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Letter to the Editor

Reply to the letter to the Editor: "*N*-Acetyltransferases and the susceptibility to benzidine-induced bladder carcinogenesis"

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Dear Sir,

We thank Drs. Wang and King for their interest in our article on NAT2 acetylation and bladder cancer risk in workers exposed to benzidine.[1] In their letter to the editor,[2] Drs. Wang and King indicated that our results contradict the conclusion reached by Cartwright *et al.*[3] As we noted in our article,[1] the apparent contradiction is explainable, at least in part, by the fact that workers in that report are likely to have been exposed to 2-naphthylamine, a monoarylamine, as well as to benzidine, a diarylamine (unpublished findings).[3] In contrast, workers in the factories we studied were exposed primarily to benzidine and related compounds.[1] Cartwright *et al.* were aware of the implications of studying NAT2 acetylation and bladder cancer among workers exposed to more than one aromatic amine, as they noted in their initial report that "...different aromatic amines have different activation pathways despite close molecular similarities."[3]

Drs. Wang and King question our comments on the role of peroxidatic activity of prostaglandin H synthase (PHS) in benzidine carcinogenesis. They state that mono *N*-acetylation of benzidine effectively precludes the activation of benzidine by PHS. However, Zenser and colleagues have demonstrated that the peroxidatic activity of PHS converts *N*-acetylbenzidine (ABZ) to *N*′-hydroxy-*N*-acetylbenzidine (N'HA)[4] and that in the presence of dGMP *N*′-(3′-monophospho-deoxyguanosin-8-yl)-*N*-acetylbenzidine (dGp-ABZ) is formed.[5]

Drs. Wang and King have suggested that *N*′-hydroxy-*N*′-glucuronide-*N*-acetylbenzidine is likely to play a central role in benzidine bladder carcinogenesis, based on their findings in the rat heterotopic bladder model.[6] Rothman *et al.*[7] demonstrated that acidic urine pH increased the level of both free ABZ and the dGp-ABZ adduct in exfoliated urothelial cells of workers exposed to benzidine and benzidine-based dyes, and that ABZ strongly correlated with adduct levels. These observations are consistent with the hypothesis that *N*-acetylbenzidine-glucuronide may have an important role in benzidine carcinogenesis, as Zenser *et al.* have shown that this compound is extremely acid labile with its half-life reduced to several minutes under acidic conditions in urine.[8,9] In contrast, it is unlikely that DNA adduct formation would have been influenced by urine acidity if *N*′—OH—*N*-acetylbenzidine-*N*′-glucuronide was the only adduct forming agent, because this glucuronide is much more stable under acidic pH conditions, with a long half-life.[8,9] As such, the observations of Drs. Wang and King, based on the heterotopic bladder model, may not be generalizable, as this model does not mimic urine flow and urine pH, key components of aromatic amine carcinogenesis in human [a substantial proportion of humans have urine pH at or below pH 6.0 (N. Rothman, unpublished data)].[10] Also, the lower relative carcinogenicity of *N*′-hydroxy-*N*-acetylbenzidine in this model may be explained by the relatively high pH (7.1–7.4) of the fluid in the heterotopic bladder, conditions under which *N*-hydroxy arylamines are known to be unstable. In addition, *N*′-hydroxy-*N*-acetylbenzidine itself may be excreted unconjugated in urine and it is known to react rapidly with DNA at acidic pH to form dGp-ABZ (this is how this adduct was originally characterized).[11] Thus, *N*′-hydroxy-*N*-acetylbenzidine could be formed not only from PHS but also in liver from ABZ metabolism, then enter the circulation, and be filtered into the urinary bladder lumen, where it could be rapidly absorbed and further activated by NAT1 in the urinary bladder epithelium.

Accordingly, we agree with Drs. Wang and King that other enzyme systems may also contribute to *N*-acetylbenzidine carcinogenicity and that it is possible that *N*′-hydroxy-*N*-acetylbenzidine can lead to bladder carcinogenesis through the formation of an *N*′-acetoxy ester. Thus, Figure 2 in our article, which summarized and compared key pathways in the metabolism of monoarylamines and diarylamines, has been revised as shown in Figure 1.

We would like to respond to their comment that we failed to report the identification of *N*′-hydroxy-acetylbenzidine-*N*′-glucuronide in human urine. Our study was an observational case-control study of bladder cancer patients and controls, who had

The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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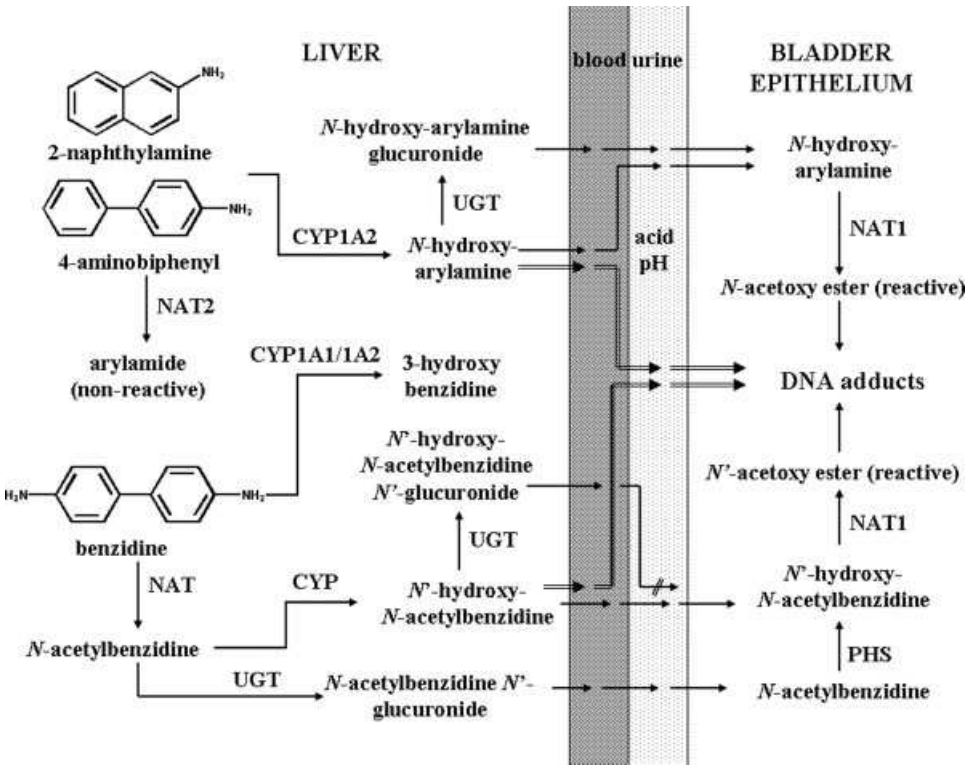

FIGURE 1 – Comparison of key pathways in the metabolism of monoarylamines and diarylamines. CYP1A1/CYP1A2, cytochrome P-450 1A1/1A2; NAT1/NAT2, *N*-acetyltransferase 1/2; UGT, UDP-glucuronosyltransferase; PHS, prostaglandin H-synthase.

worked previously in factories where benzidine was present but who were not exposed to benzidine at the time of our study; therefore, benzidine metabolites would not have been present in their urine.[1] The Rothman *et al.* study[7] enrolled workers exposed to benzidine and benzidine-based dyes at the time of the study and measured benzidine, and mono- and diacetylated benzidine, but did not measure *N*′-hydroxy-*N*′-acetylbenzidine-glucuronide. We agree that analysis of additional benzidine metabolites and their respective glucuronides, and the exploration of their correlation with DNA adducts in this study, would have been of interest, although as pointed out by Drs. Wang and King, some of these compounds may be so unstable that it is not feasible to measure them. The Rothman *et al.* analysis of benzidine, ABZ and DABZ before and after acid treatment provided an indirect measurement of the *N*-glucuronides of benzidine and ABZ, demonstrating their importance and presence *in vivo*.[7]

We agree with Drs. Wang and King's statement that benzidine and monoamino aromatics may be carcinogenic through similar pathways. Published evidence[12–14] suggests that the second amino group of benzidine is still susceptible to *N*′-oxidation and *N*′-glucuronidation; therefore, the metabolic fate of mono- and diarylamines may not be that different, with the major difference being that a monoacetylated benzidine derivative can still be bound to DNA. Studies in rodents suggest that *N*-acetylbenzidine is an "active" metabolite (*i.e.,* given that it can be further metabolized into reactive metabolites that can alter DNA), while *N*,*N*′-diacetylbenzidine is most likely a detoxified metabolite.[15]

Finally, we maintain that the revised figure does not change our conclusions that slow *N*-acetylation by NAT2 decreases the risk for benzidine-induced bladder cancer. As indicated in our article, these results suggest the existence of key differences in the metabolism of mono- and diarylamines, and their interaction with genotype, to affect individual susceptibility to bladder cancer.

Yours sincerely,

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