Dear Editor,

We received the letter by Rainiers et al regarding our previous work (1) with mixed feelings. We are grateful to these authors for bringing to our attention the misspelling of the reagent used to monitor hepatic oxidative stress in vivo during ischemia/reperfusion (I/R) by two photon confocal imaging. However, we regret that 2′,7′-dichlorofluorescein appeared in the experimental procedures when in fact we actually used 2′,7′-dichlorofluorescein diacetate (Sigma, catalogue number D6883), also known as 2′-7′-dichlorodihydrofluorescein diacetate, a cell-permeable non-fluorescent probe that is de-esterified intracellularly and turns to highly fluorescent 2′,7′-dichlorofluorescein upon oxidation, used to ultimately detect ROS/RNS. We hope this unfortunate misspelling may have not confounded or mislead researchers working in I/R liver injury.

We appreciate the effort by Rainiers et al to confirm the inability of 2′,7′-dichlorofluorescein to detect ROS/RNS, although most investigators working in the field of oxidative stress for almost 30 years (2) know that 2′,7′-dichlorofluorescein diacetate or derivatives such as chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (Invitrogen, catalogue number C6827) are the reduced substrates to be used in these experiments, as reported in an outstanding number of papers, including ours (3-6). While we apologize for the mistake, we regret the attempt of Rainiers et al to invalidate the conclusions of our study based in the misspelling on the name of the probe, not in the protocol used. In the confocal microscopy data we observed a clear mitochondrial depolarization monitored with TMRM following I/R, particularly in mice exhibiting increased cholesterol loading. More important, the degree of hepatocellular damage monitored by transaminases and H&E staining increased substantially following I/R in
mice exhibiting high hepatic cholesterol content, which was reduced by strategies that decrease cholesterol loading, such as statins. We believe our results report novel findings in the field of liver I/R injury, which may have important clinical implications for the near future. Finally, we agree with Rainiers et al that care should be taken to avoid mistakes or misspellings in research articles. In this regard, we welcome the initiative of some journals to include detailed description of commercial products used in the study such as the catalogue or CAS number, which certainly will minimize future confusions.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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