Y.Y.Ü. Vet. Fak. Derg. 1996, 7(1-2): 74-77

Effect of Ryanodine Decreasing the Positive Inotropic Effect of Monensin

İsmail MERAL

Yüzüncü Yıl Üniversitesi, Veteriner Fakültesi, Fizyoloji Anabilim Dalı, Van, TÜRKİYE

Geliş tarihi: 03 Mart 1996

Monensin'in Oluşturduğu Pozitif İnotropik Etkiyi Azaltmada Ryanodin'in Etkisi

Özet: Bu çalışmada erkek kobaylardan izole edilen papillar kaslar kullanılarak ryanodin'in monensin zehirlenmesini gidermedeki etkinliği araştırıldı. Papillar kasların kasılma şiddeti (KŞ), kasılma hızı (KH) ve gevşeme hızı (GH) monensin veya monensin+ryanodin tatbikinden bir saat sonra ölçüldü. Monensin'in, tatbikinden bir saat sonra KŞ ve KH'yı artırtığı ancak GH'yı değiştirmediği saptandı. Ancak monensin+ryanodin tatbik edilen kasların KŞ'si ve KH'sının sadece monensin tatbik edilenlerden daha düşük olduğu bulundu. Bu bulgular ışığı altında, ryanodinin hücre içerisindeki kalsiyum konsantrasyonunda oluşturduğu bu azalmadan dolayı monensin zehirlenmesini gidermede kullanılabileceği sonucuna varıldı.

Anahtar Kelimeler: Monensin, Ryanodin, Kobay, Kalsiyum, Papillar kas.

Summary: This experiment was carried out to investigate the ability of ryanodine to counteract the toxic effect of monensin by using adult male guinea-pig papillary muscles. Contraction force (CF), contraction velocity (CV) and relaxation velocity (RV) were measured one hour after the monensin only and monensin+ryanodine treatments. Monensin treatment significantly increased the CF and the CV but did not change the RV. However, the CF and the CV of monensin+ryanodine treated group were significantly lower than the CF and the CV of monensin only treated group. It was concluded that ryanodine's ability to unload Ca^{2+} from cardiac myocytes could make it useful in monensin poisoning and in heart failure cases where Ca^{2+} saturation of mitochondria has occurred.

Key Words: Monensin, Ryanodine, Guinea-pig, Calcium, Papillary muscles.

Introduction

Our laboratory, for the past four years, has been working with a drug (ryanodine) to counteract the toxic effects of monensin. During this time the market positive inotropic effect of monensin on cardiac muscle and the ability to increase the functional survival time of isolated guinea-pig papillary muscle was observed (1).

Monensin is used in birds as a coccidiostat (2, 3) and is used in cattle as a bloat preventive (4, 5) also a growth promoter (6, 7). Other animals (8) have been kept on monensin for a year without any detectable ill effects. The biochemical pathogenesis of monensin-induced myotoxicity have been shown, by many workers, to be caused by intracellular Ca^{2+} overloading, especially mitochondrial overloading (9, 10). This problem can be controlled by dosage and apparently this compound has a useful margin of safety (1).

Monensin has also a strong positive inotropic effect in cardiac muscle (11, 12). This effect occurs because, as an ionophore, monensin has strong affinity for $[Na^+]_o$. The monensin-induced increase in $[Na^+]_i$ facilitates the entry of Ca^{2+} into the cell by a Na^+ (out) / Ca^{2+} (in) exchange mechanism (13). This Ca^{2+} shift is the primary factor mediating the cellular response. Another factor modifying the cellular response includes the alteration of the pH of intracellular components (pH_i) since monensin increases the pH_i by transferring H^+ out of the cell (14).

It has been proposed (15, 16) that ryanodine causes a decrease in the extent, or a slowing, of sarcoplasmic reticulum Ca^{2+} release in cardiac muscle. Since an increase in intracellular Ca^{2+} concentration (13) is the primary reason for monensin poisoning, ryanodine's ability to unload Ca^{2+} from cardiac myocytes could make it useful in monensin poisoning and in heart failure cases where Ca^{2+} saturation of mitochondria has occurred.

The primary purpose of this study was to utilize a papillary muscle preparation to investigate the ability of ryanodine to counteract the toxic effects of monensin by measuring the contraction force (CF), contraction velocity (CV) and relaxation velocity (RV).

Materials and Methods

Preparation of Papillary Muscles

Fifteen male guinea-pigs, 500-600 g, were heparinized (1000 IU per animal, IP injection) 30 minutes before being decapitated. Hearts were removed rapidly and put in a beaker filled with ice-cold KRB of the following composition [mmol/l]: Na^+, 115.9: Ca^{2+}, 2.2: K^+, 4.0: Mg^{2+}, 1.3: Cl^-, 126.9: $H_2PO_4^-$, 2.1: HCO_3^-, 21.7: glucose, 10.9. The pericardium was removed, and the aorta and the

pulmonary artery were excised. The hearts were transferred to a second beaker of the same solution, and then rapidly put in a glass pan filled with Krebs solution and bubbled with 100 % O_2. Two papillary muscles with diameters of approximately 1.0-1.5 mm were dissected from the right ventricle of each guinea-pig and each muscle was mounted in an organ bath containing a Krebs solution with 2.2 mmol/l $CaCl_2$. The Krebs solution was perfused with a mixture of 95 % O_2 and 5 % CO_2 and maintained at 37 ^{0}C. The pH was maintained between 7.50 - 7.60. The muscles were attached to a capacitance transducer (Harvard isometric capacitance transducer, Harvard apparatus, inc., South Notick, MA) electrically connected to a Beckman recorder (R611). The tendon end of each muscle was attached to the transducer by a silk suture and the opposite end was held by a plastic clamp placed in the muscle chamber. Muscles were stimulated at a frequency of 0.2 Hz by using a pair of platinum field effect electrodes. The transducers to measure force were calibrated for each experiment by using weights (1 g equaled a displacement of 40 mm). Frequency was calibrated by an oscilloscope.

Contractility Experiment, experimental design and statistical analysis

Three treatments were used; control (0.18% alcohol), monensin only treated (10 μmol/l) and monensin (10 μmol/l) + ryanodine (5 μmol/l) treated groups. Ten papillary muscles were used for each group. Each animal provided two muscles, individual muscles were the experimental unit for the experiment. Experimental units (a single muscle) were randomly allocated to treatments. Chemicals were administered after an equilibration period of 35 min. Contraction force, contraction velocity and relaxation velocity were measured one hour after the treatment. The data were expressed as mean ± standard error (SE) and analyzed using analysis of variance (ANOVA). Least significant difference (17) was used to test for differences among means for which ANOVA indicated a significant ($P \leq 0.05$) F ratio.

Results

Fig. 1 and Fig. 2 respectively show the representative tracings of the monensin- or monensin+ryanodine-induced changes in the CF and CV of guinea-pig papillary muscles. Monensin increased both the CF and the CV significantly ($P<0.05$) when compared to the control group. Both the CF and the CV of monensin+ryanodine treated group were significantly lower than ($P<0.05$) the CF and the CV of monensin only treated group.

Fig. 3 shows the changes in the RV of guinea-pig papillary muscles induced by the monensin- or monensin+ryanodine treatment. Both treatment did not change the RV of the guinea-pig papillary muscles one hour after the treatment.

One of the major problems with the guinea-pig papillary muscles was that a change that occurred in one guinea-pig in 40 minutes did not occur in another until 60 minutes, therefore the changes were very unpredictable but in most cases these changes had the same general pattern.

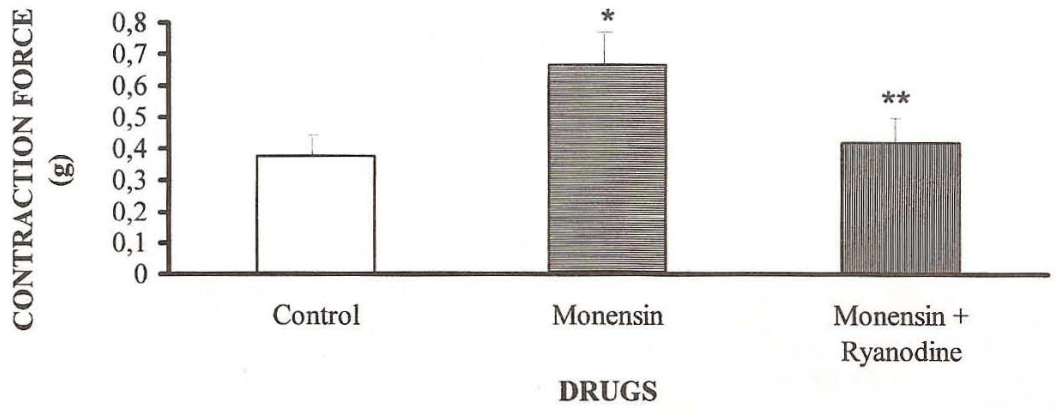

Figure 1. Effects of monensin only and monensin+ryanodine treatment on the contraction force (g) of guinea-pig papillary muscles. Mean ± SE is shown (n=10). *denotes a significant difference between control and treatment group. **denotes a significant difference between monensin and monensin+ryanodine group.

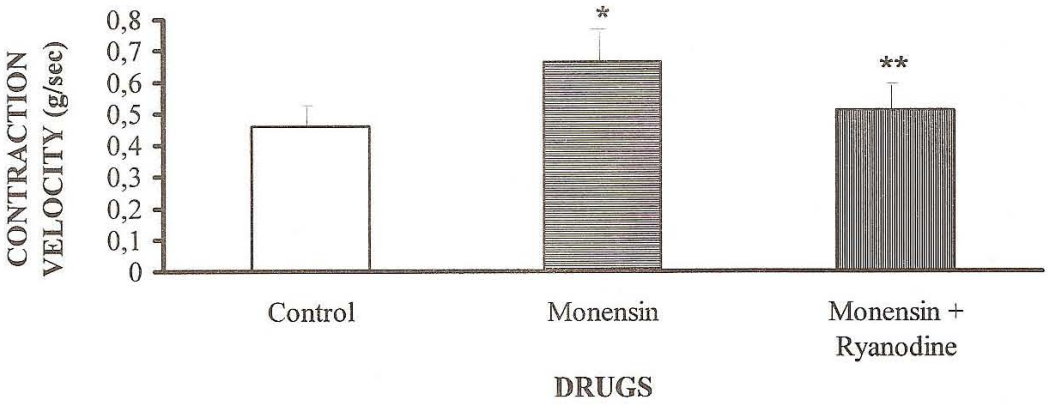

Figure 2. Effects of monensin only and monensin+ryanodine treatment on the contraction velocity (g/sec) of guinea-pig papillary muscles. Mean ± SE is shown (n=10). *denotes a significant difference between control and treatment group. **denotes a significant difference between monensin and monensin+ryanodine group.

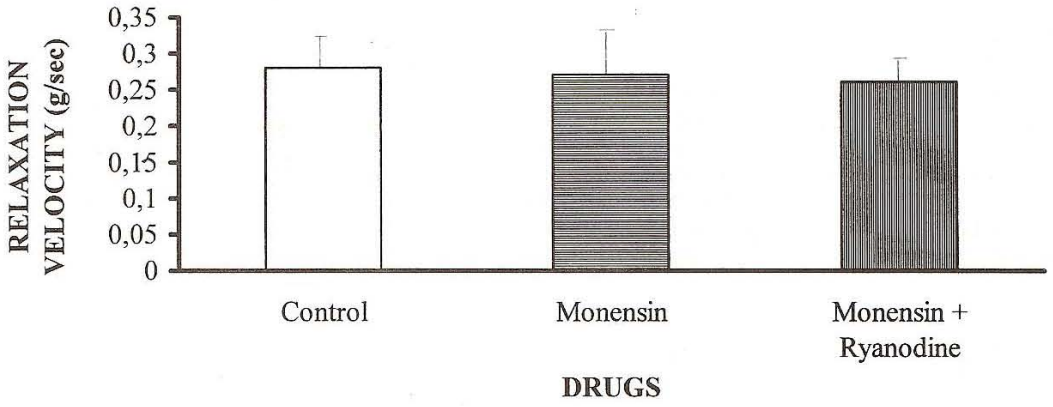

Figure 3. Effects of monensin only and monensin+ryanodine treatment on the relaxation velocity (g/sec) of guinea-pig papillary muscles. Mean ± SE is shown (n=10).

Discussion

This study was undertaken to utilize a papillary muscle preparation to investigate the ability of ryanodine to counteract the toxic effects of monensin by measuring the CF, CV and RV. We found that Monensin only treatment increased both the CF and the CV significantly and did not change the RV. However, monensin+ryanodine treatment significantly lowered the CF and the CV of monensin only treated group. These findings are consistent with our previous findings that have suggested a ryanodine induced increase in the heart rate and blood pressure of monensin only treated groups (18).

The positive inotropic effect of monensin depends on extracellular Na^+ concentration. Monensin binds Na^+ outside of the cell and carries it into the cell and increases $[Na^+]_i$ and thus $[Ca^{2+}]_i$ and the CF (13). Monensin also increases the pH_i due to the increased exit of a H^+ when monensin is transported out of the cell (14). However, Meral et al, 1996 (1) demonstrated that monensin can increase the $[Ca^{2+}]_i$ in the absence of the extracellular Na^+. This indicated that monensin actually may cause a Ca^{2+} release from the sarcoplasmic reticulum or may be a Ca^{2+} as well as

a Na^+ ionophore. Since ryanodine causes a decrease in the extent of sarcoplasmic reticulum Ca^{2+} release in cardiac muscle, our findings made the idea stronger that monensin may cause a Ca^{2+} release from the sarcoplasmic reticulum.

To be able to demonstrate whether monensin-induced increase in $[Ca^{2+}]_i$ is due to an influx or a release from the SR, we measured the CV. An increase in the rate of Ca^{2+} release from the SR would increase the CV. We found that 10 μmol/l monensin increased the CV significantly. This result indicated that monensin causes a Ca^{2+} release from the SR. However, we do not know weather this increase in intracellular Ca^{2+} concentration is due to Ca^{2+}-induced Ca^{2+} release or direct release of Ca^{2+} from the SR. The decrease in the CV after ryanodine administration also indicated the role of SR on monensin-induced increase of intracellular Ca^{2+} concentration.

The RV is affected by two mechanisms. First, a SR Ca^{2+}-ATPase that pumps Ca^{2+} into the SR thus decreases $[Ca^{2+}]_i$. Second, a Na^+ (in) / Ca^{2+} (out) exchange. Under steady-state conditions, the amount of Ca^{2+} entering the cell equals that which exits the cell, and the amount released by the SR equals that sequestered by the SR (15). If an abrupt change in this balance of fluxes takes place, then the Ca^{2+} content of the SR will be affected. Monensin did not change the RV of guinea-pig papillary muscles one hour after administration. However, in our previous experiment we found that monensin increased the RV two hours after the administration (1). This indicated that it takes time for monensin to increase the RV.

We concluded that, since an increase in intracellular Ca^{2+} concentration is the primary reason for monensin poisoning, ryanodine's ability to unload Ca^{2+} from cardiac myocytes could make it useful in monensin poisoning and in heart failure cases where Ca^{2+} saturation of mitochondria has occurred.

References

1. Meral, İ. (1996) Utilization of fatigued and non-fatigued papillary muscles and ventricular myocytes on the comparison of inotropic, chronotropic and intracellular calcium changes induced by monensin and digoxin. Iowa State University, Veterinary Collage, the USA (Doktora tezi).
2. Fahim M. and Pressman B. (1981) Cardiovascular effects and pharmacokinetics of the carboxylic ionophore monensin in dogs and rabbits. Life Sci. 29: 1959-1966.
3. Van Vleet JF. and Ferrans, VJ. (1984) Ultrasonic alterations in skeletal muscle of pigs with acute monensin myotoxicosis. Am. J. Pathol. 114, 3: 461-471.
4. Thornton JH., Owens FN., Lemenager RP. and Tatusek R. (1976) Monensin and ruminant methane production. J. Anim. Sci. 43: 336 (Abstr).
5. Potter EL., Cooley CO., Richard LF., Roun AP. and Rathmacher RP. (1976) Effect of monensin on performance of cattle fed forage. J. Anim. Sci. 1976; 43, 665-669.
6. Mitema ES. and Sangiah S. (1988) Effects of some calcium modulators on monensin toxicity. Vet. Hum. Toxicol. 30, 5: 409-413.
7. Mollenhauer HH., Morre DJ. and Rowe LD. (1990) Alteration of intracellular traffic by monensin; mechanism, specificity and relationship to toxicity. Biochim. Biophys. Acta. 1031: 225-246.
8. Todd GC., Novilla MN and Howard CL (1984) Comparative toxicology of monensin sodium in laboratory animals. J. Anim. Sci. 58: 1512-1517.
9. Ozaki H., Kishimoto T., Karaki H. and Urakawa N. (1982) Effects of the Na ionophore monensin on the contractility response and the movement cations in the vascular smooth muscle of rabbit aorta. Naunyn-Schmiedeberg's Ach Pharmacol. 321: 140-144.
10. Shlafer M. and Kane P. (1980) Subcellular actions and potential adverse cardiac effects of the cardiotonic ionophpre monensin. J. Pharmacol. Exp. Ther. 214: 567-573.
11. Pressman BC. (1976) Biological applications of ionophores. Ann. Rev. Biochem. 45: 501-529.
12. Sutko JL., Besch HR., Bailey JC., Zimmerman G. and Watanabe AM. (1977) Direct effects of the monovalent cation ionophores monensin and nigericin on myocardium. J. Pharmacol. Exp. Ther. 203: 685-700.
13. Elsasser TH. (1984) Potential interactions of ionophore drugs with divalent cations and their function in the animal body. J. Anim. Sci. 59, 3: 845-853.
14. Shlafer M. and Kane P. (1980) Subcellular actions and potential adverse cardiac effects of the cardiotonic ionophore monensin. J. Pharmacol. Exp. Ther. 214: 567-573.
15. Barry WH. and Bridge JHB. (1993) Intracellular calcium homeostasis in cardiac myocytes. Circulation. 87, 6: 1806-1815.
16. Sutko JL., Ito K. and Kenyon JL. (1985) Ryanodine: a modifier of sarcoplasmic reticulum calcium release in striated muscle. FASEB. 44, 15: 2984-2988.
17. Snedecor GW. and Cochran WG. (1989) Statistical methods, Eight edition. pp. 227.
18. Meral İ. (1996) Investigation of a counteragent for monensin toxicity. Tr. J. Vet. Ani. Sci. (Baskıda).