Letters to the Editor

Measuring DNA Damage Among Smokers

To the Editors: Ellahueñe et al. (1) wrote in the abstract that ''The objective of this study was to establish if the alkaline SCGE assay in whole blood cells gives similar results as the same method in isolated lymphocytes, because whole blood cells are simpler and more economical to use, specifically in human genotoxic biomonitoring.'' It sounds strange because either isolated lymphocytes or whole blood is indicated as a source of human cells in the widely cited protocol of SCGE assay, implying that both options are equally suitable for biomonitoring (2). Successful application of whole blood cells in the comet assay was well documented—I randomly found some articles from Medline concerning both humans (3-5) and mice (6).

The data concerning the influence of smoking on DNA damage of blood cells are very contradictory (7). Two recently published articles showed opposite results (8, 9). In both investigations the subjects smoked about 13 cigarettes per day, but in the first one the age of the subjects was 28.9 years (20 males), whereas in the other it was 36 years (5 males and 5 females). It is well known that the brand and the number of cigarettes consumed, diet, and geographic variation in smoking habit can be important variables in investigations on DNA damage in cells of smokers (7). The authors (1) studied only six smokers, but data on the number of cigarettes consumed per day and the gender are absent. Hence, very important data are not presented on these factors which should be considered in the study of DNA damage (7).

The authors wrote that ''the tail moment, expressed in arbitrary units, was calculated as: (tail length × percentage of migrated DNA) / 100.'' In Table 1 the data on three mentioned variables are presented. Calculations have shown that all data on ''Tail moment'' do not correspond to other data. For example, ''0 sampling time'' 0.15 (tail length) × 0.03 (DNA%) = 0.0045. According to the authors' definition, it should be divided by 100. However, the authors presented another figure as tail moment—0.54. I guess the sign (/ 100) is wrong and should be (× 100). But again in this case ''Tail moment'' is 0.45, and not 0.54. Another example: sampling time 16 hours, CP—0.19 × 0.1 = 0.019 × 100 = 1.9. However, the authors indicated 3.37! It is extremely strange that all the digits on tail moment do not correspond to the calculations!

In my opinion, the article has a lot of shortcomings that need to be clarified.

Armen K. Nersesyan
Environmental Toxicology
Institute of Cancer Research
Borschkegasse 8a
Vienna, Austria

References

1. Ellahueñe MF, Perez-Alzola LP, Farfan-Urzua M, et al. Preliminary evaluation of DNA damage related with the smoking habit measured by the comet assay in whole blood cells. Cancer Epidemiol Biomarkers Prev 2004;13:1223–9.
2. Collins AR, Dusinska M. Oxidation of cellular DNA measured with the comet assay. In: Armstrong D, editor. Methods in molecular biology, vol 186. Totowa, NJ: Humana Press; 2002. p. 147–60.
3. Danadevi K, Rozati R, Saleha Banu B, Hanumanth Rao P, Grover P. DNA damage in workers exposed to lead using comet assay. Toxicology 2003;187: 183–93.
4. Garaj-Vrhovac V, Kopjar N. The alkaline Comet assay as biomarker in assessment of DNA damage in medical personnel occupationally exposed to ionizing radiation. Mutagenesis 2003;18:265–71.
5. Garaj-Vrhovac V, Zeljezic D. Assessment of genome damage in a population of Croatian workers employed in pesticide production by chromosomal aberration analysis, micronucleus assay and Comet assay. J Appl Toxicol 2002;22:249–55.
6. Devi KD, Banu BS, Grover P, Jamil K. Genotoxic effect of lead nitrate on mice using SCGE (comet assay). Toxicology 2000;145:195–201.
7. Moller P, Knudsen LE, Loft S, Wallin H. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. Cancer Epidemiol Biomarkers Prev 2000;9:1005–15.
8. Speit G, Witton-Davies T, Heepchantree W, Trenz K, Hoffmann H. Investigations on the effect of cigarette smoking in the comet assay. Mutat Res 2003;542:33–42.
9. Hininger I, Chollat-Namy A, Sauvaigo S, et al. Assessment of DNA damage by comet assay on frozen total blood: method and evaluation in smokers and non-smokers. Mutat Res 2004; 558:75–80.

In Response: In response to the Letters to the Editor concerning our article ''Preliminary evaluation of DNA damage related with the smoking habit measured by the comet assays in whole blood cells,'' we wish to clarify some points:

1. Although we know that isolated lymphocytes or whole blood has been used in widely cited protocols of SCGE, we used a modified experimental design with three sample times for each individual.
2. In relation to the additional references suggested in the letter, we would point out that, due to the great number of articles that have been published using the comet assay, we selected only some of those articles; we are sure that we unintentionally omitted some interesting ones. With respect to refs. 8 and 9, from 2003 and 2004, respectively, they were published later than the date we sent the article to the journal (November 19, 2003).
3. We are completely in accord with the asseveration that ''The data concerning the influence of smoking on DNA damage of blood cells are very contradictory.'' That is the reason for our study. Knowing that the age, the gender, the diet, etc., are important variables for DNA damage, we selected only healthy, young (19-23 years old) male smokers (gender was stated in Introduction) who smoked at least 10 cigarettes per day (and not >20) to homogenize the sample for this study.
4. Data from Table 1 are not really in error, rather there is a misunderstanding. The direct calculations the writers of this letter made might give the wrong results because the tabulated data are the mean values for the seven mice used at each time. For each animal, we scored 20 cells, for each cell we calculated tail length, DNA percentage, and tail moment and then calculated the means of 20 cells for each variable in each mouse. Finally, we calculated the mean for each time (seven mice). This was explained in the review process and we added a paragraph in Results and changed the Table 1 legend to explain this; but apparently, this is still confusing. We apologize to any readers who were confused by the format of data presentation in our article.
5. Finally, it is true that the sign ''/100'' is wrong; the correct sign is ''×100.'' We apologize for this error and thank the letter writers for correcting this mistake.

We thank the writers of this letter for their interest in our work and hope the additional information we have provided is helpful to people who have read this article.

Manuel F. Ellahueñe