Lucigenin Chemiluminescence Assay for Superoxide Detection

To the Editor:

The recent review by Tarpey and Fridovich published in Circulation Research mostly contains interesting and sound material; however, I would like to comment on the section entitled, “Superoxide Detection, Chemiluminescence Reactions.” Basing mainly on their own data, authors maintain that lucigenin-amplified chemiluminescence (LucCL) overestimates the rate of superoxide production due to the redox cycling of lucigenin. Taking into account that LucCL is the most sensitive method of superoxide detection, which is especially useful for the detection of low superoxide concentrations in vascular systems (see, for example, References 3 through 5), such an assertion (if true) devalues numerous previous experimental findings.

Fortunately, it is not true. And our conclusion is not due to “the triumph of hope over reality” (page 229) but is based on reliable experimental data. We have shown earlier that lucigenin cannot take part in redox cycling with molecular oxygen because of its positive one-electron reduction potential (about 0.19 V). (Spasojevic et al recently measured the two-electron reduction potential of lucigenin as −0.14 V. However, it was shown long ago that two-electron reduction potentials are not equal to one-electron reduction potentials.) In addition to theoretical considerations, it is possible to demonstrate the reliability of the LucCL method on the basis of experimental data. We compared the results of superoxide measurement by LucCL and cytochrome c reduction in several enzymatic and cellular systems, and we found out that there are excellent correlations among the results obtained for the xanthine- and NADH-xanthine oxidase systems, neutrophils, and monocytes (correlation coefficients are of 0.930 to 0.994). More than that, we showed that if the direct reduction of lucigenin takes place (for example, in NADH-xanthine oxidase system), it will result in a decrease, and not an increase, in superoxide production due to the competition between one-electron reduction of oxygen and lucigenin.

Hence, we believe that lucigenin-amplified chemiluminescence remains a reliable and sound assay of superoxide detection.

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