughout the whole experiment regardless of the route of administration, although a doubling of the intramuscular dose was required to achieve this efficacy. Moreover, death was also significantly delayed in the drug-treated animals subjected to the 35 mm Hg bleeding. Since all the animals received the same anesthetic procedure, we assume that pentobarbital anesthesia, which has been shown to reduce mortality rate in a rodent model of HS (20), had no considerable contribution to the observed differ-

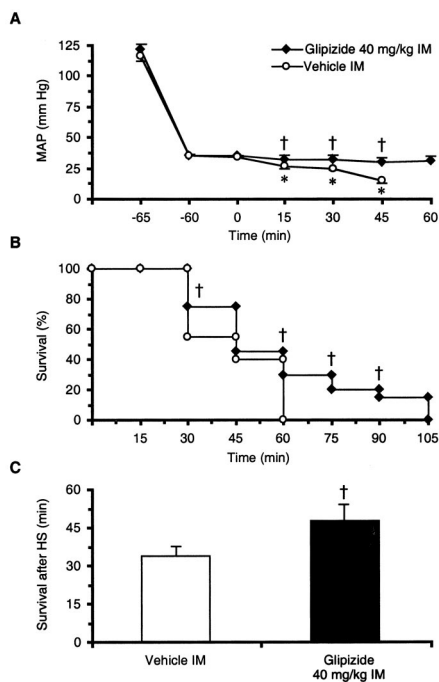


Figure 3. Effects of intramuscular (IM) administration of glipizide sodium salt on (A) mean arterial pressure (MAP) and (B and C) survival of anesthetized rats subjected to a 35 mm Hg hemorrhagic shock (HS). Rats were subjected to a 5-min blood withdrawal to reach the target MAP, which was maintained constant for 60 mins. At the end of the hemorrhage (T_0), glipizide sodium salt or vehicle was injected with the same dose repeated 30 mins later. Data are mean \pm SEM, $n = 20$ animals/group. * $p < .05$ vs. intragroup posthemorrhage baseline (T_0); † $p < .05$ vs. vehicle im group.

ences in survival. Furthermore, in awake rats subjected to volume-controlled HS, parenteral administration of glipizide sodium salt also markedly extended short-term survival in the absence of any additional pharmacologic intervention.

What could be the possible explanation of the observed effects of glipizide sodium salt in HS? There is convincing experimental evidence that tissue acidosis, hypoxia, and ATP depletion contribute to systemic opening of K_{ATP} channels leading to pathologic vasodilation and further impairment of perfusion in regional vascular beds (6–8, 10, 12). By inhibiting K_{ATP} channels, glipizide sodium salt may induce hemodynamic stabilization and attenuate end-organ dysfunction during the postshock phase, resulting in improved survival. The favorable effects of the drug also may be mediated through the normalization of intracellular ATP concentrations induced by increased production and release of

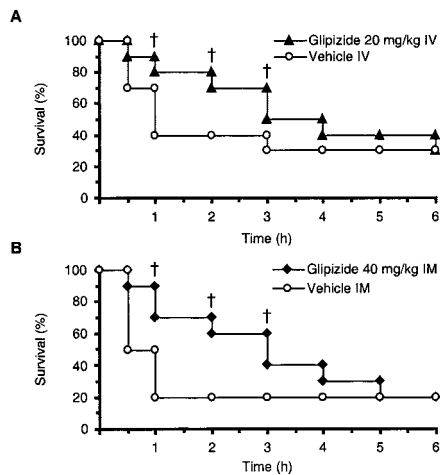


Figure 4. Effect of (A) intravenous (IV) and (B) intramuscular (IM) administrations of glipizide sodium salt on survival of awake rats subjected to blood withdrawal of 4.25 mL/100 g over 20 mins. At the end of the hemorrhage (T_0), glipizide sodium salt or vehicle was injected with the same dose repeated 30 mins later. † $p < .05$ vs. corresponding vehicle group; $n = 10$ animals/group.

insulin (16, 21). Administration of insulin after HS has been shown to restore depleted intracellular ATP concentrations (22). In turn, the normalization of intracellular ATP concentrations may reverse the ischemia-induced opening of K_{ATP} channels (21, 23). Indeed, the present findings that treatment with glipizide sodium salt increases MAP and postpones the onset of end-organ failure in anesthetized rats subjected to pressure-controlled hemorrhage are consistent with previous reports on other sulfonylureas (4, 5, 14). In rodent and porcine models of pressure-controlled HS, intravenously injected glibenclamide improved MAP (4) and counteracted deterioration of regional hemodynamics and metabolism when administered in combination with lactated Ringer's solution (5). Furthermore, in an ovine model of severe uncontrolled bleeding after puncture of abdominal aorta, glibenclamide increased MAP, attenuated development of acute renal failure, and also improved survival (14). Apparently, vasoconstriction in response to glipizide sodium salt does not represent a nonspecific mechanism, since the drug had no such effect in nonshocked rats in the present study.

Our observation that in HS (unlike in the nonshocked animals) glipizide sodium salt had no effect on the changes in blood glucose concentrations is in agreement with a recent study of uncontrolled hemorrhage in

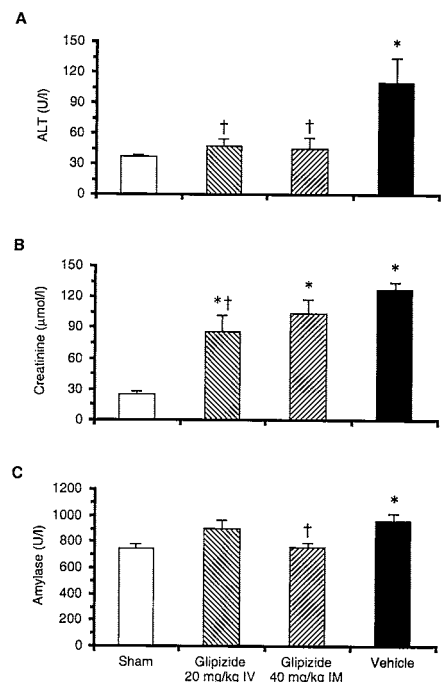


Figure 5. Plasma concentrations of (A) alanine aminotransferase (ALT), (B) creatinine, and (C) amylase in anesthetized, sham-operated rats ($n = 5$ animals/group) and in anesthetized rats three hours after a 40 mm Hg hemorrhagic shock ($n = 6$ animals/group). In the latter animals, glipizide sodium salt or vehicle was injected either intravenously (IV) or intramuscularly (IM) at the end of a 60-min hemorrhage period, with the same dose repeated 30 mins later. Data are mean \pm SEM * $p < .05$ vs. sham-operated animals; † $p < .05$ vs. vehicle-treated animals.

sheep (14). This point is important since HS is known to be associated with a marked hyperglycemia due to a combination of decreased cellular glucose utilization and increased mobilization of glucose by the liver (24). Exogenous (and possibly endogenous) glucose is crucial in protecting against multiple organ failure in HS (22, 25). In fact, it has been suggested that immediately after hemorrhage, glucose acts as a nonpermeant solute drawing fluid into the circulation (25). Thus, a reduction in circulating glucose by glipizide sodium salt would have been considered a potential side effect of the therapy of HS.

Why does the drug not decrease blood glucose concentrations in HS, whereas it produces pronounced hypoglycemia in the nonshocked rats? Part of the answer may be related to the pharmacokinetics of glipizide sodium salt during HS. It is well known that HS leads to a marked decrease in skeletal

Table 3. Effects of Glipizide Sodium Salt in Anesthetized, Nonshocked Rats

	0 Mins	30 Mins	60 Mins	90 Mins	120 Mins
Mean arterial pressure, mm Hg					
Glipizide 20 mg/kg iv	123.4 ± 5.1	128.9 ± 5.0	115.2 ± 9.7	107.2 ± 5.5	106.9 ± 5.2
Glipizide 40 mg/kg im	119.2 ± 10.5	108.2 ± 8.3	121.2 ± 6.3	112.6 ± 5.2	102.3 ± 5.9
Sham	119.6 ± 11.7	124.7 ± 8.3	120.7 ± 8.9	117.1 ± 10.0	120.3 ± 9.6
Heart rate, beats/min					
Glipizide 20 mg/kg iv	328 ± 20	323 ± 14	315 ± 16	304 ± 21	325 ± 18
Glipizide 40 mg/kg im	332 ± 26	311 ± 24	333 ± 24	313 ± 23	301 ± 23
Sham	342 ± 37	359 ± 36	354 ± 23	323 ± 34	341 ± 23
Blood glucose, mg/dL					
Glipizide 20 mg/kg iv	67.0 ± 0.9	44.8 ± 3.2 ^{a,b}	44.4 ± 2.9 ^{a,b}	52.4 ± 6.3 ^{a,b}	51.2 ± 6.4 ^{a,b}
Glipizide 40 mg/kg im	70.8 ± 1.4	46.8 ± 2.9 ^{a,b}	47.2 ± 3.4 ^{a,b}	48.8 ± 4.6 ^{a,b}	56.4 ± 4.2 ^{a,b}
Sham	62.6 ± 3.4	64.2 ± 5.1	67.4 ± 3.5	72.2 ± 3.6	77.0 ± 4.3 ^c

Rats received either no additional treatment (sham-operated) or administration of glipizide sodium salt. The latter was injected either intravenously (iv) or intramuscularly (im) at T₀, with the same dose repeated 30 mins later. Data are mean ± SEM, n = 5 animals/group.

^ap < .05 vs. intragroup baseline; ^bp < .05 vs. sham group.

Table 4. Plasma Concentrations of Glipizide (µg/mL) in Awake, Nonshocked Rats (n = 5 Animals/Group) and in Anesthetized Rats Subjected to a 40 mm Hg (n = 10 Animals/Group) and a 35 mm Hg (n = 20 Animals/Group) Hemorrhagic Shock

	5 Mins	15 Mins	30 Mins	60 Mins	120 Mins	180 Mins	240 Mins	360 Mins
Non-shocked rats								
Glipizide 20 mg/kg iv	54.8 ± 9.1	51.2 ± 8.7	46.2 ± 6.5	40.5 ± 4.8	31.0 ± 4.0	19.3 ± 4.6		12.2 ± 1.7
Glipizide 40 mg/kg im	42.5 ± 3.4	50.1 ± 2.7	59.8 ± 2.4	61.3 ± 4.7	51.5 ± 4.2	41.2 ± 4.7		25.0 ± 2.6
40 mm Hg hemorrhagic shock								
Glipizide 5 mg/kg iv			123.4 ± 7.3	131.2 ± 7.1	101.8 ± 0.4	76.8 ± 9.1		
Glipizide 20 mg/kg iv			119.4 ± 4.2	129.8 ± 6.5	132.0 ± 10.1	116.6 ± 12.3	108.2 ± 6.0	
Glipizide 10 mg/kg im			8.7 ± 0.9	23.7 ± 3.2	43.3 ± 7.7	61.1 ± 0.5		
Glipizide 40 mg/kg im			24.9 ± 4.3	40.3 ± 5.3	50.1 ± 5.8	59.2 ± 5.7	68.0 ± 10.0	
35 mm Hg hemorrhagic shock								
Glipizide 40 mg/kg im			15.7 ± 2.9	37.5 ± 2.7				

In nonshocked rats, glipizide sodium salt was injected either intravenously (iv) or intramuscularly (im) at T₀. In rats subjected to hemorrhagic shock, glipizide sodium salt was injected either intravenously (iv) or intramuscularly (im) at the end of a 60-min hemorrhage period (T₀), with the same dose repeated 30 mins later. Data are mean ± SEM.

muscle blood flow (1). Thus, one would expect that plasma concentrations of the drug should be lower after the intramuscular administration to the animals subjected to HS compared with the nonshocked rats. Our direct measurements of glipizide plasma concentrations, indeed, confirm this assumption. Moreover, in the shocked rats, mean and peak plasma concentrations after the intramuscular injection of 40 mg/kg were approximately 50% lower than the concentrations in response to 20 mg/kg given intravenously. Overall, after the intramuscular injections, plasma concentrations of glipizide were the highest at the end of the experiment. All of these findings indicate a slow systemic absorption of the K_{ATP} blocker from the poorly perfused skeletal muscle during HS.

Nevertheless, some other mechanism also must contribute to the observed effects, as even the intravenous administration of glipizide sodium salt, which resulted in high plasma concentrations,

failed to reduce blood glucose concentrations during HS. We think that part of the answer is the relatively well-characterized phenomenon of “insulin resistance” occurring during shock and trauma, that is, a reduced ability of the organs to respond to insulin and to mobilize glucose (26, 27). The mechanisms and mediators of insulin resistance are multiple and likely to involve cytokines as well as a β-adrenoceptor-mediated pathway (27). In the current therapeutic scenario, insulin resistance works to our advantage, as it does not allow hypoglycemia to develop during the initial phase of the pharmacologic resuscitation.

The skeletal muscle vascular bed exhibits good autoregulatory properties between 50 and 100 mm Hg, but below 50 mm Hg it behaves in a purely passive manner (28, 29). Even in our very severe experimental model, despite the poor perfusion of the skeletal muscle at 35 mm Hg, we found that glipizide sodium salt entered the systemic circula-

tion in concentrations that improved short-term outcome. There may be a positive feedback cycle at work here. Namely, small amounts of the drug initially released from the severely ischemic skeletal muscle increase MAP, which increases skeletal muscle perfusion and thus further enhances the release of glipizide from the muscle. We did not measure glipizide concentrations in the skeletal muscle, but it is possible that glipizide can deposit in this tissue for prolonged periods of time, especially in low-perfusion states. If intramuscular administration of a K_{ATP} blocker enters clinical practice for the acute management of severe HS, it is likely that during definite treatment (volume resuscitation, pharmacologic therapy, closure of the wound), the skeletal muscle blood flow will increase (1). The latter may lead to a second phase of the drug release from the skeletal muscle into the circulation. Obviously, during the definite medical treatment period, the patient’s plasma

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glucose concentrations would need to be carefully monitored and adjusted as necessary.

Several isoforms of the K_{ATP} channels are present in various tissues, including the vascular smooth muscle isoform, the heart isoform, and the pancreatic isoform present in β -cells (21, 23, 30). The molecular composition of these channels is well known: Native K_{ATP} channels are a complex of a regulatory protein containing sulfonylurea receptor (SUR) and an inward-rectifying K^+ channel (Kir) serving as a pore-forming subunit. To date, three isoforms of SUR (SUR1, SUR2A, and SUR2B) have been identified. The molecular structure of K_{ATP} channels is a heteromultimeric assembly of these complexes: SUR1 with Kir6.2 (pancreatic type), SUR2A with Kir6.2 (cardiac type), and SUR2B with Kir6.1 (vascular smooth muscle type) (22, 23, 30). It is widely assumed that in shock, the beneficial site of action of the K_{ATP} channel blockers is the vascular smooth muscle (6), although this notion has never been proven directly, and the potential contribution of the myocardial K_{ATP} channel has yet to be clarified. It is now possible to develop selective ligands that preferably bind to the vascular or the pancreatic K_{ATP} channels (31, 32). In theory, it is conceivable that a vascular-selective K_{ATP} channel inhibitor may be superior to glipizide for the management of HS, although this question remains to be tested experimentally. Ultimately, inhibitors of K_{ATP} channels should not be considered as replacements for conventional therapy of HS but eventually may serve as an

initial therapeutic measure to gain time until definitive treatment.

CONCLUSIONS

In rats subjected to severe controlled hemorrhagic shock, parenteral administration of the water-soluble glipizide sodium salt attenuates vascular and end-organ dysfunction and prolongs short-term survival. Despite poor perfusion of the skeletal muscle in shock, the intramuscular route of administration produces effective plasma concentrations of the drug, and the intramuscular and the intravenous treatments exert comparable beneficial effects. Based on the current findings, further investigations are justified to test whether the intramuscular administration of glipizide sodium salt can extend the "golden hour" period and improve the outcome after severe hemorrhage in trauma victims.

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