Preeclamptic Superoxide-Anion Production: Is There an Increase or a Failure of Reduction?

To the Editor:

We read with interest the article by Tsukimori et al\textsuperscript{1} analyzing the relationship between the superoxide-anion (O$_2^-$) production of neutrophils during pregnancy in women with preeclampsia and essential hypertension. The results of the study demonstrated that n-formyl-methionyl-leucyl-phenylalanine stimulated O$_2^-$ production of neutrophils during pregnancy was increased in women with preeclampsia compared with healthy pregnant women. There was no significant difference in the neutrophil O$_2^-$ production in normal pregnancy between pregnant and postpartum women. In association with this, the authors' previous study shows that there is no significant difference in O$_2^-$ production in nonpregnant and healthy pregnant women.\textsuperscript{2}

Several studies take up a question with the O$_2^-$ production in pregnancy and preeclampsia, but the results are contradictory. Our unpublished results come to the conclusion that O$_2^-$ production of neutrophils (stimulated by n-formyl-methionyl-leucyl-phenylalanine or phorbol-12,13-dibutyrate) in women with preeclampsia is significantly increased compared with healthy pregnant women; and in normal pregnant women, the granulocytes’ O$_2^-$ production significantly decreases compared with nonpregnant and postpartum women concordant with the Crocker et al\textsuperscript{3} study presented earlier. Furthermore, in our examinations, there was no significant difference in O$_2^-$ production between nonpregnant and preeclamptic pregnant women. The average O$_2^-$ production of the granulocytes in nonpregnant women was 1.184 nmol/min per 3 $\times$ 10$^5$ cells, in healthy pregnant women was 0.472 nmol/min per 3 $\times$ 10$^5$ cells, and in preeclamptic patients was 1.106 nmol/min per 3 $\times$ 10$^5$ cells, stimulated by n-formyl-methionyl-leucyl-phenylalanine. These discrepancies give a chance for contradictory conclusions.

We assume that the cause of this deviation is the HEPES buffer, used by the authors for >1 hour at 37°C for incubating neutrophils. Incubation of cells in HEPES buffer for >60 minutes or less, depending on protocol conditions, could exposed cells to a nonphysiological surrounding toward the end of the incubation period. The nonphysiological environment includes the alkaline pH, toxic, and pharmacological effects as principals. HEPES interferes with the function of some ion channels, including calcium transport, which is important in O$_2^-$ production.\textsuperscript{4,5} Contrarily, the Hank’s buffer, used by us, is of a very simple composition and does not cause such a nonphysiological environment.\textsuperscript{5}

We would like the authors of the statement to respond to our concerns. The number of studies in connection with O$_2^-$ is increasing even in obstetrics. Minute differences in laboratory methods may result in contradictory conclusions. A standardized method might be helpful to solve this problem.

Disclosures

None.

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