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Scientific comment

on

Antioxidants can increase melanoma metastasis in mice

Le Gal et al. (2015) *Science Translational Medicine* 7(308), 308re8.

Le Gal and colleagues (2015) published a report in *Science Translational Medicine* investigating the effects of high oral doses of N-acetylcysteine (NAC) in drinking water on the formation of melanoma metastases in a triple-transgenic mouse model of chemical-induced melanoma. The authors report additional cell culture experiments with NAC and Trolox in melanoma cell lines. The findings of this paper attracted ample attention by the media, where the employed test compounds, the reported data and the conclusions drawn by the authors were inaccurately presented and unsubstantiated conclusions drawn and conveyed to the general audience. This false presentation of the report in the media led to unjustified concern in the general public.

The **Society of Nutrition and Food Science (SNFS)** therefore would like to summarize the most important findings from this work and evaluate the conclusions that can and cannot be drawn from the presented experiments.

Study design and major findings

Animal experiment

Le Gal and co-workers (2015) used a genetically modified mouse model and induced melanoma formation by application of a chemical onto the skin of the mice. After three weeks, tumour-bearing mice were randomized into a control group (n=14) and a group (n=17) receiving high doses of N-acetylcysteine (corresponding to 665 to 1330 mg/day for a 70 kg human) in their drinking water. No differences between groups were observed regarding the number and volume of primary tumours, but a statistically higher number of lymph node metastases was seen in NAC-treated (4 metastases per animal) compared to control mice (2 metastases per animal). The authors then determined glutathione (GSH) and the ratio of GSH to glutathione disulphide (GSSG, the oxidized form of GSH) in the primary tumours and lymph node metastases of four animals per group and found that the known GSH precursor NAC did not increase GSH or the GSH/GSSG ratio in the primary tumour, but in the lymph node metastases.

Cell culture experiments

Le Gal et al. (2015) then performed additional cell culture experiments in order to obtain insights into potentially underlying mechanisms. Seven melanoma cell lines were incubated with supraphysiological concentrations of NAC (200 µmol/L; maximum plasma concentrations in healthy humans given 1200 mg NAC were 2.9 µmol/L (Borgström & Kågedal 1990) and ca. 15 µmol/L when administered as effervescent tablets containing 600 mg NAC (Liu et al. 2010)) and Trolox (20 µmol/L) and cell proliferation, migration, and invasion were studied. Cell proliferation did not differ from control in NAC- and Trolox-treated cells, while migration and invasion were increased. Treatment with both compounds increased the GSH/GSSG ratio, which indicated enhanced de-novo synthesis of GSH. Blocking GSH biosynthesis with a chemical reduced the enhanced migration of the cultured melanoma cells to the level observed in untreated control cells. The redox state of the cells (the balance of oxidative and antioxidative processes) was not affected by incubation with high concentrations of NAC or Trolox. Two signalling molecules involved in the regulation of cell migration (RhoA and Rac1) were activated, compared to control, in one melanoma cell line incubated with NAC or Trolox (number of observations not specified). Signalling molecules involved in invasion phenotypes were not modified by incubation with NAC or Trolox in comparison to control cells.

What is the relevance of these findings for humans?

The test compounds studied are of little biological relevance in the context of nutrition

NAC is a drug known to act as a precursor of glutathione (GSH), an endogenous antioxidant, and might thus act as an indirect antioxidant through the induction of GSH biosynthesis (Rushworth & Megson 2014). GSH, however, also serves as a water-soluble endogenous molecule that is attached to xenobiotics (substances foreign to the body) in order to enhance their elimination from the organism. Thus, increased GSH content can, among other things, confer antioxidant activity and/or enhance the elimination of xenobiotics, including carcinogens (Forman et al. 2009).

Trolox is a synthetic water-soluble compound, exclusively used for research purposes, that shares the chromanol ring structure of α-tocopherol, the major form of the fat-soluble vitamin E. Even though Trolox is often described as a vitamin E-derivative, it does not have the same biological activity or even chemical characteristics (Kamal-Eldin & Appelqvist 1996). Vitamin E resides in cellular membranes, where it protects membrane lipids from oxidation. As a water-soluble compound, Trolox partitions into aqueous environments rather than the lipid-phases of membranes (Barclay et al. 1995). Since vitamin E and Trolox have very different chemical properties and are present in different cellular compartments, the observed (biological) activities of one cannot be used to derive conclusions about the other.

Neither NAC nor Trolox are vitamins or antioxidant nutrients and therefore the data obtained in the present study can not be used to generalise about the biological activities of vitamins or dietary antioxidants, as has been extensively done in the media. The experiment only reports on the activity of NAC, and to a smaller extent also Trolox, and can therefore only help us to understand potential biological activities of these two compounds.

The tested compounds did not act as antioxidants in the reported experiments

Le Gal and co-workers (2015), throughout the entire text, state to have studied effects of 'antioxidants'. An antioxidant is 'any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate' (Halliwell 1995). In their paper (Figure S4), the authors provide data showing that the presence of oxidized species in melanoma cells did not differ between control, NAC- and Trolox-treated cells, which is direct evidence that 'antioxidant effects' (prevention of oxidation) were not involved in the observed differences in cell migration.

Since neither NAC nor Trolox delayed or prevented the oxidation of an oxidizable substrate, it is incorrect to refer to the test compounds as antioxidants in the context of the present work. Based on the authors' data, the observed differences in cell migration are likely due to non-antioxidant modulation of the activity of signalling molecules. In fact, NAC and Trolox are both known to act on additional cellular targets, including the tumour necrosis factor-α/nuclear factor κ B signalling pathway (Zafarullah et al. 2003, Sung et al. 2012), which is known to be involved in the regulation of tumour cell migration and invasion (Wu & Zhou 2010), and have been suggested in independent studies to reduce cancer cell migration and invasion (Supabphol et al. 2009, Sung et al. 2012).

It remains unexplained why the authors insist on referring to NAC and Trolox as antioxidants in the context of their work, as neither exerted antioxidant activity in their experimental models (Le Gal et al. 2015).

Do 'antioxidants' and specifically N-acetylcysteine and Trolox promote cancer?

As Le Gal et al. (2015) point out in the discussion of their paper, their work is in contradiction to previously published data. Thus, there are studies to suggest that both NAC and Trolox may reduce migration and invasion of certain types of cancer cells (e.g. Supabphol et al. 2009, Sung et al. 2012). Nevertheless, their study provides interesting preliminary data that should be followed up in independent experiments using sound scientific and statistical methods. Such experiments should also test antioxidant compounds that are, contrary to the experimental chemical Trolox and the drug N-acetylcysteine, of nutritional relevance. Furthermore, antioxidant activity, that is the involvement of redox chemistry in the reported phenomena, should be ascertained by sound methodology if conclusions are drawn regarding the biological activities of antioxidants.

The results reported by Le Gal et al. (2015) cannot be applied to human nutrition and are thus no reason for concern regarding the intake of dietary antioxidants and vitamins.

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Conclusions

Since Le Gal and colleagues (2015) did not study nutritionally relevant compounds, vitamins, or agents with antioxidant activity, at least not in the reported experiments, their findings should not be misinterpreted as cause for concern or a reason to avoid the intake of dietary antioxidants and vitamins. The reported results do not provide novel information regarding the safety of dietary antioxidants and vitamins. Hence, there is no reason, based on the current findings, to question the previously documented safety of antioxidants and vitamins in the diet or dietary supplements.

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