To characterize and predict the QT interval prolongation and proarrhythmia potential of drug candidates, investigations into the challenges of using multi-electrode array system and human stem cell-derived cardiomyocytes are on-going. Sometimes, discussions arise on whether assay results should be interpreted using total (applied) or unbound concentrations to understand the compounds cardiovascular liabilities. To provide useful suggestions, we measured the unbound concentrations of 59 drugs which include CiPA selected 29 compounds in the iCell Cardiomyocytes Maintenance Medium (CMM, Cellular Dynamics International) including fetal bovine serum by equilibrium dialysis method using Rapid Equilibrium Dialysis device (ThermoFisher Scientific). Then, unbound fraction (fu) values at low and high concentrations (100-fold differences) in CMM were calculated. When saturation of binding at the high concentration was observed, an additional experiment was done at a middle concentration. Most of compounds showed similar fu values at low and high concentrations. On the other hand, several compounds such as Mibefradil, Thioridazine, Bepridil, Prenylamine, Tolterodine, Haloperidol, and Dronedarone showed more than 2-fold higher fu values at high concentrations than those at low ones. Tamoxifene and Amiodarone showed very low fu values (less than 0.01) which indicated very strong protein binding. Further analysis of the relationships between the fu values in CMM and human plasma was conducted. Good correlation in rank order with non-linier correlation was obtained. These results indicated that the actual unbound concentrations in CMM will be very useful to consider and interpret the relationship to unbound plasma concentrations which caused CV events in human.