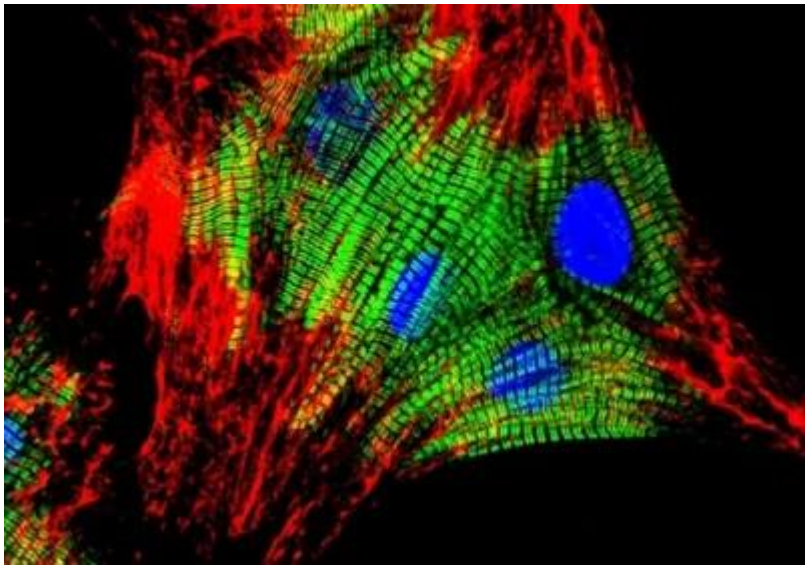
In mRNA-1273 (Moderna) and BNT162b2 (Pfizer/Biontech) COVID-19 vaccines, a messenger RNA (mRNA) sequence determines the structure and assembly of the immunogen, the SARS-CoV-2 spike (S) glycoprotein. The mRNA is protected from degradation by lipid nanoparticles (LNPs) and taken up by the cells as an LNP-mRNA complex through simple endocytosis. In this *in vitro* study, the authors from Germany and Hungary investigated the effects of mRNA-1273 and BNT162b2 vaccines on the function, structure, and viability of isolated rat left ventricular cardiomyocytes. Additionally, the human AC16 cardiomyocyte cell line of ventricular origin was used to study the uptake of LNP-mRNA complexes and the translation of the encoded S protein.

The authors emphasized that inadequate cardiac safety testing and exploration of the adverse event profile of mRNA vaccines led to adverse cardiac events, classified as myocarditis and/or pericarditis. A common theory about the underlying pathophysiological mechanisms of myocardial injury is a possible immunological cross-reaction. It was suggested that antibodies directed against epitopes of the S protein, as a result of vaccination, may also react with epitopes of the α-myosin heavy chain. The authors, however, point out that the α-myosin heavy chain is a sarcomeric protein almost exclusively expressed in atria and cannot directly interact with circulating antibodies due to its intracellular localization.



About the study

The scientists investigated the direct effect of mRNA-1273 and BNT162b2 COVID-19 vaccines on the function, structure, and viability of isolated rat cardiomyocytes over 72 hours. The cellular and molecular effects of the two mRNA vaccines were studied on the left ventricular cardiomyocytes isolated from the hearts of three-month-old male Wistar rats. At a stimulation frequency of 2 Hz, the function of cardiomyocytes was analyzed by determining the relative cell shortening, contraction velocity, and relaxation velocity. Since this model allows accurate quantification of myocyte contraction parameters up to 48 hours after isolation, the 'functional state' after 72 hours was estimated qualitatively.

Confocal laser scanning microscopy was used to image the sarcomere structure of isolated cardiomyocytes. The uptake of LNP-mRNA complexes and the translation of the encoded S protein were investigated in the AC16 human cardiomyocyte cell line of ventricular origin.

Results

The effects of mRNA-1273 and BNT162b2 on rat cardiomyocytes

After 24 h, no functional or morphological differences were detected between cells treated with mRNA-1273 or BNT162b2 and untreated control cells. The culture dishes incubated with mRNA-1273/BNT162b2 and untreated controls showed regularly contracting cells (percentages of 75% and 77%, respectively).

After 48 hours of incubation with mRNA-1273 or BNT162b2, the sarcomere structure examination revealed no irregularities in the structure of the parallel myofibrils in any group.

The effects of mRNA-1273 vaccine

After 48 h of incubation with mRNA-1273 vaccine, the number of contracting myocytes decreased and quantification of contraction parameters was no longer possible. The mRNA-1273 vaccines induced arrhythmic and completely irregular, partially 'peristaltic' contractions of myocytes. Myocardial cells that contract regularly were reduced to only 10%, whereas the percentage of arrhythmic and irregularly beating cells was approximately

52%.

The analysis of the calcium transients in cardiomyocytes treated with mRNA-1273 showed arrhythmic, localized, and irregular transients.

According to the authors, arrhythmic and completely irregular contractions detected after incubation with mRNA-1273 vaccine, together with the irregular and localized calcium transients, indicate significant dysfunction of ryanodine receptor 2 (RyR2) and direct impairment of sarcoplasmic reticulum-dependent calcium release. The RyR2 regulates the calcium release from the sarcoplasmic reticulum in cardiomyocytes and plays an integral role in excitation-contraction coupling. Numerous studies have identified that dysfunction of RyR2 causes arrhythmias, ventricular tachycardia, and sudden cardiac death.

Reduced doses of the mRNA-1273 to 1.0 µg/ml failed to improve these results. A further reduction to 0.3 µg/ml increased the proportion of myocardial cells that regularly contract, but it was only transiently effective within 48 hours.

After 72 hours, cells incubated with mRNA-1273 vaccine almost completely ceased their function. The control dishes had 41% contracting cardiomyocytes.

The effects of the mRNA BNT162b2 vaccine

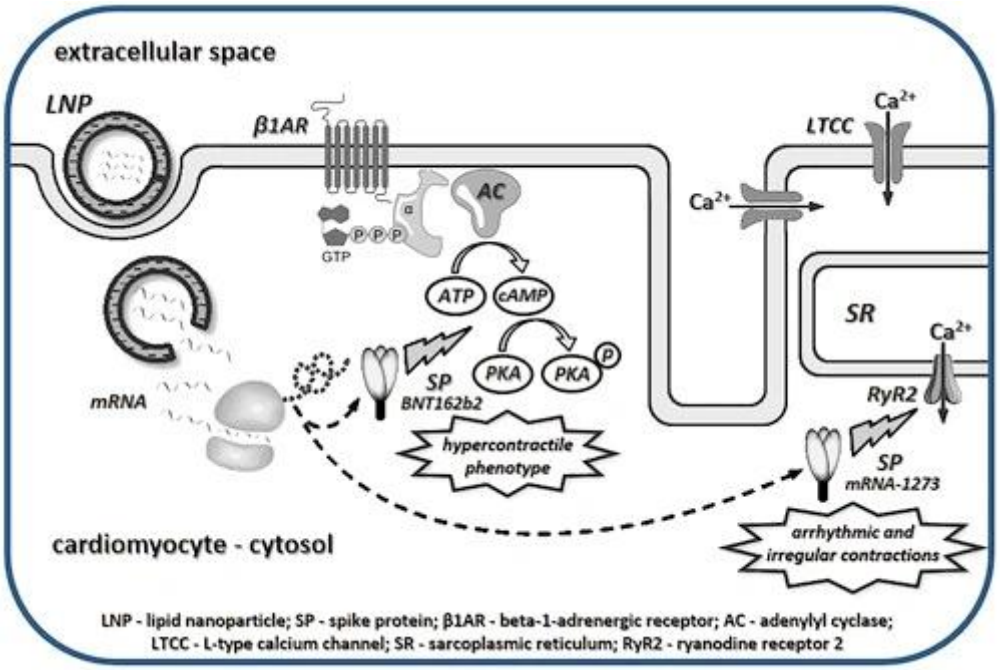
After 24 or 48 hours, cells treated with BNT162b2 contracted rhythmically and uniformly. However, they exhibited an increase in relative cell shortening (+22.6%), contraction velocity (+31.9%), and relaxation velocity (+32.1%) compared to control myocytes.

The analysis of the calcium transients revealed a rhythmically and uniformly detectable systolic release and diastolic decrease of calcium in BNT162b2-treated cells and untreated control myocytes.

As positive inotropic (enhanced contractility) and lusitropic (faster relaxation) effects are mediated mainly through the activation of protein kinase A (PKA), researchers evaluated myocyte PKA activity. After 48 h of incubation with BNT162b2, myocyte PKA activity increased to levels comparable to those in untreated cells stimulated with isoprenaline, an agonist of β-adrenoceptors. The BNT162b2 vaccine significantly increased PKA activity, the intensity and dynamics of calcium transients in BNT162b2-treated myocytes, and all contraction parameters described above. The authors stated that cell contraction pattern and sustained PKA activation that developed after the BNT162b2 application were largely

consistent with the functional changes found in cardiomyocytes (or myocardium) after the catecholamine stimulation and could correspond histopathologically to catecholamine-induced cardiomyopathy. The permanent stimulation of β-adrenergic signaling mechanisms increases the energy demand of the myocardium.

After 72 hours of incubation with the BNT162b2 vaccine, the proportion of beating myocytes decreased to 27% compared to untreated controls. In contrast, mRNA-1273 had no impact on PKA activity.



Original figure from the article of Schreckenberg R, et al. Br J Pharmacol. 2024;181:345–361.

The uptake of mRNA and the translation of the vaccine-encoded S protein in rat or human cardiomyocytes

The analysis of LNP-mRNA complex uptake and translation of the encoded S protein in rat cardiac tissue showed that mRNA was detected in all sections of the left and right ventricular myocardium, the septum, and both atria

Cell-specific uptake was found in cardiomyocytes and non-myocytic rat cells, such as endothelial cells and fibroblasts. The fraction of non-myocytic cells consistently showed higher mRNA levels than myocyte fraction. After 48 h of incubation with mRNA-1273 or BNT162b2 vaccines, positive results were found exclusively for the intracellular fraction of treated cells, whereas the supernatant was always negative.

The mRNA uptake and the translation of the encoded S protein were also investigated in the AC16 human cardiomyocyte cell line of ventricular origin. The results showed efficient uptake of LNP-mRNA complexes and the translation of the encoded S protein. In contrast to results found in isolated rat cardiomyocytes, the S protein was detected in both, the cell fraction and supernatant of the human cardiomyocyte cell line. The authors noted that this cell culture system does not permit any definitive conclusions regarding the duration or extent of the S protein production, however, all results for AC16 cells point to a highly efficient and potentially long-lasting translation.

Conclusion

In this study, mRNA-1273 and BNT162b2 COVID-19 vaccines were shown for the first time to induce cardiotoxic effects with disturbances of normal contractile function in rat cardiomyocytes. The effects of the vaccines differed fundamentally in their pathophysiological mechanisms, which were very specific.

The mRNA-1273 vaccine induced arrhythmic and irregular contractions by largely disrupting sarcoplasmic calcium release, whereas the BNT162b2 vaccine induced a cell contraction pattern by chronic activation of PKA, consistent with catecholamine-induced cardiomyopathy. Impairment of RyR2 and persistent activation of PKA are risk factors for sudden cardiac death, ventricular tachyarrhythmias, and contractile dysfunction. Both mechanisms provide a possible explanation for persistent cardiac symptoms observed in individuals diagnosed with long COVID syndrome.

The cardiotoxic effects and functional disturbances induced by mRNA-1273 and BNT162b2 are pathophysiologically consistent with cardiomyopathy, and in contrast to the clinically diagnosed adverse effects, predominantly categorized as myo-and/or pericarditis.

The authors suggested that the effects of mRNA-1273 and BNT162b2 vaccines on the function of the cardiac conduction system cells, which are histologically distinct from working myocardial cells, should be investigated in further studies. They also recommended

that the risk-benefit ratio of mRNA-based vaccines should be re-evaluated, taking into account these results and the hidden cardiotoxic effects of the vaccines. In addition, these results should be considered in the future diagnosis and treatment of cardiac symptoms associated with mRNA-based COVID-19 vaccination.

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Journal Reference

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