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A computational study toward the "personalized" activity of alternariol – Does it matter for safe food at individual level?

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ABSTRACT

Mycotoxins in food may threat public health at a global scale. However, for most of them, the current body of knowledge does not support a proper risk assessment and more data are needed to clarify their toxicity. In particular, the assessment of "personalized" action may succeed in understanding and counteracting the effects of many toxicants. Therefore, the assessment of "personalized" toxicology of mycotoxins might deserve attention to foster the understanding of their mechanisms of toxicity and to eventually improve the assessment of risk. This work dealt with the early warning analysis of possible differences in eliciting androgenic stimuli by alternariol, a widespread mycotoxin produce by *Alternaria* species, when mutations on the androgen receptor occur. It was applied a computational study based on docking simulations, pharmacophore modeling and molecular dynamics to assess the capability of alternariol to interact with the androgen receptor bearing the M749I substitution – which confers insensitivity to androgens stimulation. The results collected pointed to possible "protective" effects against alternariol suggesting: i) the likely existence of inter-individual responses to alternariol stimulation; ii) the meaningfulness of collecting data on "personalized" response to mycotoxins toward a more precise paradigm addressing the risk assessment at the individual level.

1. Introduction

Improving the safety of food is known since decades as critical to ameliorate and ensure humans and animals wellbeing at a global scale (Miyagishima et al., 1995). In this context, counteracting the presence of toxic compounds in foodstuff is among the top-priority tasks to get in line with the forthcoming needs of food production worldwide. Among the others, contaminants of natural origin represent a huge threat due to their multiple adverse effects and the difficulties in implementing effective strategies to reduce their contamination along the production chains (Rather et al., 2017). In particular, mycotoxins, the secondary metabolites of filamentous fungi, are among the most widespread and unavoidable contaminants in food and feed with a strong prevalence in grains and plant-based products (Dellafiora and Dall'Asta, 2017). Mycotoxins exposure through diet may pose a severe threat for human health, and regulations to limit the occurrence of some of them in food and feed (e.g. zearalenone, aflatoxins, fumonisins, ochratoxin A and deoxynivalenol) have been set in many countries (Marroquín-Cardona et al., 2014; Matumba et al., 2017). Regulations must rely on sound scientific evidences and additional toxicological data on the less characterized mycotoxins, such as those emerging or modified, have been claimed at international level toward a more informed body of knowledge to support risk assessment studies (EFSA, 2014; Gruber-Dorninger et al., 2017; Lorenz et al., 2019).

Currently, in many countries differences in the allowed maximum level of contamination of some mycotoxins exist (e.g. aflatoxin B1, deoxynivalenol, fumonisisn, ochratoxin A, patulin and zearalenone in Europe) depending on the age of population groups to whom the potentially contaminated food is intended for, with strictest levels for infant and young children (European-Commission, 2012). However, personalized recommendations dealing with the specific risks subjects may be exposed to are still missing. In this regard, according to the "personalized" toxicology paradigm, xenobiotics such as drugs and toxicants may result in diverse outcomes depending on environmental factors and/or individual components at both genetic and epigenetic levels (Fischer et al., 2013; Gordon, 2003; Hattis, 1996; Jager, 2013). Accordingly, the severity of mycotoxins action is likely to change on individual basis too. However, the inter-individual variability of

Abbreviations: AOH, alternariol; AR, androgen receptor; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; EE, ethinyl-estradiol; MD, molecular dynamic; MENT, 7α-methyl-19-nortestosterone; RMSD, root-mean-square deviation; RMSF, root-mean-square fluctuation; wt, wild type

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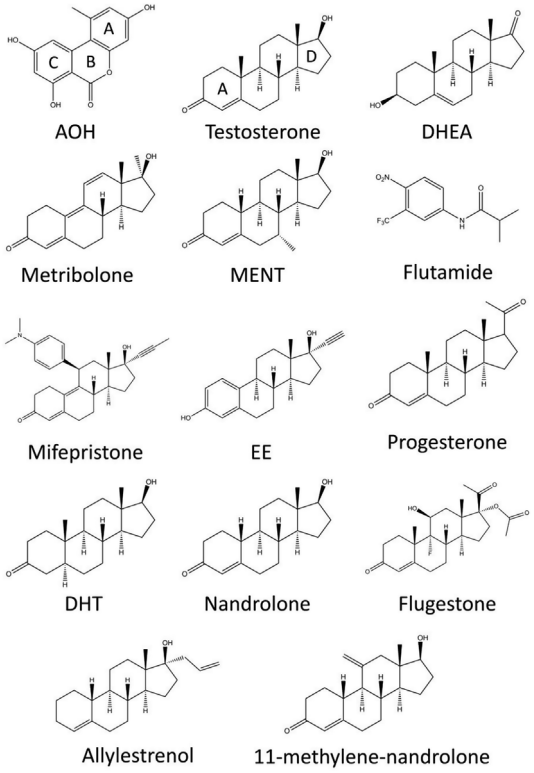

Fig. 1. Chemical structures of molecules analyzed in this work.

mycotoxins action is still a largely overlooked issue and safe levels of exposure (if any) for subjects with specific genetic and/or epigenetic heritages still have to be defined. In this light, the relevance of inter-individual differences in determining either enhanced or reduced susceptibility to mycotoxins need to be assessed beforehand to better design and interpret risk assessment studies, as well as to plan personalized nutritional/pharmacological interventions.

In the context of advancing the understanding of "personalized" action of mycotoxins, the work presented here dealt with the early warning analysis of possible differences in the androgenic activity of alternariol (AOH; Fig. 1) due to a mutation occurring at the androgen receptor (AR). AOH is among the most abundant mycotoxins produced by *Alternaria* spp. (Myresiotis et al., 2015) with a prevalent occurrence in cereals and cereal-based products, tomato and sauces, sunflower seeds and oil, fruits, beer and wine (EFSA, 2011). AOH belongs to the group of emerging mycotoxins and additional toxicity data have been claimed toward a more informed background of knowledge to support risk assessment (EFSA, 2011). AOH exhibits cytotoxic potential and it may cause oxidative stress and DNA damage (Dellafiora et al., 2015a; Fehr et al., 2009; Tiessen et al., 2013). Recently, AOH has been found endowed of weak estrogenic activity (Dellafiora et al., 2018b; Lehmann et al., 2006) and it proved to act as full agonist of AR (Stypuła-Trębas et al., 2017). AR belongs to the steroid hormone group of nuclear receptors and it is a ligand-induced transcriptional factor promoting the transcription of specific genes under the control of androgens (Claessens et al., 2001). The binding of endobiotic ligands, such as testosterone or its potent 5α-reduced metabolite dihydrotetsosterone (DHT) (Fig. 1), at the ligand binding domain induces protein activation via homo-dimerization and translocation into the nucleus, where the recruitment of cofactor proteins and the activation of transcriptional machinery take place (Saeed et al., 2016).

Several AR mutations have been found responsible of altered susceptibility to the ligand-dependent stimulation (Shi et al., 2002). Also, most of them have been found recurring in AR-dependent malignancies with diverse roles in pharmacoresistance and/or in androgens insensitivity (Brooke and Bevan, 2009; Lallous et al., 2016; McPhaul, 2002). Among them, the M749I substitution, which is a mutation at the steroids ligand binding site recurrent in latent prostate cancer (Hay and McEwan, 2012), showed insensitivity to steroids hormones, including DHT (Shi et al., 2002), possibly conferring protection from prostate cancer (Takahashi et al., 1995). This work aimed at assessing the possible effects of M749I mutation on the AOH-dependent stimulation by using a computational study, a technique that is getting more and more consensus in the high-throughput molecular characterization of toxicologically relevant compounds (Dellafiora et al., 2015b, 2017; Schrey et al., 2017; Selvaraj et al., 2018). Specifically, a molecular modeling approach based on pharmacophoric modeling, docking studies and molecular dynamics within both the wild type (wt) and mutated AR was used to assess the possible effects of M749I mutation on the AOH-dependent stabilization of the active conformation of AR.

2. Material and methods

2.1. Models preparation

The model for the wt human AR ligand binding domain derived from the good-resolution DHT-binding crystallographic structure deposited in the RCSB PDB databank (http://www.rcsb.org) (Berman et al., 2000) with ID code 4OEA (Hsu et al., 2014). The structure was processed using the Sybyl software, version 8.1 (www.certara.com) checking the consistency of atom and bond types assignment and removing the co-crystalized ligand, as previously reported (Dellafiora et al., 2015b). The model for the M749I AR was derived from the wt model introducing the M749I mutation using the Mutate Monomers option in the Byomolecule module of Sybyl, version 8.1 (www.certara.com). To avoid improper atomic coordinates, it was applied a mild local minimization 3 Å around the mutated residue using the Powell algorithm (500 iterations, 0.05 kcal/(mol·Å).

2.2. Pharmacophoric modeling

The binding site of both AR models was defined using the Flapsite tool of the FLAP software while the GRID algorithm was used to investigate the corresponding pharmacophoric space (Baroni et al., 2007; Carosati et al., 2004). The DRY probe was used to describe potential hydrophobic interactions, while the sp2 carbonyl oxygen (O) and the neutral flat amino (N1) probes were used to describe the hydrogen bond acceptor and donor capacity of the target, respectively.

2.3. Docking simulations

GOLD (Genetic Optimization for Ligand Docking) software was chosen to perform docking studies as it already succeeded in computing protein-ligand interactions. The explorable space of binding site to dock ligands was set within a 10 Å sphere around the center of pocket. Software setting and docking protocol previously reported were used (Dellafiora et al., 2018b). As an exception, the use of external scoring functions was omitted as the GOLD's internal scoring function GOLD-Score succeeded in analyzing the reference set of compounds (*vide infra*).

2.4. Molecular dynamic simulations

Molecular dynamic (MD) simulations were done to investigate the dynamic of AOH interaction with the ligand binding site of both wt and M749I AR, in comparison to those of the endogenous agonist DHT. The best scored binding poses calculated by docking simulation were used as input for MD. The model used for docking simulation showed an unresolved peripheral loop (residues 843–851) and the structural continuity was obtained by means of homology modeling using the software Modeller (version 9.19) (Webb and Sali, 2006). In particular, the

primary structure of AR was set as the query and the 3D AR model used for docking simulation was set as the template to reconstitute loop continuity. MD simulations were performed using GROMACS (version 5.1.4) (Abraham et al., 2015) with CHARMM27 all-atom force field parameters support (Best et al., 2012). All the ligands have been processed and parameterized with CHARMM27 all-atom force field using the SwissParam tool (Zoete et al., 2011). Crystallographic waters kept in the docking studies were removed and protein-ligand complexes were solvated with SPCE waters in a cubic periodic boundary condition, and counter ions (Na^+ and Cl^-) were added to neutralize the system. Prior to MD simulation the systems were energetically minimized to avoid steric clashes and to correct improper geometries using the steepest descent algorithm with a maximum of 5000 steps. Afterwards, all the systems underwent isothermal (300 K, coupling time 2psec) and isobaric (1 bar, coupling time 2 psec) 100 psec simulations before running 50 nsec simulations (300 K with a coupling time of 0.1 psec and 1 bar with a coupling time of 2.0 psec).

2.5. Statistical analysis

Each docking run was performed in quintuplicate and data are expressed as the mean of at least four replicates ± standard deviation (SD). GOLD implements a genetic algorithm that may introduce variability in the results in a system-dependent manner possibly causing outliers. Therefore, outlier points were removed to a maximum of one for each run after being identified with the modified Thompson Tau Test, as already reported (Dellafiora et al., 2018a). Data were statistically compared by one-way ANOVA ($\alpha = 0.05$), using IBM SPSS Statistics for Linux, version 25 (IBM Corp., Armonk, NY).

3. Results and discussion

3.1. Assessing procedure reliability

As previously reported, docking simulations may reliably estimate the biological activity of small molecules computing their interaction with targets of biological/toxicological concern (e.g. (Maldonado-Rojas and Olivero-Verbel, 2011; Rollinger et al., 2006)). However, a fit-for-purpose cross-docking validation was done to check the case-specific model capability to reliably discriminate the agonistic activity of ligands. To do so, a reference set of compounds including positive and negative controls was defined and used to challenge the wt AR model. Specifically, the subset of positive controls included testosterone, nandrolone, DHT, metribolone, 11-methylen-nandrolone and 7α-methyl-19-nortestosterone (MENT), which are well-known true agonists (Sonneveld et al., 2006). Conversely, the subset of negative controls included progesterone, mifepristone, ethinyl-estradiol (EE), flutamide, flugestone and allylestrenol, which are known to be unable to trigger significant androgenic stimuli (Sonneveld et al., 2006). In addition, the capability of procedure to generate reliable geometries of binding was assessed comparing the calculated binding architectures of testosterone, DHT and metribolone with those resolved so far by crystallographic studies.

The procedure proved to be reliable in predicting both the adrogenic activity and the binding architecture of ligands as: i) all the positive controls included in the reference set were significantly discriminated from the negative controls ($p < 0.05$, Games-Howell *post hoc*), according to their capability to trigger androgenic stimuli (Fig. 2); ii) the computed poses of testosterone, DHT and mifepristone were in strong agreement with the binding architectures reported by crystallographic studies (Fig. 3). However, due to the lack of correlation between the computational scores of positive controls and their androgenic activity reported in the literature (Sonneveld et al., 2006), the model presented here proved to be reliable in predicting androgenic activities only in qualitative terms, in agreement with previous studies using similar approaches (e.g. (Dellafiora et al., 2018a)).

Moreover, it was also assessed the capability to correctly predict the effects of M749I mutation in preventing the interaction of ligands with M749I AR. To this end, the model was challenged using a set of compounds collected from the literature that were experimentally proved unable to activate the M749I AR. The set included DHT, flutamide, progesterone and dehydroepiandrosterone (DHEA), according to Shi and coworkers (Shi et al., 2002). As shown in Fig. 2, all of them recorded scores significantly lower than those of positive controls and in the range values of negative controls, thereby proving the reliability of the M749I AR model.

On the basis of these results, calculating the interaction with the AR pocket proved to be a reliable analytical method to qualitatively estimate the androgenic activity of compounds on both wt and M749I AR.

3.2. Docking of AOH

Once assessed the procedure reliability, the model was challenged with AOH. As shown in Fig. 2, AOH recorded a score significantly higher from those of negative controls and in the range of scores of positive controls pointing to its capability to positively interact with the agonist AR conformation, in agreement with its full agonist activity previously reported (Stypuła-Trębas et al., 2017). AOH docked the binding site with the typical pocket occupancy of steroids and other (poly)aromatic compounds within AR and its proximate paralogous nuclear receptors. In more detail, the calculated pose of AOH resembled the crystallographic pose of DHT and other steroids with the A ring overlapped to the steroid's A ring (Fig. 4A) engaging with polar contact Asn705, Gln711 and Arg752. From the pharmacophoric point of view, the binding pose was found quite complying with the distribution of polar and hydrophobic space (Fig. 4B). However, both α-pyrone moiety and hydroxyl group in position 7 were found improperly placed into the hydrophobic region of the site likely causing degrees of hydrophobic/polar interferences. This pharmacophoric mismatch might partially explain the molecular basis underlying the mild androgenic activity found experimentally (Stypuła-Trębas et al., 2017). It is worth mentioning that the pose of AOH was in strong agreement with the one of the mycotoxin zearalenone within the alpha isoform of estrogen receptor (Fig. 4C), a well-described nuclear receptor paralogous to AR, further supporting the likeliness of the calculated pose of AOH.

The capability of AOH to interact with the M749I was then calculated. As shown in Fig. 2, the score of AOH was significantly lower than those recorded within the wt AR model ($p = 0.001$, according to Games-Howell *post hoc*), and in the range of values recorded by negative controls ($p = 0.19$ to flutamide, according to Games-Howell *post hoc*). The close inspection of the binding pose in comparison to that obtained into the wt model revealed that the local pocket reshaping was responsible for worsening the pocket fitting. In particular, the M749I substitution slightly changed the space available for ligands (Fig. 5A and B) preventing the accommodation of AOH as observed into the wt AR (Fig. 5C) and hampering the formation of polar contacts with Gln711 and Arg752. Specifically, such contacts are crucial to dock the pocket (De Jesus-Tran et al., 2006) and are recurrent in almost all the public AR crystallographic structures resolved so far, suggesting the unlikelihood of such an unconventional pose in reality.

On the basis of the results presented above, a significant interaction between AOH and M749I AR was thought not likely, pointing to the possible resistance of such mutated form to the AOH-dependent stimulation.

3.3. Molecular dynamic study

The dynamic of interaction of AOH within AR was compared to that of DHT, taken as reference for AR agonists, and to those of both ligands into the M749I AR, assuming they can reach the pocket. The protein showed in the four complexes (namely, AOH-wt AR, DHT-wt AR, AOH-M749I AR and DHT-M749I AR) a quite comparable geometrical

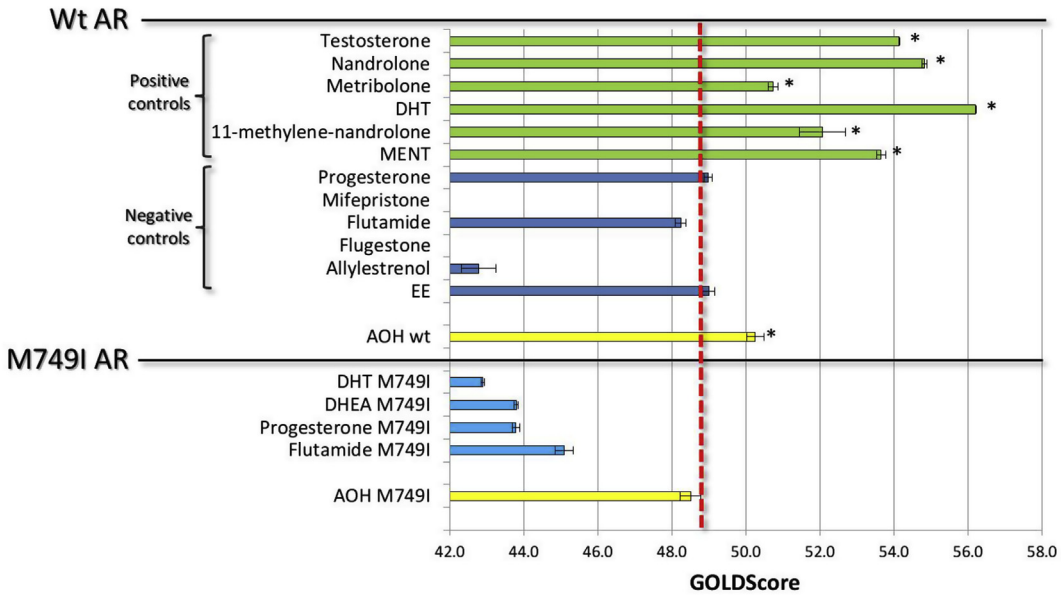

Fig. 2. Docking results. Positive controls and AOH within wt AR recorded scores significantly higher than those of negative controls, AOH and other steroids within M749I AR. Significance to negative controls: *p < 0.05 (according to Games-Howell *post hoc*).

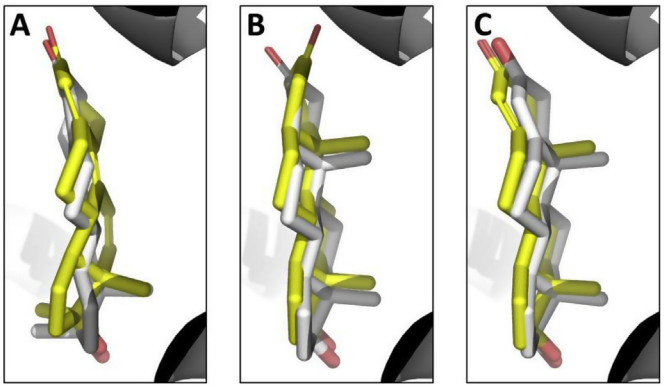

Fig. 3. Comparison between calculated pose (yellow) and crystallographic binding architecture (white) of metribolone (**A**), DHT (**B**) and testosterone (**C**). The crystallographic coordinates of metribolone, DHT and testosterone derived from the PDB structures 1E3G (Matias et al., 2000) 4OEA (Hsu et al., 2014) and 2AM9 (De Jesus-Tran et al., 2006), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

stability, assessed via the root-mean-squared analysis of protein backbone atomic coordinates. In more detail, protein RMSD variations in the four systems were found of slight intensity and converging to each other indicating that the protein atomic coordinates were similar to the respective initial structures along the all MD simulations (Fig. 6A). On this basis, ligands docked at the mutated binding site were thought unable to impair the overall geometrical stability of AR at the timescale under analysis, in agreement with previous data reporting the overall stability of AR structure (Sakkiah et al., 2018).

Conversely, the analysis of ligands RMSD fluctuations in the four complexes revealed the more pronounced mobility of AOH, to a major extent, and DHT, to a minor extent, in the mutated complexes in comparison to the respective complexes with wt AR (Fig. 6B). It is worth to note that DHT within the wt AR recorded lower and more stable scores than AOH, in agreement with the well documented capability to fit the pocket and reflecting its strong androgenic activity (Sonneveld et al., 2006). Accordingly, it could be inferred the importance of a geometrically stable interaction within the pocket to promote androgenic stimuli. Therefore, the findings collected pointed to possible disrupting effects of M749I on the capability of ligands, and especially AOH, to stably dock the mutated pocket.

In addition, the formation and persistence of hydrogen bonds network between the ligands and both the wt and M749I AR was measured and compared. As shown in Fig. 6C, DHT within wt AR showed the formation of either one or two (both most frequently) or three (less frequently) hydrogen bonds uniformly distributed along the all dynamic at the timescale under analysis. AOH in complex with wt AR showed the predominant formation of one hydrogen bond uniformly distributed along the dynamic, with a slight less frequent formation of two hydrogen bonds than in the case of DHT-wt AR complex, though distributed along the all simulation. Conversely, the establishment of either three or four hydrogen bonds was mainly found at the beginning or at the very beginning of the simulation, respectively. Given the weak activity of AOH in comparison to endogenous steroids (Stypuła-Trębas et al., 2017), this result pointed to the importance of establishing and maintaining multiple hydrogen bonds over the time to bind and stimulate AR, as shown by DHT. On the basis of these results, the lower capability of AOH than that of DHT to form long-lasting and multiple hydrogen bonds may provide a rationale to explain the mild androgenic activity found experimentally. Moreover, it is worth to note that the interaction between DHT and M749I AR, which is supposed to not occur in reality (Hay and McEwan, 2012), was characterized by one hydrogen bond well-distributed along the simulation, while showing two hydrogen bonds sporadically – even less frequently than in the case of AOH-wt AR – and three hydrogen bonds only at the very beginning of dynamic. These findings further supported the above mentioned statement concerning the importance of keeping long-lasting and multiple hydrogen bonds to stimulate androgenic stimuli. Therefore, the disrupting effects of M749I mutation in maintaining such a network of interactions could provide a mechanistic basis to explain, at least in part, the insensitivity of M749I AR to DHT stimulation.

Concerning the AOH-M749I AR, the formation of one hydrogen bond was found well-maintained along the all simulation. However, the presence of either two or three hydrogen bonds was found sporadically and mainly limited at the first half of simulation (both less frequent and worse distributed than in the case of AOH-wt AR). Moreover, the formation of two hydrogen bonds in AOH-M749I AR was found less frequent and worse distributed than in the case of DHT-M749I AR. The formation of four hydrogen bonds was not found, though detected in the AOH-wt AR complex. Therefore, on the basis of the results reported

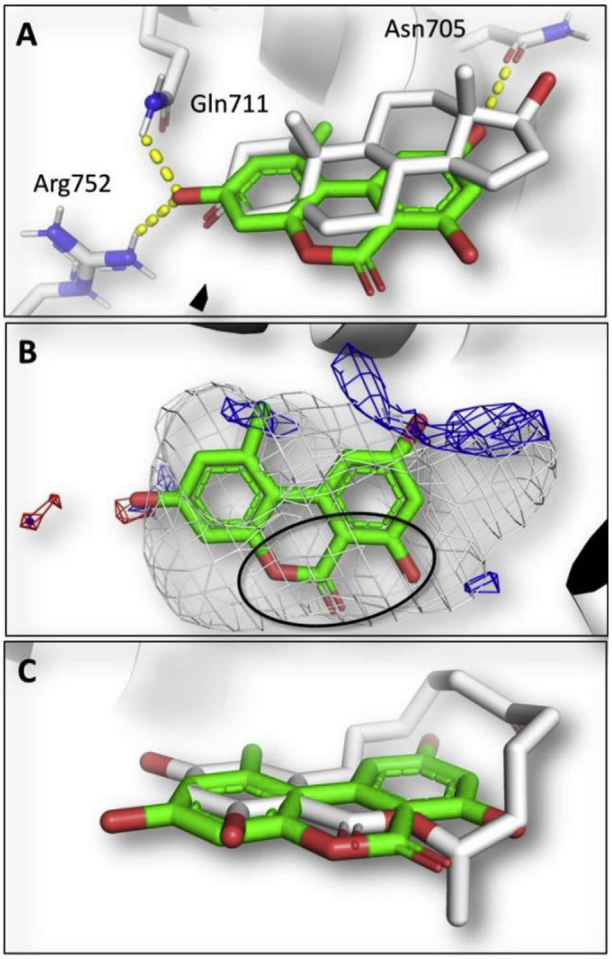

Fig. 4. Binding pose of AOH within wt AR. Protein is represented in white cartoon while residues involved in polar interactions are shown in white sticks. Ligands are represented in sticks. **A.** Calculated pose of AOH (green) in comparison to the crystallographic pose of testosterone (white) (De Jesus-Tran et al., 2006). Yellow dotted lines represent hydrogen bonds. **B.** AOH docked within the pharmacophoric space of the wt AR pocket. Grey, red, and blue meshes indicate regions sterically and energetically favorable to receive hydrophobic, hydrogen bond acceptor, and hydrogen bond donor groups respectively. The black circle indicates the improper arrangement of polar groups within hydrophobic environment. **C.** Calculate pose of AOH (green) within wt AR superimposed to the crystallographic pose of zearalenone (white) within alpha isoform of estrogen receptor (Nwachukwu et al., 2017). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

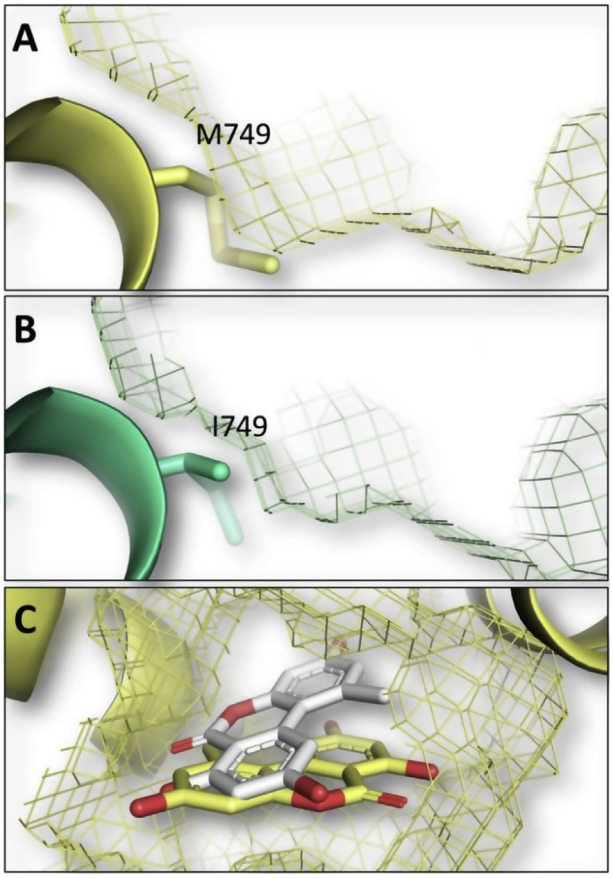

Fig. 5. Pocket reshaping and different pocket occupancy of AOH due to M749I mutation. Protein is represented in cartoon, while amino acid side chains and ligands are represented in sticks. The shape of the binding site is represented by mesh. **A.** wt AR. **B.** M749I AR. **C.** Comparison between the calculated pose of AOH within wt AR (yellow) and M749I AR (white). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

above, the network of hydrogen bonds was thought not supporting a favorable interaction given the overall worsening of frequency, lasting and number of hydrogen bonds in comparison to the other known complexes under analysis.

In addition to that, the local mobility of protein residues in the four complexes was monitored analyzing the root-mean-square fluctuation (RMSF) of protein backbone. As shown in Fig. 7, AOH-M749I AR complex in comparison to the wt AR complexes recorded higher mobility in regions with a well-documented key role in AR regulation (namely, the regions including the residues 726–732, 780–786 and 885–890). In particular, the region formed by the residues 780–786 was a part of the binding site and the higher mobility recorded therein might point to the local instability of pocket. This finding, together with the AOH instability discussed above, further proved unlikely the stable docking of AOH into the M749I AR pocket. Notably, enhanced movement in this region previously related to inactivation of AR (Sakkiah et al., 2018), further distrusting an agonist-like activity of AOH within the mutated AR. Furthermore, a higher mobility was observed also in the regions forming the so define AF2 regulatory domain, which includes both the residues ranges 726–732 and 885–890. In more detail, AF2 domain consists of a hydrophobic surface cleft deputed to recognize co-regulator proteins and it plays a crucial role in AR activation (He et al., 2004). The functionality of AF2 domains strictly depends on the so defined helix 12 (H12), which must adopt precise position and orientation to complete the AF-2 surface (Warnmark et al., 2003). In particular, H12 exhibits significant mobility in the absence of ligand and it is misplaced in response to non-agonist AR conformations (Shen and Balk, 2009). Thus, the dynamic properties of H12 may play a relevant role in determining the activation ability of AR and the increase of its flexibility may result in reduced activation of AR (Elhaji et al., 2006). In this light, AOH-M749I AR showed an enhanced mobility in the regions forming the AF2 domain (i.e. residues ranges 726–732 and 885–890), and specifically in a part of H12 (residues range 885–890), in comparison to that observed in wt AR complexes. It is worth to note that enhanced movements in both the residues ranges 726–732 and 885–890 were found specifically related to AR inactivation (Elhaji et al., 2006; Sakkiah et al., 2018). Concerning the interaction between DHT and M749I AR, it is important to note the relevant increase of mobility in the residues range 885–890. Therefore, on the basis of what reported before, the enhanced movements of H12 may further explain the mechanistic basis underlying the insensitivity of M749I AR to DHT stimulation.

Taken together, all the results above mentioned pointed to the

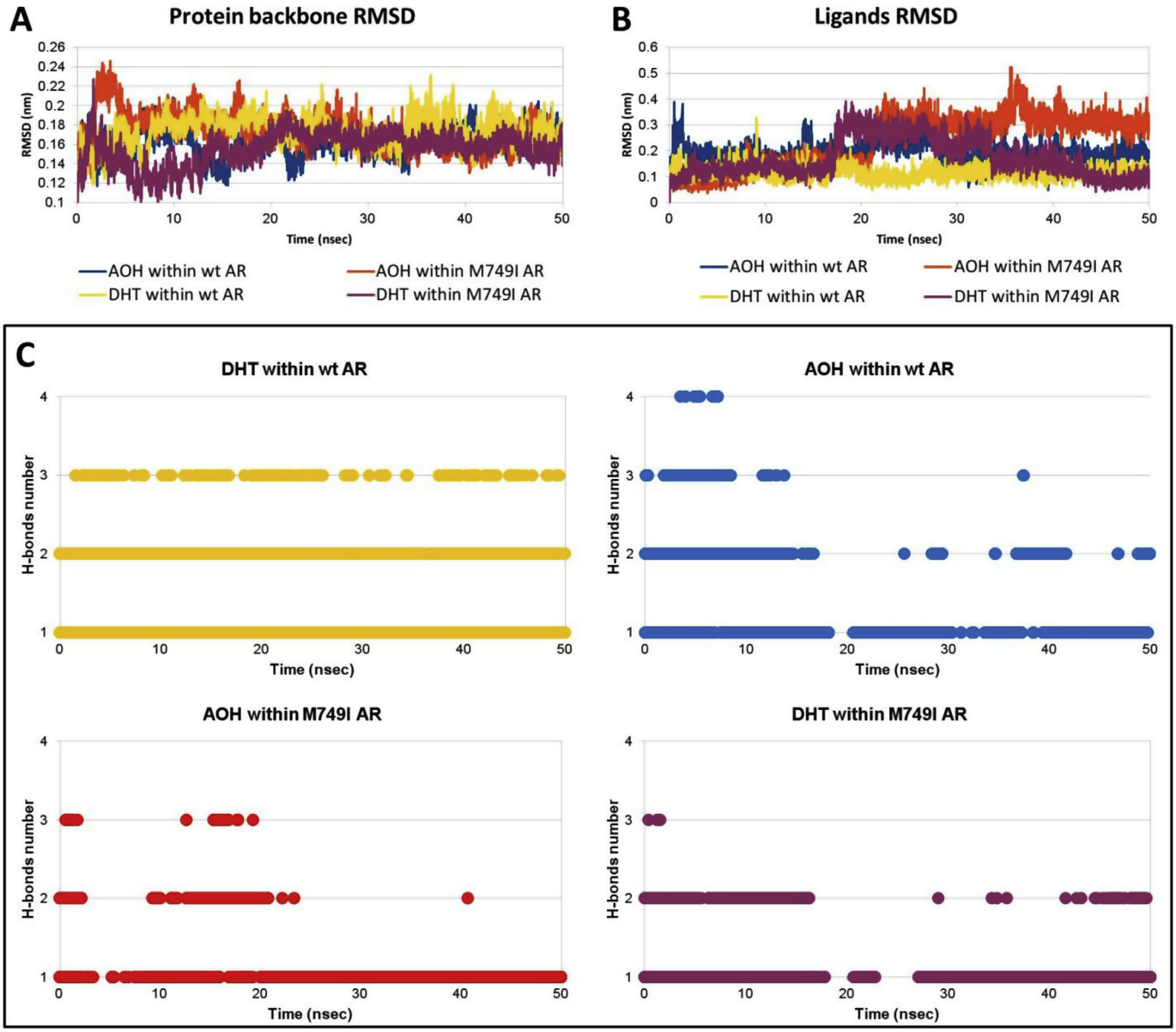

Fig. 6. Conformational changes and formation of hydrogen bonds within both wt AR and M749I AR in complex with AOH or DHT. **A.** RMSD plot of wt AR and M749I AR in complex with AOH or DHT. **B.** RMSD plot of AOH and DHT within wt AR or M749I AR. **C.** Number of hydrogen bonds formed in the four complexes along the timescale under analysis.

incapability of AOH to favorably dock the M749I AR suggesting its inability to geometrically stabilize the canonical agonistic conformation of AR.

4. Conclusions

The contamination of food and feed by mycotoxins may pose a severe health threat and actions to establish tolerable levels of contamination (if any) need to be urgently put in act to safeguard the public health at a global scale. The Scientific Community has investigated the toxicology of mycotoxins over decades and the data collected so far have converged in the body of knowledge currently used in risk assessment studies. Nevertheless, the data available nowadays are not sufficient to properly assess the risk of all the mycotoxins of possible concern in food and feed production. As an example, the paucity of toxicological data on AOH actually hinders risk assessment studies. Indeed, keeping in mind that AOH potentially represents a severe foodborne threat widespread worldwide, the current lack of data eventually results in delaying both the definition of “safe” levels of contamination and the decisions making in terms of allowed limits. In addition, the understanding of the mechanisms and modes of action is still in its infancy for most mycotoxins (including AOH) posing a high degree of uncertainty in characterizing the real threat mycotoxins may pose to consumers in their lifetime. As a matter of fact, epidemiological studies on mycotoxins have described conflicting scenarios and a solid consensus on the real effects on exposed populations is still missing. Ideally, the precise and complete comprehension of the mechanisms and modes of action underlying mycotoxins effects shall help in interpreting *in vivo* evidences, deterministically interconnecting the exposure to the effects in humans at the individual scale. In this regard, “personalized” responses to mycotoxins exposure due to subject-specific differences are likely to occur, as for many other xenobiotics. Therefore, the possible “personalized” activity of mycotoxins deserves investigations toward a more comprehensive paradigm of analysis, also in the light of improving both *in vitro-in vivo* extrapolation and interpretation of epidemiological data.

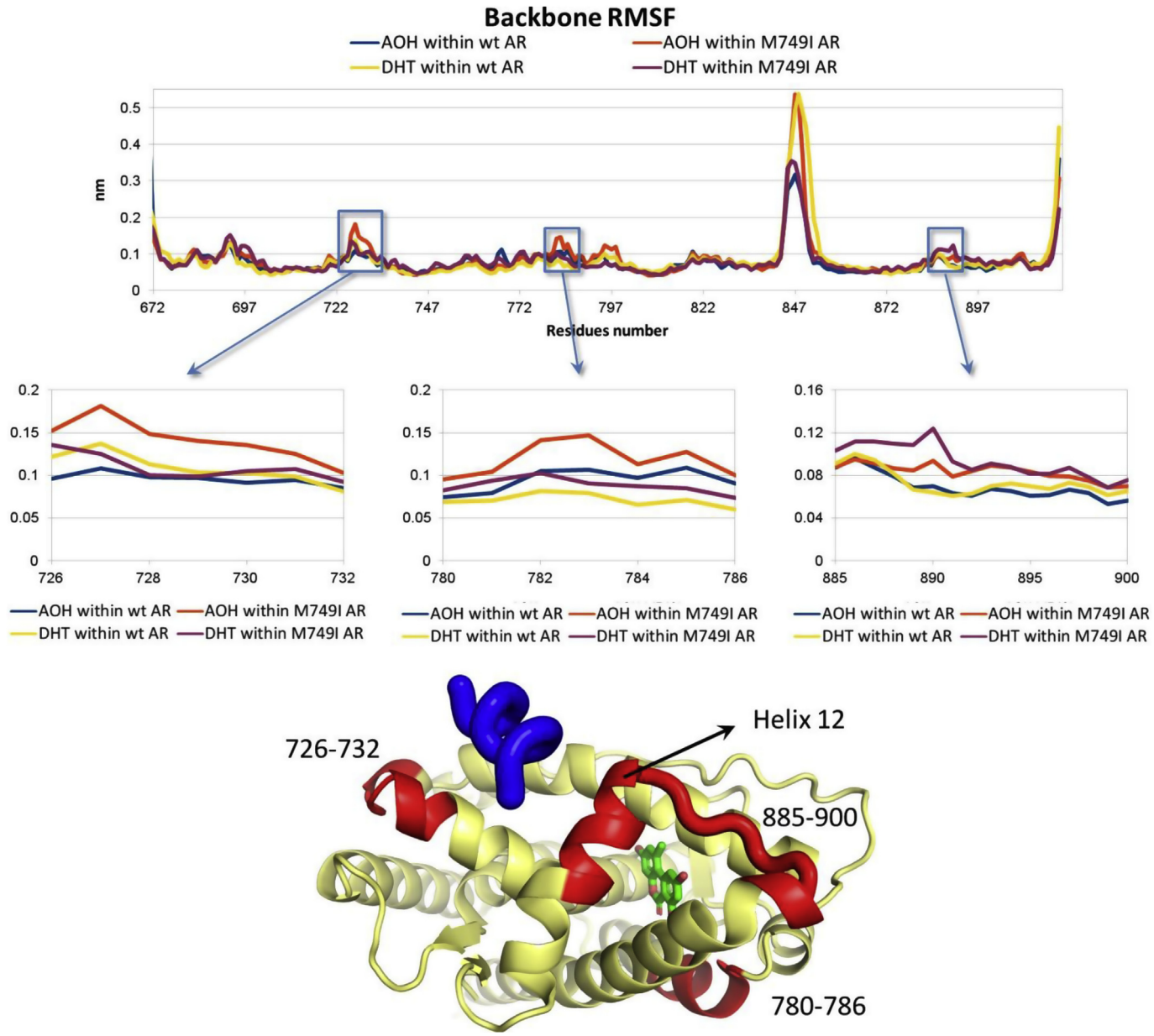

Fig. 7. RMSF plot of protein residues in the four complexes under analysis. The fluctuations of the regions with a proved relevance for AR activation are shown in the close-ups reported below the overall RMSF plot. The three-dimensional representation at the bottom shows the location of such regions (colored in red), while in blue ribbon is shown the placement of co-regulatory proteins at the AF2 domain, according to crystallographic studies (PDB code 1XJ7) (Estebanez-Perpina et al., 2005). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In this frame, as a proof of concept, the present work dealt with the possible "personalized" androgenic effects of AOH in relation to the capability to stimulate the activity of the mutated AR bearing the M749I substitution. A molecular modeling approach was used to study the AOH-AR binding event at a molecular level providing mechanistic insights to explain, at least in part, the structural basis likely underlying the insensitivity of M749I AR to androgens stimulation. All the data collected on the interaction between AOH and M749I AR pointed to possible "protective" effects of such specific mutation. Therefore, the agonist-like action of AOH could be thought unlikely. On the one side, our work deemed likely the existence of mutation-dependent differences potentially responsible for a divergent inter-individual AOH androgenicity, though its toxicology still needs to be characterized from a "personalized" perspective either *in vitro* or *in vivo*. In particular, the results collected pointed to possible protective effects, but the occurrence of mutations able to increase AOH androgenicity cannot be excluded, as found for other compounds (Shi et al., 2002). On the other side, these results pointed out the meaningfulness of broadly collecting data on "personalized" response to mycotoxins toward a more precise paradigm to deal with their risk assessment at the individual level.

Conflicts of interest

The authors declare no conflict of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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