Anti-Arrhythmic Effect of Chronic Acetaminophen Treatment in the Aging F344XBN Rat Involves Diminished Myocardial Fibrosis and Altered Micrornas Regulation

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Abstract

There is a growing need for pharmacological agents to manage cardiovascular disease in the rapidly increasing elderly population. Acetaminophen has been shown to have cardioprotective effect against ischemia-reperfusion injury by acting as an antioxidant. Recently, we reported that chronic acetaminophen ingestion significantly attenuates age associated increases in cardiac ROS and apoptosis. Here, we examined the effect of chronic acetaminophen treatment on incidents of arrhythmias, Cx43 expression, myocardial fibrosis and miRNA expression in the aging Fischer344XBrown Norway (F344XBN) rat hearts. Aging male F344XBN rats (27-mo; n=8) were treated with acetaminophen (30mg/kg/day p.o.) for six months. Serial electrocardiography was performed during the study. There was an increase in incidence of premature atrial and ventricular arrhythmias with age, which were attenuated by acetaminophen treatment. Histological and immunohistochemical analysis revealed increased myocardial fibrosis and altered localization of gap junction protein Cx43 respectively, while 6-months acetaminophen ingestion is able to inhibit such alterations to certain extent. Aging in F344XBN rats was associated with decreased expression of Cx43 however acetaminophen treated hearts levels were slightly higher than age-matched control animals. The expression of mir-1, mir-133, mir-214, mir-30a and mir-30d was 66-, 64-, 54-, 73- and 74-% lower, respectively in 33-month control animals compared to that of 6-month animals (p<0.05). Acetaminophen treatment significantly increased the expression of mir-1 and mir-214, while the other miRNA remain unchanged. These results indicate that acetaminophen may prevent the incidence of age-associated arrhythmias and that this alteration is associated with diminished fibrosis and changes in cardiac miRNA expression. WC=271

Introduction

Cardiovascular disease remains the foremost cause of death and morbidity worldwide and is especially prevalent in the elderly. Nearly half the deaths occurring from cardiovascular causes take place suddenly due to fatal ventricular arrhythmias [1]. Aging by itself represents a major risk factor for cardiovascular disease and is associated with structural and functional alterations in the cardiovascular system including increased left ventricular (LV) wall thickness, increased LV mass, impaired LV ejection and higher incidence of arrhythmias [2-4]. Echocardiographic studies have demonstrated that aging in the Fischer 344/NiaHsd X Brown Norway/BiNia (F344XBN) rat is characterized by age-associated alterations in cardiac structure and function [5,6] while others have reported that cardiac aging in these animals is associated with increases in the amount of cardiac reactive oxygen species (ROS) [7,8] and apoptosis [9]. It has also been established that myocardial aging is characterized by left ventricular (LV) fibrosis caused by the progressive reduction in cardiomyocyte number thereby decreases myocardial compliance ultimately leading to increased risk of ventricular dysfunction and arrhythmias [10-12].

The factors that regulate age-associated cardiac remodeling have not been elucidated. The “free radical theory of aging” proposes that aging allows for gradual damage to biomolecules via free radical reactions [13]. In addition, the theory characterizes aging by increased levels of reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. The basis for these changes are still not fully understood, but age-related increases in ROS may be due to altered mitochondrial electron transport leakage [14,15], xanthine oxidase [16], nitric oxide synthase [17-19], or NADPH oxidases [20-22]. Indeed, elevation of ROS levels with age have been shown to influence several important factors including cardiac hypertrophy, structure and function of blood vessels, inflammation, apoptosis and alteration of the extracellular matrix profile [23-26]. Whether interventions aimed at decreasing age-related increases in ROS may be beneficial for the treatment of age-associated cardiac arrhythmias or dysfunction is not well understood [27].

The molecular mechanism(s) governing the cardiac remodeling are not well understood. Recently, data has suggested that microRNAs (miRNAs) - small ~ 22-nucleotide noncoding RNAs that inhibit transcription or translation by interacting with the 3′ untranslated region (3′UTR) of target mRNAs [28] - may play a role in the regulation of cardiac differentiation and disease [29]. In this context, several miRs including miR-1, 21, 29, 30, 195, 133, 206 and 208 appear to play an important role in the process of cardiac remodeling, putatively by regulating changes in gene expression that accompany pathological cardiac hypertrophy and contractile dysfunction [30-33]. The factor(s) that regulate the expression of miRNA are not clear however recent studies have suggested that alterations in cellular ROS levels may be involved [34-36]. Similarly, how aging may affect the expression of cardiac miRNA has not been elucidated.

Acetaminophen is a potent antioxidant that has been used therapeutically for several decades [37], but its potential cardioprotective properties have only recently begun to be studied. It is thought that acetaminophen can help protect cells and tissues from the damaging effects of peroxynitrite [38], myeloperoxidase [39], cyclooxygenase [40], and other peroxides [41]. Recent work has also suggested that that acetaminophen exhibits potent cardioprotective activity in preventing ROS-associated cardiac damage in the rat and dog heart [42-45]. Whether the chronic administration of acetaminophen is effective for the treatment of age-associated cardiac remodeling is unknown. Using the tissues from the same animals employed in this study we have previously reported that chronic acetaminophen ingestion significantly attenuates age associated increases in cardiac ROS and apoptosis [46]. Here we are presenting data showing the effect of aging and chronic
low dose acetaminophen treatment on the expression of cardiac miRNA and the development of cardiac arrhythmias in the aging F344×BN rat. Our data suggest that aging in F344×BN rats is associated with increased incidence of cardiac arrhythmias, lowered expression of gap junction protein connexin-43 (Cx43), increased myocardial fibrosis and altered regulation of miRNA, while 6-months chronic acetaminophen ingestion is able to inhibit some of those changes.

Materials and Methods

Animals

All procedures were performed in accordance with the Marshall University Institutional Animal Care and Use Committee (IACUC) guidelines, using the criteria outlined by the American Association of Laboratory Animal Care (AALAC). F344×BN rats aged 6 and 27 months, were purchased from the National Institute on Aging colony at Harlan. Adult F344×BN rats at 27-months of age were chosen for the study since they do not exhibit significant cardiovascular deficit at this age [6,7,47,48] and roughly coincide with humans in the 5th decade of life according to the probability of survival curves generated by the NIA [49]. Rats were housed two per cage in an AALAC approved vivarium with a 12 hour light-dark cycle and temperature maintained at 22 ± 2°C, and fed ad libitum. All animals were allowed to acclimatize for 2 weeks before initiation of any treatment or procedures. All animals were examined for precipitous weight loss, failure to thrive or unexpected gait alterations and animals with apparent abnormalities or tumors were removed from the study. Periodic weight measurements were taken throughout the duration of the study.

Materials

McNeil Pharmaceuticals (Fort Washington, PA) provided acetaminophen pure compound, a non-aspirin pain reliever that is not classified as an NSAID, with analgesic and antiinflammatory effects, which was used in the study. Antibodies against Cx43 and rabbit IgG antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Precast 10% SDS-PAGE gels were procured from Cambrex Biosciences (Baltimore, MD, USA), and enhanced chemiluminescence (ECL) Western blot detection reagent was acquired from Amersham Biosciences (Piscataway, NJ, USA). Restore Western blot stripping buffer was obtained from Pierce (Rockford, IL, USA) and 3T3 cell lysates were from Santa Cruz Biotechnology. All other chemicals were purchased from Sigma (St Louis, MO, USA).

Acetaminophen treatment, electrocardiography and tissue collection

F344×BN rats (27-month; N = 6) were given acetaminophen (30 mg / kg body weight / day, Sigma-Aldrich, Inc., St. Louis, MO) for 6 months in drinking water. Rats were treated until the age of 33-months, which coincides with humans in the seventh decade of life. Age-matched F344×BN rats were maintained as controls. Heart rate and cardiac rhythm was measured before commencement of treatment and at the end of the treatment using needle electrodes under ketamine-xylazine (4:1) cocktail (50 mg/kg, I.P) anesthesia.

Tissues were collected under anesthesia using a ketamine-xylazine cocktail (40:10 mg/kg i.p.) and supplemented as necessary for reflexive response. After midline laparotomy, the heart was removed and placed in Krebs-Ringer bicarbonate buffer (KRB) containing: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 24.2 mM NaHCO₃ and 10mM d-glucose, (pH 7.4) equilibrated with 5% CO₂ / 95% O₂ and maintained at 37°C. Isolated hearts were quickly massaged to remove any blood from the ventricles, cleaned of connective tissue, weighed, and immediately snap frozen in liquid nitrogen. Liver and kidneys were excised immediately after removing the heart and either snap-frozen under liquid nitrogen or stored in 5% buffered formaldehyde.

Histology

Tissue specimens were serially sectioned (6μm) using an IEC Minotome cryostat and collected on poly-lysine coated slides. After fixing in acetone, (−20°C for 2 min) sections were stained with hematoxylin and eosin, mounted and covered. Morphometric evaluation was performed with the use of a computerized imaging analysis system (Olympus MicroSuite™ Basic). Masson’s trichrome staining of serially sectioned cardiac tissue was performed according to the manufacturers guidelines (Poly Scientific R & D Corp., Bay Shore, NY).

Immunohistochemistry

Immunostaining for Cx43 was visualized by immunofluorescence as outlined by the antibody manufacturer. Briefly, sections were washed three times with phosphate-buffered saline (PBS) containing 0.5% Tween-20 (PBS-T), pH 7.5. After incubation for 30 min in a blocking solution (5% BSA), sections were incubated with specific antisera diluted in PBS-T (antibody dilution of 1:100) for 1 h at 24°C in a humidified chamber. After washing three times with PBS, sections were incubated with FITC anti-rabbit IgG (1:200) for 30 min at 24°C in a humidified chamber. DAPI was also included in the secondary antibody solution at a concentration of 1.5μg/ml in order to visualize cell nuclei. After a final PBS wash and mounting, specimens were visualized by epifluorescence using an Olympus fluorescence microscope (Melville, NY, USA) fitted with a x40 objective. Images were recorded digitally using a CCD camera and analyzed using Olympus MicroSuite™ Basic from Olympus America (Melville, NY, USA).

Immunoblot analysis

Tissues were pulverized in liquid nitrogen using a mortar and pestle until a fine powder was obtained and washed three times with ice-cold PBS as described previously [50]. Samples were then suspended on ice in 100μl of TPER (2 ml/g tissue weight; Pierce, Rockford, IL, USA) supplemented with 100 mM NaF, 1 mM Na3VO4, 2mM PMSF, 1μg/ml aprotinin, 1μg/ml leupeptin, and 1μg/ml pepstatin. After lyses, homogenates were centrifuged at 10,000 x g at 13,000 x g for 15 min. Aliquots (60μg of total protein for each sample) were separated on a 10% SDS-PAGE gel. Transfer of protein onto nitrocellulose membranes was performed using standard conditions. To verify transfer of proteins and equal loading of lanes the membranes were stained with Ponceau S. Semiquantitative immunodetection of specific proteins was performed using established methods as described previously [51]. A total of three SDS-PAGE gels were run for each experimental set to evaluate changes in dependent variable tissue content and basal phosphorylation where applicable. Immunoblots were stripped with Restore Western blot stripping solution (5% BSA), sections were incubated with specific antisera for 30 min, washed three times with phosphate-buffered saline containing 0.5% Tween-20 (PBS-T), pH 7.4, and blocked for 2 h at room temperature. The membranes were then probed with specific antibodies, rinsed, and reprobed. Randomization of antibody incubation was utilized to minimize potential experimental error associated with membrane stripping.

miRNA and mRNA Analyses

Total RNA was harvested from cardiac tissues using TRIzol Reagent, (Invitrogen) and X μg of total RNA was converted to cDNA by using (X) according to manufacturer’s protocol. Reverse transcription was performed using the QuantimIR RT kit (System Bioscience) according to manufacturer’s protocol. Sybergreen-based real-time qPCR was performed by using a 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA) and gene-specific primers for the miRNA of interest designed according to the QuantimIR RT kit’s guidelines.
incidence of premature atrial (PAC) and ventricular (PVC) contractions as shown in Figure 1A. Figure 1B shows representative ECG traces from control and acetaminophen treated rats, where the untreated control shows frequent supra-ventricular tachycardia.

**Aging is associated with decreased connexin-43 levels**

Compared to 6-month animals, Cx43 expression in the 33-month control animals was 25% lower ($P<0.05$) (Fig 2A). Acetaminophen treatment for 6-months significantly increased Cx43 levels by 8% ($P<0.05$) (Figure 2A). These results were corroborated by immunohistochemical analysis of these hearts. Consistent with previous findings [56,57], gap junctional Cx43 immunoreactivity was primarily observed at the intercalated discs (Figure 2B). When compared to hearts from 6-mo rats, Cx43 immunoreactivity at the intercalated disc regions of the membrane was markedly lower in 33-mo control rat hearts. Conversely, the cytosolic Cx43 immunoreactivity was significantly higher with age, appearing to be clustered inside the fibers of old hearts (Figure 2B).

**Aging is associated with increased cardiac fibrosis**

It is thought that the intrinsic properties of the myocardium change during aging due to connective tissue alterations [58]. In agreement with this possibility, we found increased perivascular fibrosis as well as subcellular alterations of the cardiomyocytes and their junctions in the left ventricles of the aged control animals suggesting a deterioration of heart function. Similarly, qualitative histological analysis on heart sections of 33-month acetaminophen treated rats revealed the presence

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**Figure 1:** Chronic acetaminophen treatment decreases incidence of cardiac arrhythmias: (A) Percentage incidence of premature atrial (PAC) and ventricular (PVC) contractions observed in the 33-mo control and 33-mo APAP-treated rats (n=8). (B) Representative echocardiographs (Lead II) recorded from 6-mo control, 33-mo control and 33-mo APAP-treated rats (n=8).

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of fibrosis, although the incidence of these findings appeared to be less than in untreated controls (Figure 3).

**Prolong acetaminophen ingestion is not associated with hepatotoxicity**

Prolonged use of acetaminophen at high dosages has been shown to result in hepatotoxicity because acetaminophen produces free radicals and depletes cellular glutathione stores [59,60]. To address this possibility, as previously reported [61], we also investigated the effect of chronic acetaminophen treatment on major organs such as liver and kidney. Consistent with other acute and chronic studies with similar or higher acetaminophen dosages [62], our data suggested that chronic acetaminophen (30 mg/kg body weight/day) ingestion for up to 6 months does not cause hepatotoxicity in aged rats [63-68].

**Aging is associated with alterations in cardiac miRNA expression**

In order to evaluate whether miRNAs play a significant role in the aging process, a highly sensitive, semi-quantitative RT-PCR method was employed to analyze the expression of miRNA thought to be involved in cardiac remodeling. The relative expression is presented. Compared to 6-month animals the amount of mir-1, mir-133, mir-214, mir-30a and mir-30d was 66-, 64-, 54-, 73- and 74-% lower, respectively in 33-month control animals (Figures 4-6). The amount of mir-206, mir-21, mir-24, mir-195 and mir-29a was not altered with aging.

**Acetaminophen treatment is associated with alterations in miRNA expression.**

Compared to 33-month control animals, acetaminophen treatment increased the expression of mir-1 and mir-214 expression levels by 29- and 53 %, respectively, (P<0.05) (Figures 4 and 6). The amount of mir-133, mir-30a and mir-30d did not change with treatment.

**Discussion**

Multiple experimental models have been used to assess the effects of aging on ROS and CVD, including dogs, the Fisher 344 (F344) and...
Figure 4: Aging decreases myocardial mir-1 and mir-133 expression: Quantitative RT-PCR analyses of microRNA expression in 6-mo control, 33-mo control and 33-mo APAP-treated rat hearts using SYBR green I. Relative expression of mir-1 (A) and mir-133 (B) between groups was calculated after normalization to U1 mir expression in the same samples.

*Significantly different from 6-mo control. †Significantly different from 33-mo control. For all comparisons, P < 0.05; n = 4

Figure 5: Aging decreases myocardial mir-30a and mir-30d expression: Quantitative RT-PCR analyses of microRNA expression in 6-mo control, 33-mo control and 33-mo APAP-treated rat hearts using SYBR green I. Relative expression of mir-30a (A) and mir-30d (B) between groups was calculated after normalization to U1 mir expression in the same samples.

*Significantly different from 6-mo control. †Significantly different from 33-mo control. For all comparisons, P < 0.05; n = 4

Figure 6: Chronic acetaminophen treatment alters myocardial mir-214 expression: Quantitative RT-PCR analyses of microRNA expression in 6-mo control, 33-mo control and 33-mo APAP-treated rat hearts using SYBR green I. Relative expression of mir-214 between groups was calculated after normalization to U1 mir expression in the same samples.

*Significantly different from 6-mo Control. †Significantly different from 33-mo control. For all comparisons, P < 0.05; n = 4

Aging in the F344XBn heart is associated with increases in cardiac arrhythmias

Nearly half the deaths occurring from cardiovascular causes take place suddenly due to fatal ventricular arrhythmias [1]. Similar to previous studies, we observed an age-associated increase in the incidence of ventricular arrhythmias in the F344XBn rat [5-7]. Of interest is our finding that 33-months of acetaminophen treatment appeared to decrease the incidence of PAC and PVC (Figure 1). Recent data has implicated a link between the loss of the gap junction protein and cardiac dysfunctions.

Aging in the F344XBn heart is associated with changes in miRNA expression.

Fischer 344/NiaHsd X Brown Norway/BiNia (F344XBn) aging rat models, and other species [69-74]. A recent review of aging rat models concluded that the F344XBn rat strain seems to exhibit “physiological aging” to a greater degree than others [49,75,76]. Indeed, work has demonstrated that aging in the F344XBn is characterized by increases in cardiac ROS [7,46] and apoptosis [9]. Other data has suggested that acetaminophen may function as a potent antioxidant [42,43]. Given the possible linkage between increases in cardiac ROS and cardiac dysfunction, we postulated that ingestion of the acetaminophen would attenuate detrimental changes in the aging F344XBn heart. The main findings of this study were that chronic acetaminophen ingestion is capable of decreasing age-associated increases in cardiac arrhythmias and that aging in the F344XBn heart is associated with changes in miRNA expression.
Cx43 and fatal arrhythmias. This finding makes intuitive sense given that Cx43 is thought to be critical for maintaining normal conduction through the myocardium [77,78]. For example, using a heart-specific Cx43 conditional knockout mouse model in which cardiac Cx43 abundance decreases rapidly within the first few weeks of age, Danik and colleagues have demonstrated a decrease in cardiac conduction velocity and a dramatic increase in susceptibility to inducible lethal ventricular tachyarrhythmias [79]. To ascertain if alterations in Cx43 levels may play a similar role in the aging F344XBN heart we examined the regulation of Cx43 using immunoblot analysis. The expression of Cx43 is significantly lowered with aging and acetaminophen treatment is able to slightly decrease the attenuation of Cx43 expression in treated animals (Figure 2A). This finding is consistent with previous work investigating the effects of aging on Cx43 [80]. Interestingly, acetaminophen treatment gave rise to a slight, but significant increase, in the amount of Cx43 expression. Further, immunohistochemical analysis revealed that the distribution of membrane Cx43 appears to be morphologically well preserved at the intercalated discs in the young 6-month animals. Conversely, the cytosolic Cx43 immunoreactivity was markedly lower at the intercalated disc regions of the membrane in 33-month control rat hearts and appeared to be clustered inside the fibers of old hearts (Figure 2B).

Although these results, taken together, suggest that chronic acetaminophen ingestion may be able to modulate the expression of Cx43 it is unlikely that the magnitude of the differences we see in the regulation of Cx43 can, by itself, explain the decrease in age-associated arrhythmias we observed in the acetaminophen treated animals. Indeed, previous data has suggested that Cx43 protein losses of up to ~40% are insufficient by themselves to induce cardiac arrhythmias [79]. How acetaminophen may decrease cardiac arrhythmias in the aging F344XBN heart is presently unclear. Given the putative link between cardiac ROS levels and arrhythmias [81], it is likely that the anti-oxidant capacity or due to alterations in signaling pathways that control cardiac fibrosis warrants further investigation.

And also whether the decrease in cardiac fibrosis is directly responsible for the diminished cardiac arrhythmias we observe in acetaminophen treated rats is not clear at this time.

Aging in the F344XBN heart is associated with decreases in mir-1, mir-133, mir-214, mir-30a and mir-30d

Previous work by our laboratory and others have demonstrated that aging in the male F344XBN is associated with increases in heart mass, left ventricular hypertrophy, increased incidence of cardiac arrhythmias, decreased ventricular compliance and impairment in systolic and diastolic function [5,6] as well as increased expression of oxidative-nitrosative stress [7] and cardiomyocyte apoptosis [83]. Using semi-quantitative real time RT-PCR we demonstrate decreases in the amount of mir-1, mir-133, mir-214, mir-30a and mir-30d with aging (Figure 4-6). The physiological significance of these findings awaits further experimentation. Previous data has suggested that mir-1 and mir-133 expression is upregulated, downregulated, and unchanged during cardiac remodeling [30,32,78,84-91]. Why aging may decrease the expression of mir-1, mir-133, mir-214, mir-30a and mir-30d levels is not understood. The divergent results likely reflect differences in disease models, extent of disease, myocardial regions sampled, assay platforms, normalization methods, sample size, control samples, and statistical analyses.

Recent reports have implicated mir-1 as participating in the etiology of ventricular arrhythmias as it is thought that this miRNA post-transcriptionally represses the expression of Cx43 [77,78]. Interestingly, the increase in arrhythmias we observe with aging are associated with decreases in Cx43 (Figure 3), and also decrease in mir-1 expression (Fig 4). This result is in opposition to the previous findings suggesting that decreases in mir-1 expression will lead to increase in Cx43 expression. Indeed, the role that mir-1 plays in regulating Cx43 in the aging heart is still unclear as treatment associated increases in mir-1 expression (Figure 4) would lead one to predict that Cx43 levels would be decreased. Clearly this postulate was opposite to what we found as acetaminophen treatment led to slight yet significant increases in Cx43 expression. In addition to its role in regulating Cx43, it is also possible that mir-1 acts as an inhibitor of cardiac hypertrophy. For example, the expression of mir-1 in culture inhibits serum-induced hypertrophy and is associated with inhibited RasGAP, Cdk9 and fibronectin expression [89]. Recent data has also shown that cardiac-specific over expression of mir-1 in the embryonic heart inhibits cardiacmyocytes proliferation and prevents expansion of the ventricular myocardium [92]. As such, it is possible that the decrease in mir-1 we see with aging and increase in the aged acetaminophen treated animals may indicate a role for mir-1 to limit cardiac remodeling. However, the mechanism(s) underlying these alterations is currently unclear but may be related to the antioxidant properties of acetaminophen. Indeed, recent experiments have shown that the expression of several mir-RNA species can be modulated by changes in cellular ROS [93]. For example, the expression of mir-21 is increased following the exposure of cultured myocytes to increased hydrogen peroxide [94] while mir23b is down regulated in vascular smooth muscle cells following exposure to hydrogen peroxide [93]. Further experiments perhaps using other methods, pharmacological treatments or time points of observation will no doubt be useful in increasing our understanding.

Similar to many micro-RNAs, the role of mir-133 in cardiac remodeling has yet to be established. Recent work has demonstrated that increased mir-133 resulted in the suppression of protein synthesis and inhibition of hypertrophic growth in cultured neonatal cardiac myocytes [30]. In this same study these authors also showed in mice that antisense RNA oligonucleotides targeting mir-133 resulted in cardiac hypertrophy and reinduction of the fetal gene program [30]. Other studies have suggested that mir-133 may act as antiapoptotic factor by repressing the translation of caspase-9 [36]. Given these postulates it is possible that the age-associated decrease in mir-133
we observe may play a role in the underlying mechanism for age associated left ventricular hypertrophy. Alternatively, mir-133 has also been implicated in the pathogenesis of cardiac arrhythmias as it has been shown that this molecule can regulate the ether-a-go-go related gene (ERG) which encodes a key portion of the cardiac potassium channel [95]. Whether the changes in mir-133 we observe are related to factors controlling cardiac hypertrophy or cardiac rhythm will require further investigation. Recent studies have also implicated a role for mir-30 and mir-133 in the control of cardiac fibrosis. Liu et al., showed that mir-133 knockout mice develop severe fibrosis and heart failure [87]. Recently, Duisters et al., investigated the regulation of connective tissue growth factor (CTGF), a key molecule in the process of fibrosis at the post transcriptional level and showed that the expression of mir-133 and mir-30 was inversely related to the amount of CTGF in rodent models of heart disease and in human patients with pathological left ventricular hypertrophy [85]. Similar to their findings the age-related increases in fibrosis we observe in F344XBN rat hearts is associated with diminished expression of mir-133, mir-30a and mir-30d (Figures 4 and 5). Conversely, the diminished myocardial fibrosis we see in acacetaminophen treated aged rats is not associated with any alterations in the expression of mir-133, mir-30a and mir-30d. The role of mir-214 in cardiac remodeling is not yet fully investigated. Recent study suggests that mir-214 is unregulated during pathological hypertrophy; however, transgenic mice over-expressing mir-214 caused no abnormal phenotype in the heart [32]. Our results indicate that mir-214 expression significantly decreases with age in aged control rats and remain unchanged in acetaminophen treated aged rats compared to the young 6-month animals (Figure 6). Why expression of mir-214 is altered differently in aged hypertrophied hearts compared to other models of pathological hypertrophy remains unclear and needs further investigation.

In conclusion, the results of this study have shown that chronic acetaminophen treatment decreases the incidence of age-associated arrhythmias and that this alteration is associated with increased Cx43, decreased myocardial fibrosis and alterations in cardiac miRNA expression. Given that acetaminophen is well tolerated in humans, FDA approved for over the counter sale, economical, and widely available, different animals and methods may be warranted to expand upon these observations.

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