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Analysis of the interactions between environmental and food contaminants, cadmium and deoxynivalenol, in different target organs

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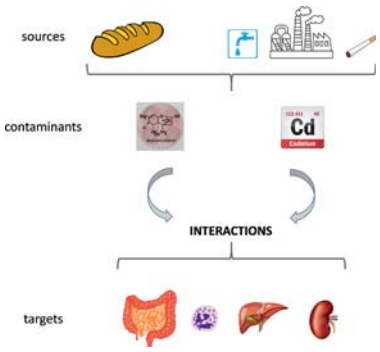
HIGHLIGHTS

- Consumers can be exposed to a cocktail of deoxynivalenol and cadmium.
- Interaction observed in renal cells exposed to different ratios of DON/Cd ranged from moderate antagonism to nearly additive.
- In intestinal cells, interactions ranged from nearly additive to antagonism.
- In blood and liver cells, interactions ranged from synergy to antagonism depending on the cytotoxicity level.
- Consequences of combined exposure to environmental and food contaminants are specific to the target organ.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium (Cd), a common and widespread toxic heavy metal, and mycotoxins such as deoxynivalenol (DON) are frequent contaminants of the food supply. Most of the data on their toxicity concern their effects when present alone. However, consumers can be exposed to a cocktail of DON and Cd. To improve the understanding of their combined toxicity, the effects of DON and Cd alone or in combination were investigated in different human cell lines from the kidney (HEK-293), intestine (Caco-2), blood (HL-60) and liver (HepG2). Cytotoxicity was assessed through ATP measurement and types of interactions determined by the Isobologram-Combination index method. HEK-293 cells were exposed to increasing doses of DON, Cd and their combination at different ratios (DON/Cd of 2/1; 1/1; 1/2 and 1/8). Regardless of the ratio, the type of interaction observed in HEK-293 cells ranged from moderate antagonism to nearly additive with increasing cytotoxicity. In Caco-2 cells, the interactions ranged from nearly additive to antagonism whatever the ratio. At ratio 1/1, in HL-60 and HepG2 cells, interactions ranged from synergy to antagonism depending on the cytotoxicity level. Using human cells lines, this study indicates that the consequences of combined exposure to environmental and food contaminants are specific to the target organ. Further studies are needed to confirm these data in vivo.

1. Introduction

Exposure to chemical contaminants is an important concern worldwide. Most of the toxicological data concern the effects of chemical contaminants when present alone; however, humans are usually exposed to multiple toxic compounds, which might impact health via food or

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the environment (Heys et al., 2016). In recent years, combined toxicity studies have been carried out to assess the effects of mixtures of food contaminants such as mycotoxins (Alassane-Kpembi et al., 2017a; Ruiz et al., 2011), endocrine disrupters and carcinogens (Le Magueresse-Battistoni et al., 2017) or a mixture of air pollutants (Coyle et al., 2006).

Deoxynivalenol (DON) is the most prevalent mycotoxin naturally present in grains and other food commodities. It is produced by toxigenic *Fusarium* species that are common pathogens of cereal crops under a temperate climate (Kokkonen et al., 2010). Humans are mainly exposed to DON through cereal-derived food. A European survey showed that 61%, 89% and 47% of wheat, maize, and barley samples, respectively, are contaminated with DON (Schothorst and Van Egmond, 2004). In Europe, a tolerable daily intake (TDI) of 1 μg/kg body weight per day for DON was established; however, young children can be exposed to DON at levels close to or even higher than the TDI (EFSA, 2013). Intoxication symptoms of DON may include reduced weight gain, a decrease in food consumption, neuro-endocrine changes and alteration of intestinal and immune functions (Payros et al., 2016). Following acute exposure, vomiting and bloody diarrhea have been observed (Pestka, 2010). There is no evidence for carcinogenic properties of DON and it was classified into group 3 by the International Agency for Research on Cancer (IARC) (IARC, 1993; Payros et al., 2017).

Cadmium (Cd) is one of the common and widespread toxic heavy metal found naturally in the Earth's crust and is usually present in the environment as an inorganic salt. Its presence in the environment is a consequence of both natural and anthropic processes. Natural sources of Cd include volcanic activity, weathering consumption of rocks, sea aerosols, forest fires and mobilization of Cd adsorbed in soils and landfills (ATSDR, 2012). The anthropic origin of Cd derives from batteries, pigments, plastic stabilizers, pesticides and fertilizers, and photovoltaic devices, as well as from rubber processing, the galvanization process, fossil combustion and waste incineration (ATSDR, 2012). Cd can be released into soil, water and air, and it bio-accumulates in the organic matter by entering the food chain. Grain and cereal products, fish and offal are the major contributors of Cd exposure in food (IARC, 2012; Kabata-Pendias and Pendias, 1984). A high amount of Cd also accumulates in tobacco leaves, and tobacco smokers are exposed by inhalation. Acute intoxication of Cd may lead to liver, lung, and testis damage, while chronic intoxication may result in pulmonary damage, disturbance of metabolism, dysregulation of blood pressure, obstruction of kidney function, and structural damage of bones (Godt et al., 2006). Cd and Cd compounds have been classified as carcinogenic to humans (group 1) by IARC (2012).

The toxicity of a mixture cannot always be predicted based on the toxicities of its individual compounds (Alassane-Kpembi et al., 2017a; Sarigiannis and Hansen, 2012). Exposure to toxic mixtures could result in antagonistic, additive or synergistic effects. Various studies have analyzed the interactions between mycotoxins and reported synergistic, additive or antagonistic effects. For example, the combination of DON, nivalenol and their acetyl derivatives resulted in synergistic in vitro cytotoxicity at low doses, while additive and antagonistic interactions were observed at higher doses (Alassane-Kpembi et al., 2013, 2015). Ex vivo, the interaction of DON and NIV in the pro-inflammatory response was synergistic at low doses (Alassane-Kpembi et al., 2017b). In vivo exposure to mixtures of DON and other mycotoxins showed different types of interactions, the most frequent being less than additive (Grenier and Oswald, 2011). A few studies have also investigated the interactions between mycotoxins and other toxins. For example, synergy was observed between DON and colibactin, a genotoxin produced by some strains of *Escherichia coli* (Payros et al., 2017). Similarly, a synergistic interaction was shown between ochratoxin A, a mycotoxin produced by *Penicillium* and *Aspergillus* and aristolochic acid, a nephrotoxic and genotoxic plant product (Stiborova et al., 2015). As far as Cd is concerned, some in vivo and in vitro studies have focused on the interactions between Cd and pesticides (Xu et al., 2017) or between Cd and other pollutants (Muthusamy et al., 2016).

Humans can be simultaneously exposed to DON and Cd through inhalation or water and food consumption. However, to the best of our knowledge, the combined toxicity of Cd and mycotoxins has never been addressed. The aim of the present study was to assess the combined effects of DON and Cd on different target organs using cell lines from the kidney, intestine, blood and liver.

2. Materials and methods

2.1. Cell culture and reagents

Human embryonic kidney 293 cells (HEK-293) and human liver hepatocellular carcinoma (HepG2) cells were maintained in EMEM (Sigma, St Quentin Fallavier, France) supplemented with 10% fetal calf serum (FCS) (Eurobio, Courtaboeuf, France), 1% penicillin/streptomycin (Eurobio) and 0.5% L-glutamine (Eurobio). Human colon carcinoma Caco-2 cells were maintained in DMEM-glutamax (Gibco, Life Technologies, Courtaboeuf, France) supplemented with 10% FCS, 1% amino acid non-essential (Sigma) and 0.5% gentamycin (Eurobio). Human promyelocytic leukemia cells (HL-60) were maintained in IMDM (Sigma) supplemented with 20% FCS, 1% penicillin/streptomycin.

All cell lines were incubated in a humidified atmosphere at 37 °C and 5% CO_2 in culture flasks. Adherent cells (Caco-2, HEK-293 and HepG2) were passaged by trypsinization (0.5% trypsin in 0.5 mM EDTA) when they were sub confluent. HL-60 were passaged by dilution when the concentration reached 1×10^6 cells/ml.

DON and Cd chloride were purchased from Sigma and were dissolved to 5 mM in water. Stock solutions were stored at −20 °C before dilution in complete cell culture medium (Table 1).

Adherent cells were seeded in 96-well plates and incubated 24 h in complete media before treatment. HEK-293 and Caco-2 cells were seeded with a density of 5000 cells/well. Because of their slower growth HepG2, with a density of 15,000 cells/well (Cervera et al., 2011; Lee et al., 1999; Sambuy et al., 2005). HL-60 suspension cells were treated immediately after being seeded at the density of 25,000 cells/well. The stock solutions of DON and Cd chloride were diluted in serum-free medium and all cell types were treated with serial dilutions of each compound or their mixture for 48 h.

The CellTiter-glo® luminescent cell viability assay (Promega, Charbonnières-les-Bains, France) was used as previously described (Tannous et al., 2017). This test is based on the measure of ATP, a widely accepted marker of viable cells (Riss et al., 2013). The luminescent signal produced by the luciferase reaction, reflecting the presence of metabolically active cells, was read using a multiplate reader (TECAN, Lyon, France). The results were obtained by calculating the percentage of viable cells (ratio luminescence in treated samples/luminescence in non-treated samples).

2.2. Data analysis

The dose-effect relationships of the individual and combined mycotoxins were biometrically modeled using the Median-Effect Equation of the Mass Action Law as already described (Alassane-Kpembi et al., 2013, 2015).

$$f_a/f_u = (D/D_m)^m$$

D: dose of the toxin
f_a: fraction affected by D
f_u: fraction unaffected (i.e., $f_u = 1 - f_a$)
D_m: median-effect dose (e.g., EC50)
m: coefficient signifying the shape of the dose–effect relationship ($m = 1$, $m >$ and $m < 1$ indicate hyperbolic, sigmoidal, and flat