

## Combined effects of ionizing radiation and cardio-active drugs on human iPSC-derived cardiomyocytes\*

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### Motivation

There are multiple cardio-active pharmaceuticals available on the market, extracting various electrophysiological effects upon the human heart. These effects are well known and well characterized [1]. However, there is virtually no information on the combined effects of applied heart pharmaceuticals in conjunction with ionizing radiation. A deeper insight in these effects, however, is of great interest for occupationally exposed groups, heart patients receiving radiation treatments, as well as astronauts. Therefore we examined the influence of X-rays on drug-treated human induced pluripotent stem cell (hiPSC)-derived cardiomyocyte cultures. As a test substance isoproterenol, an analogon of the stress hormone adrenaline, was applied. The applied X-ray dose was 2 Gy. Electrophysiological parameters of the cultures were measured using microelectrode array (MEA) technology for 7 days after radiation exposure. Our results show an increase in the beat rate enhancing property of isoproterenol one day after irradiation. Direct radiation effects on the drug itself could be excluded. This poses many questions on the combined effects of ionizing radiation and stress response.

### Materials&Methods

Human induced pluripotent stem cell (hiPSC) –derived cardiomyocytes (Cellular Dynamics, Madison, WI, USA) were cultured on microelectrode array chips (Multichannel Systems, Reutlingen, Germany) for 5 days prior to the experiment (Figure 1). Electrophysiological properties of the cell cultures were measured before and after exposure. Cardiac signals could be recorded for 7 days after treatment and were analyzed in terms of beat rate, conduction velocity, field action potential duration and general spike shape, using the MATLAB-based software DrCell [2]. X-irradiation was performed with 250 kV at 16 mA at a dose rate of 2 Gy/min. First only culture medium supplemented with 500 nM isoproterenol was irradiated with 2 and 7 Gy of X-ray and subsequently applied to the cardiomyocytes. Effects of the irradiated chemical on the cells were monitored for one day. Then cultures were treated with 500 nM of isoproterenol and subsequently irradiated with 2 Gy X-rays. Electrophysiology was then monitored for another 7 days.

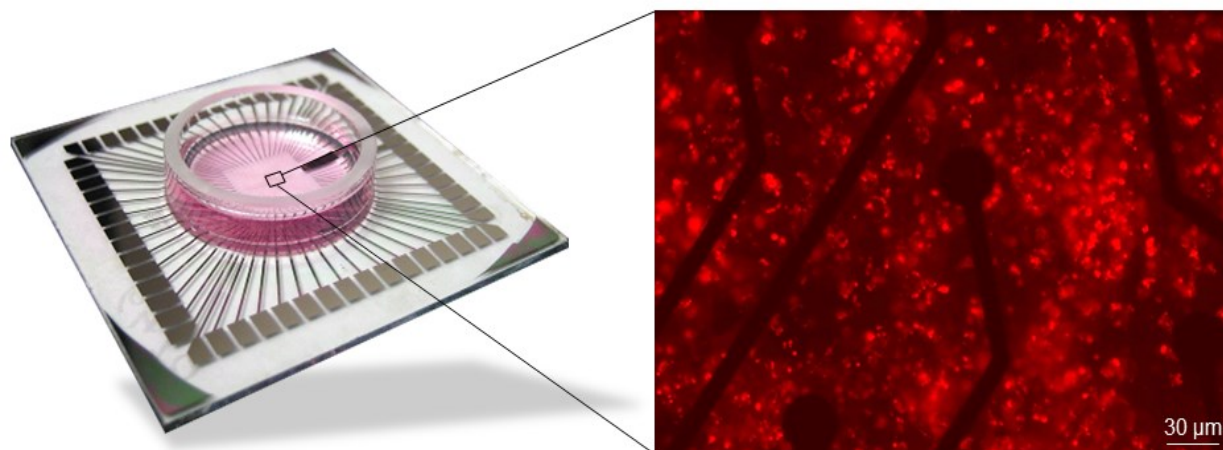


Figure 1: Human induced pluripotent stem cell-derived cardiomyocytes cultured on a microelectrode array at a 100× magnification. Myosin heavy chains are genetically marked with red fluorescent protein. Microelectrodes have a diameter of 30 µm.

## Results

In a study performed by Guo et al., it was shown that isoproterenol, has a beat rate enhancing effect on hiPSC-derived cardiomyocytes [3]. To examine if ionizing radiation alters the effectiveness of isoproterenol, culture medium containing 500 nM isoproterenol was irradiated with 2 and 7 Gy, respectively. These irradiated media were administered to the cultures and electrophysiology was monitored for 1 day. The experiments showed that irradiation with up to 7 Gy X-rays had only minor effects on the beat rate enhancing property of the pharmaceutical, 1 day after application (Figure 2).

After exclusion of possible radiation-induced effects on the chemical, cardiomyocyte cultures were first treated with isoproterenol and subsequently irradiated with 2 Gy X-rays. The cultures were measured daily for seven days after exposure. One day after treatment, the beat rate of samples exposed to 2 Gy X-ray and 500 nM isoproterenol increases above all other samples (Figure 3).

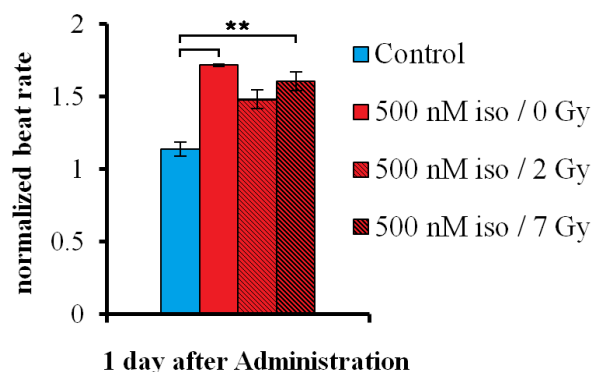


Figure 2: The beat rate enhancing property of isoproterenol is only marginally affected by X-irradiation, 1 day after administration. Data represent medians normalized to the controls before treatment. Error bars represent standard error.  $N_{\text{controls}} = 6$ ;  $N_{\text{isoproterenol } 0; 2; 7 \text{ Gy}} = 3$  (statistics were performed by two-tailed unpaired t-test, CI = 95%,  $**P < 0.01$ ).

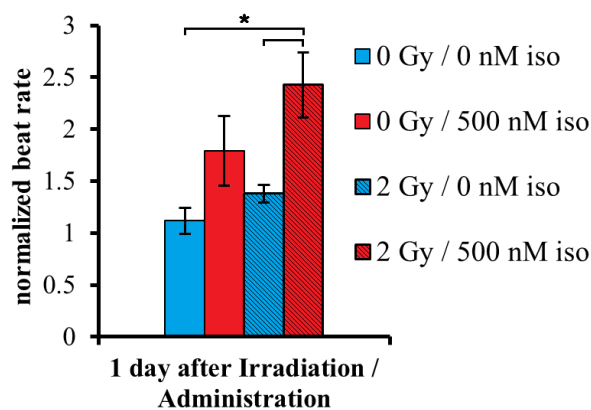


Figure 3: X-irradiation further enhances the beat rate of cardiomyocytes treated with 500 nM isoproterenol, one day after irradiation. Data represent medians normalized to the controls before treatment. Error bars represent standard error.  $N = 5$  (statistics were performed by two-tailed unpaired t-test, CI = 95%,  $*P < 0.05$ ).

## Summary

Our data indicate that the exposure to ionizing radiation of cardiomyocytes pre-treated with isoproterenol may further increase the enhanced beat rate of cardiomyocytes. This effect possibly harbours yet unknown threats to people occupationally exposed to ionizing radiation and are either taking heart pharmaceuticals or are exposed to stress, as isoproterenol chemically resembles the stress hormone adrenaline.

## References

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