Can We Prevent Malignant Arrhythmias by Targeting of Cardiac Connexin-43 ?

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Abstract. *We hypothesized that cardioprotective compounds such as atorvastatin (ATO), melatonin, omega-3 FA and red palm oil (RPO) may protect from malignant arrhythmias via modulation of myocardial connexin-43 (Cx43) channels and. Experiments were conducted on male rats that were: 1/ spontaneously hypertensive (SHR) without or with intake of omega-3 FA (300mg/day/2month), melatonin (400μg/day/6 weeks) or RPO (200mg/day/6weeks); 2/ hereditary hypertriglyceridemic (HTG) without or with ATO (1.5mg/day/2mth) and omega-3 FA treatment; 3/ hyper- (TH) and hypo-thyroid (HY) without and with RPO intake, as well as age-matched Wistar rats. Results showed that compared to Wistar rats the incidence of sVF was significantly higher in SHR, HTG and TH rat hearts that exhibited abnormal distribution and down-regulation of Cx43, while sVF was not induced in HY rat hearts with up-regulation of Cx43. Treatment of SHR, HTG and TH rats with either compounds resulted in protection from sVF that was associated with attenuation of Cx43 abnormalities and improvement of integrity of cardiomyocytes. Results point out antiarrhythmic effect of atorvastatin, omega-3 FA, melatonin and red palm oil due to, at least in part, modulation of myocardial Cx43. Further studies should elucidate pathways involved in Cx43 modulation by these compounds.*

Keywords: Connexin-43, ventricular fibrillation, antiarrhythmic compounds

1. Introduction

It has been established that rapid spreading of the electrical impulse throughout the heart is essential for synchronized contraction and it is ensured by electrical coupling of cardiomyoctes at the connexin (Cx) gap junction channels. Electrical remodeling due to cardiovascular diseases, such as hypertension, dyslipidemia or altered thyroid status has been shown to involve the changes in expression, distribution and function of cell membrane ion channels, intercellular gap-junction Cx43 channels, Ca-cycling proteins, and extracellular matrix composition [1-4]. This remodeling predisposes to arrhythmogenic mechanisms such as early or delayed afterdepolarizations, resulting in premature contraction, and re-entry of excitation, thus facilitating of life-threatening ventricular tachycardias and fibrillation (VF). Re-entry arrhythmias occur predominantly due to abnormal topology and down-regulation of Cx43 that blocks of conduction [5-7]. Taking into account our previous and studies of others [8-10] we hypothesized that cardioprotective compounds such as atorvastatin (ATO), melatonin, omega-3 FA and red palm oil (RPO) may affect Cx43 and exert antiarrhythmic effects. We aimed to explore this assumption using several rat models mimicking risk factors of human cardiovascular diseases. This study is overview of our already published findings.

2. Subject and Methods

Experiments were conducted on male rats that were: 1/ spontaneously hypertensive (SHR) without or with intake of omega-3 FA (300mg/day/2month), melatonin (400μg/day/6 weeks) or RPO (200mg/day/6weeks); 2/ hereditary hypertriglyceridemic (HTG) without or with ATO

(1.5mg/day/2mth) and omega-3 FA treatment; 3/ hyper- (TH) and hypo-thyroid (HY) without and with RPO intake, as well as age-matched healthy Wistar or Lewis rats. Basic characteristics of animals were registered and left ventricular tissue was examined for myocardial ultrastructure and Cx43 analysis using immunofluorescence, immunobloting and real-time PCR. Electrically-induced sustained ventricular fibrillation (sVF) was tested in isolated perfused heart.

3. Results

Results showed that compared to healthy rats the incidence of sVF was significantly higher in SHR [11, 12, 13], HTG [14] and TH [15] rat hearts that exhibited abnormal distribution and down-regulation of Cx43, while sVF was not induced in HY rat hearts with up-regulation of Cx43. Treatment of SHR, HTG and TH rats with either compounds resulted in protection from sVF that was associated with attenuation of Cx43 abnormalities and improvement of integrity of cardiomyocytes. For details see our published studies [11-15]. Changes in myocardial Cx43 expression and the threshold to induce VF in untreated and RPO-treated hyper- and hypo-thyroid rats are demonstrated in Fig.1.

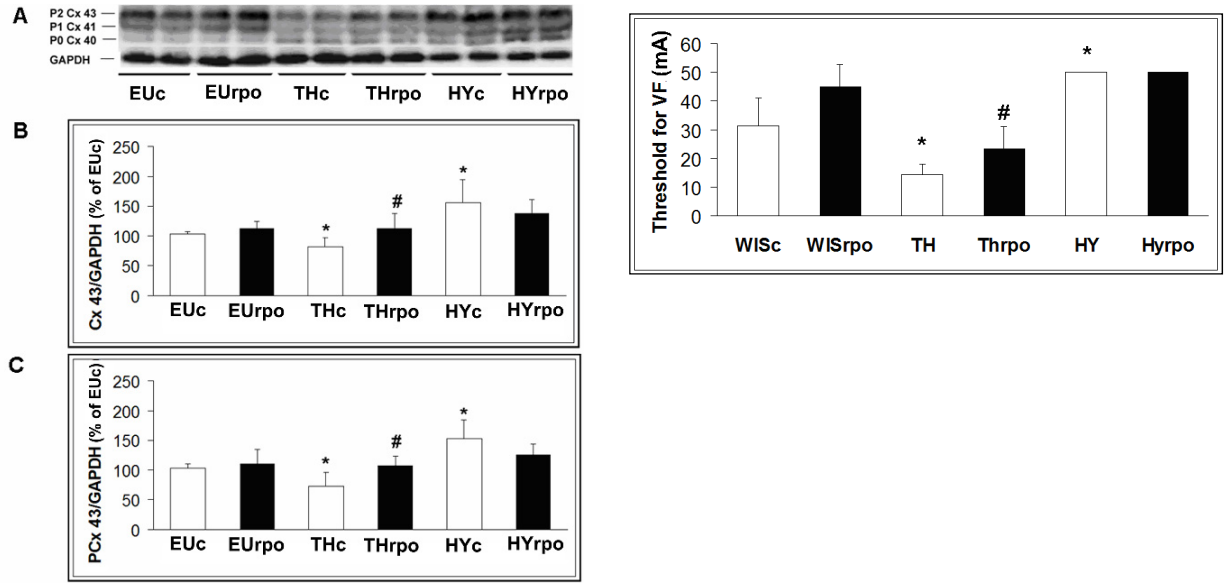

Fig. 1. Expression of myocardial total Cx43 protein (A,B) and its phosphorylated forms (A,C) normalized to GAPDH in rats with altered thyroid status (left panel), threshold to induce sVF (right panel). Abbreviations: Euthyroid control rats (Euc, n=6), EU rats treated with RPO (Eurpo, n=6), hyperthyroid rats (THc, n=6), TH rats treated with RPO (THrpo, n=6), hypothyroid rats (HYc, n=6), HY rats treated with RPO (HYrpo, n=6). Data are means ± SD, $^{*}P<0.05$ *vs*EUc, $^{\#}P<0.05$ untreated *vs* treated with RPO.

4. Discussion

We have demonstrated that the hearts of rats suffering from hypertension, hypertriglyceridemia or hyperthyroidism are more susceptible to life threatening arrhythmias, such as ventricular fibrillation (for details see references 11-15). Likewise humans exhibiting of these predisposing factors of cardiovascular diseases are in higher risk to develop malignant arrhythmias. On the other hand, some non-antiarrhythmic drugs used in treatment of cardiovascular diseases (such as statins, sartans, etc.) has been reported to exhibit pleiotropic effects and can protect from arrhythmias. Furthermore, the benefit of dietary oils intake as well as melatonin supplementation in both experimental and clinical conditions has been shown. However, the mechanisms implicated in cardioprotective and antiarrhythmic effects are still not fully elucidated. Based on our previous studies and studies of others

pointing out crucial role of intercellular Cx43 channels in myocardial synchronization we hypothesized that these compounds may modulate expression and/or phosphorylation of myocardial Cx43. The latter is crucial for Cx43 channels function. Indeed, we have demonstrated that atorvastatin, omega-3 fatty acids, red palm oil and melatonin were able to up-regulate Cx43 (i.e. increase its total expression and/or its phosphorylated forms) and hence attenuate hypertension, hypertriglyceridemia or hyperthyroidism induced abnormal distribution and expression. This was associated with protection from VF. For example, as demonstrated on Fig. 1 the down-regulation of Cx43 in hyperthyroid rats was associated with decrease while up-regulation of Cx43 in hypothyroid rats with increase of threshold for VF. The threshold was also increased after treatment of hyperthyroid rats by RPO, which increased Cx43 expression. Heart disease related deterioration of cell-to-cell communication mediated by Cx43 channels may results in unmasking of ectopic foci, enhancement of dispersion of APD and slowing of conduction[16] that promote arrhythmias. On the contrary, improvement of cell-to-cell communication by examined compounds can offer protection due to suppression of these proarrhythmic factors. Moreover, it is likely that atorvastatin, melatonin as well as omega-3 fatty acids and red palm oil might affect other ion channels functions and together with modulation of Cx43 channels to attenuate electrical instability of diseased heart and to suppress arrhythmogenesis.

5. Conclusions

In conclusion, our findings [11-15] point out antiarrhythmic effect of atorvastatin, omega-3 fatty acids, melatonin and red palm oil due to, et least in part, up-regulation of myocardial Cx43. These findings support the assumption that we can prevent malignant arrhythmias by targeting cardiac Cx43. Further studies should elucidate molecular pathways involved in Cx43 modulation by examined compounds.

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