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LETTER TO THE EDITOR

Arylamine N-acetyltransferases: a structural perspective. Comments regarding the BJP paper by Zhou *et al.*, 2013

This letter is a comment on Zhou *et al.* (2013). Arylamine N-acetyltransferases: a structural perspective. Br J Pharmacol 169: 748–760. To view this article visit http://dx.doi.org/10.1111/bph.12182

We have read with great interest the recent review published by the *British Journal of Pharmacology* entitled 'Arylamine N-acetyltransferases: A structural perspective' (Zhou *et al.*, 2013). This review highlights, from a structural point of view, the importance of the arylamine N-acetyltransferases (NAT) family of xenobiotic-metabolizing enzymes in the biotransformation of aromatic amine drugs and carcinogens and describes emerging new functions these enzymes may possess. The determination of the three-dimensional structure of prokaryotic and eukaryotic NAT enzymes has led to many advances in the understanding of their catalytic mechanisms, substrate specificity and the effect of polymorphisms on their function. In addition, the elucidation of these crystal structures has also contributed to the development of specific inhibitors of certain NAT isoforms (Sim *et al.*, 2012). Although the review by Zhou *et al.*, (2013) is topical and of great interest, several important elements described below would complete the overview of NAT structure and function.

As of 5 April 2013, the Protein Databank (PDB) contains 15 crystal structures of NAT enzymes (from eight prokaryotic NATs and two human NATs). This number of crystal structures has not changed since February 2012. **In particular, Zhou *et al.*, (2013) overlooked** three crystal structures that were published in the last 5 years: *Nocardia farcinica* NAT [PDB 3D9W, (Martins *et al.*, 2008)], *Mycobacterium marinum* NAT in complex with hydralazine drug [PDB 3LTW, (Abuhammad *et al.*, 2010)] and *Bacillus anthracis* NAT1 in complex with coenzyme A [PDB 3LNB, (Pluvinage *et al.*, 2011)]. In addition, the structure of an NAT isoform from *Bacillus cereus* has been deposited in the PDB (4DMO) and its coordinates will be available in May 2013; the crystallization of this NAT enzyme was published early last year (Kubiak *et al.*, 2012).

The structure of *M. marinum* NAT complexed with hydralazine sheds light on the binding of this drug to an NAT enzyme and reveals a novel mechanism for the acetylation reaction that results in the production of a 3-methyltriazolo[3,4-a]phthalazine ring compound (Abuhammad *et al.*, 2010). In addition, the structure of NAT1 from *B. anthracis* in complex with coenzyme A (CoA) demonstrates that the 17-residue insertion previously described as specific to human NAT enzymes (and, more broadly, to mammalian NAT isozymes) is also present in certain prokaryotic NAT enzymes (Pluvinage *et al.*, 2011). This structure also reveals a new mode of binding of the cofactor CoA when compared with the crystal structures of *M. marinum* NAT and human NAT2 (Pluvinage *et al.*, 2011). Thus, the mode of binding of acetylCoA is more diverse than originally thought and varies among NAT enzymes.

Another point pertinent to the review deals with the models for the chemical reaction that occurs at the NAT active site. The authors do describe the important work done by Wagner/Hanna's group on the hamster NAT2 enzyme showing, in particular, the existence of a thiolate-imidazolium pair at the active site (Wang *et al.*, 2004; 2005). However, the work done by Blanchard and colleagues on a prokaryotic NAT enzyme (the NAT from *Mycobacterium tuberculosis*), which identified a completely different mechanism from that observed with the hamster NAT2 (Sikora *et al.*, 2008), **also deserves attention**. In essence, Sikora *et al.* found that the enzyme has a catalytic cysteine pKa higher than 10, suggesting that the catalytic residue must be protonated at physiological pH and in the presence of substrate before nucleophile attack on acetylCoA occurs. Furthermore, these data do not corroborate the existence of a thiolate/imidazolium ion pair at physiological pH but, rather, indicate

a general base catalysis for the *M. tuberculosis* NAT enzyme. This finding suggests that the catalytic mechanism of NAT enzymes from different organisms can also vary.

This letter should not be viewed as a negative commentary on the article of Zhou *et al*., who have provided a clear and straightforward review of the exciting novel aspects of the NAT field. On the contrary, we hope our letter complements their review and provides further information on these important aspects of NAT structure and function.

Conflict of interests

None.

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