

## Reply to Letter to the Editor

### Response to comments on ‘Cytogenetic effects in children treated with methylphenidate’ by El-Zein et al.

We are writing in response to the comments of Preston et al.<sup>1</sup> regarding our paper entitled ‘cytogenetic effects in children treated with methylphenidate [1]’.

We certainly agree with the authors that the provocative findings of our study could have far reaching implications for the assessment of adverse outcomes from methylphenidate treatment. Also, it is important to state that we agree that further work is necessary before a definitive conclusion as to the genotoxicity of this drug can be drawn. In fact, this is consistent with our point of view presented in the Discussion section of our paper. We specifically noted that the study ‘should be replicated and expanded by further studies in a larger population before a definitive conclusion about the genotoxicity of methylphenidate can be attained’.

While we also agree with Preston et al. that there is published literature on the general toxicity of methylphenidate, we point out that specific studies on the genotoxicity and on the carcinogenicity of this drug, particularly in humans, are very limited. The available studies, reviewed in the recent national toxicology program (NTP)—CERHR expert panel report on the reproductive and developmental toxicity of methylphenidate [2], indicate that only one study addressed the carcinogenic risk of methylphenidate treatment in humans [3]. While this limited epidemiological study, conducted by screening pharmacy and medical records, indicated that there was no increase in reports of cancer in a small number of patients taking methylphenidate (only 529 patients), it is imperative to point out that the authors themselves urged caution in the interpretation of their findings because the small sample size for

this type of study limited the power to detect modest increases in cancer, and the study covered a relatively short time period [2,3].

As for the animal carcinogenicity studies reviewed in detail in the NTP-CERHR expert panel report [2], first, we point out that the results of the mouse study with methylphenidate indicated that male and female mice treated with the drug developed hepatocellular adenomas and carcinomas, and the incidence of hepatoblastoma, a rare neoplasm, was also increased in treated male mice. As clearly stated in the NTP-CERHR report, and based on results of the mouse study, it was concluded that ‘there is some evidence of carcinogenic activity of methylphenidate hydrochloride in male and female B6C3F1 mice’ [2]. Second, we emphasize that the B6C3F1 mouse is the standard mouse model used by the NTP in their studies to determine the carcinogenic risk of chemicals. Based on increases in hepatic neoplasms in this particular animal model, many chemicals have been classified as carcinogens by NTP. Examples of these chemicals include tetrachloroethylene (perchloroethylene), pyridine, tetrahydrofuran, and many other chemicals studied for carcinogenicity by NTP [4].

Taken together, it could be concluded that methylphenidate is carcinogenic in mice in a standard NTP cancer bioassay study, and it remains to be determined whether this drug is potentially dangerous with prolonged use in children. In our study, we decided to address this issue using multiple cytogenetic endpoints that ensured the detection of different types of genetic damage, and allowed for a better understanding of the underlying mechanisms of genetic damage associated with methylphenidate treatment.

As for the concerns mentioned regarding the design of our study, our pre- and post-treatment design should, in fact, be considered a notable strength. It is an excellent approach for assessing, in a small population, the potential effects of a treatment, as correctly pointed out by Preston et al. This design allowed each child to serve as his or her own control and avoided possible inter-individual confounders that could have been

<sup>1</sup> The Original Article and Letter to the Editor were both published in Cancer Letters Volume 230 Issue 2, pages 284–294.

difficult to evaluate if a case-control study design had been adopted. As such, our design permitted any increases in the endpoints measured in the short time of the study to be more directly attributed to the treatment. The close follow-up and frequent assessment of the patients by their pediatrician during the three-month period of the study allowed for monitoring of any potential individual confounders that might have occurred during the course of the treatment. In the population studied, there were no observed changes in the participants' health or environment that might have caused such an increase in the cytogenetic endpoints measured. Similarly, no changes in life-style habits, including dietary habits or a drastic change in weight of the participants was noted. As for the issue of not considering individuals who were switched to other medications in the current study, we point out that the inclusion of this group would have had no clear value to the study objective, which focused on investigating the potential clastogenic effect of treatment of children with a specific drug, methylphenidate.

As for concern regarding the details of the methodologies used in the study, these are all standard procedures, and, because of the policy of the journal regarding space limitation, we could not describe already well-established techniques. However, for clarification purposes, we provide assurance that blood samples obtained from both treated and untreated subjects were cultured within 24 h following blood collection. Pre-treatment samples were not held for three months and cultured later with post-treatment samples. Therefore, the argument that a pre-treatment reduction in chromosomal damage could be a result of repair or cell death during sample holding does not apply to our study. We also add for further reassurance, that the scoring of the endpoints studied was conducted on coded slides that were blindly scored together with slides from other projects. This further increases our confidence in the results generated. As for the low pre-treatment background frequency of SCEs observed in some of our subjects, we are well aware that average background levels are generally somewhat higher. We point out, however, that most of the reports in the literature are generated from adult populations, and therefore, little is known regarding the average background levels of SCEs in a population of children such as ours. We agree with the authors that this is an intriguing finding that might be worth pursuing.

In closing, we believe that the fact remains that our study clearly indicates that methylphenidate is potentially clastogenic in children. This is documented by three different cytogenetic endpoints. A final

point that we would like to make is that, while we agree with Preston et al. that an increase in chromosomal aberrations in peripheral blood lymphocytes should not be interpreted as an increased risk of cancer for any 'particular individual', we point out that a causal link between increases in chromosomal aberrations and risk of cancer does indeed exist. This is well documented in several prospective cohort studies conducted in different populations [5–9] and summarized in recent reviews [10,11]. As such, we believe that the results of our study should be regarded as a cautionary sign, and as we mentioned in our publication, these results should open the door for additional, larger studies designed to establish the safety of both methylphenidate and other amphetamine-based drugs used for the treatment of attention deficit hyperactivity disorder.

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