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LETTER TO THE EDITOR

Response to "Letter to Editor"

Ayse Nur Coskun-Demirkalp[1,2,3,4] | Akın Tekcan[5] | Mustafa Cakir[3,6] | Hamiyet Donmez-Altuntas[1,2,3]

[1]Department of Medical Biology, Faculty of Medicine, Erciyes University, Kayseri, Türkiye | [2]Institute of Health Sciences, Erciyes University, Kayseri, Türkiye | [3]Betul-Ziya Eren Genome and Stem Cell Center, Erciyes University, Kayseri, Türkiye | [4]Mucur Vocational School of Health Services, Ahi Evran University, Kirsehir, Türkiye | [5]Department of Medical Biology, Faculty of Medicine, Amasya University, Amasya, Türkiye | [6]Department of Medical Biology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Türkiye

Correspondence: Hamiyet Donmez-Altuntas (donmezh@erciyes.edu.tr)

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Thank you for the opportunity to respond to the Letter to the Editor by Jaeschke and colleagues that comments on our article, *Paracetamol induces apoptosis, reduces colony formation, and increases PTPRO gene expression in human embryonic kidney HEK 293 cells*.

We agree that HEK 293 cells are immortalized by Adenoviral 5 E1A and E1B [1]. Thus, the HEK 293 cell derivate HEK 293T cells lack normal pRb and p53 functions and these cells would consider not to be "normal." However, HEK 293 cells are widely used in cell biology research as a noncancerous kidney cell line, although HEK cells are probably not a great model for kidney cell biology [2, 3]. Although studies show that these cells have many features of neuronal cells [4, 5], they are still used as a model for kidney physiology [6, 7]. Furthermore, HEK 293 cells have been employed as a "healthy" control in comparative cytotoxicity studies with cancer cells [3, 8–11]. Therefore, our use of HEK 293 cells is consistent with accepted in vitro practice for mechanistic investigation and comparative toxicity assessment.

While we agree that the concept of killing cancer cells with high doses of paracetamol (acetaminophen) is not new [12], in our study, we firstly evaluated the relationship between paracetamol at different concentrations and tumor suppressor gene candidate PTPRO gene in HEK 293 cells [13]. Thus, we suggest that paracetamol may suppress the development of renal cancer by inducing apoptosis and reducing colony formation in a dose-dependent manner, and upregulating PTPRO gene expression in HEK 293 cells at high concentration, in addition to its renal-damaging role [13]. Furthermore, the paracetamol concentrations (5, 10, 15, 20, and 30 mM) assessed in HEK 293 cells are compatible with previous studies of 0.99−29.77 mM for 24−48 h [14], and 3.5 and 7.0 mM for 96 h [15]. In addition, our results based on in vitro paracetamol concentrations in HEK 293 cells will differ from those across in vivo dose ranges. However, a drug's in vitro behavior can be used to predict its in vivo performance [16].

We agree that paracetamol-induced apoptosis is ER-stress mediated and caspase-dependent, involving the activation of caspase-9 and caspase-3 without the presence of apoptogenic factors such as cytochrome *c* or Smac/DIABLO in kidney cells [17, 18]. Moreover, the severe overdose of paracetamol results in prolonged ER stress, which is caused by the formation of protein adducts and Cyp2E1-mediated N acetyl-p-benzoquinone imine (NAPQI) in the ER. This leads to the cleavage and activation of procaspase-12, which in turn causes apoptotic cell death in the kidney's proximal tubular cells both in vivo and in vitro [18]. However, more research into the molecular pathways of paracetamol-induced renal damage is needed. On the other hand, although our study did not assess ER stress or caspase activation for apoptosis in HEK 293 cells, we showed that paracetamol induced dose-dependent apoptosis in HEK 293 cells at all concentrations (5, 10, 15, 20, and 30 mM) in both 24 and 26 h treatments by flow cytometry [13].

In summary, we evaluated the effects of various paracetamol concentrations on apoptosis, cell cycle, and colony formation in HEK 293 cells, as well as the expression of the tumor suppressor gene candidate PTPRO gene. Our in vitro results may predict in vivo efficacy, but further in vivo research is needed to assess the potential effects of paracetamol on the onset of renal impairment or renal cancer.

Sincerely,

Ayse Nur Coskun-Demirkalp
Akın Tekcan
Mustafa Cakir
Hamiyet Donmez-Altuntas

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