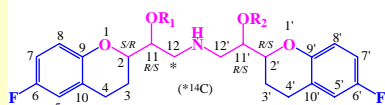


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## Introduction



*d*-Nebivolol: SRRR, R<sub>1</sub> = H, R<sub>2</sub> = H

*l*-Nebivolol: RSSS, R<sub>1</sub> = H, R<sub>2</sub> = H

GUDa: SRRR, R<sub>1</sub> = gluc, R<sub>2</sub> = H GUDc: SRRR, R<sub>1</sub> = H, R<sub>2</sub> = gluc

GUDb: RSSS, R<sub>1</sub> = gluc, R<sub>2</sub> = H GUDd: RSSS, R<sub>1</sub> = H, R<sub>2</sub> = gluc

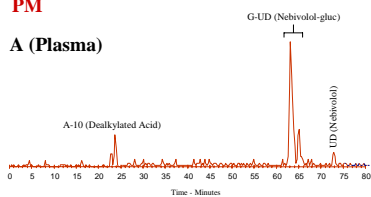
The Nebivolol drug product is a 1:1 racemic mixture of the enantiomeric pair, SRRR (*d*) and RSSS (*l*). The β<sub>1</sub>-antagonist actions are attributed primarily to the *d*-isomer<sup>1</sup> and both the *d*- and *l*-isomers further contribute to the pharmacological profile of nebivolol through vascular endothelial nitric oxide releasing capabilities.<sup>2</sup> In this study, metabolism of nebivolol in man was investigated, using *dl*-nebivolol specifically labeled with carbon-14 on the methylene group at the [R\*S\*] side of the molecule.

## Methods

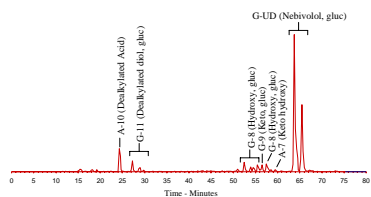
Healthy volunteers were recruited and genotyped for CYP2D6. Six subjects were qualified for study. Three extensive metabolizers (EM) and three poor metabolizers (PM) received a single oral dose of 100μg/15mg <sup>14</sup>C-nebivolol HCl. Blood, urine, and feces were collected up to 336 hours for EM or 432 hours for PM. Urine samples were centrifuged. Plasma and feces were extracted with methanol. The resultant urine supernatant and plasma and feces extracts were radio-profiled by RP-HPLC. Fractions of chromatography effluent were collected by time (0.25 min/fraction) in 96-well solid scintillator microplate. The radioactivity in each fraction was determined by Packard TopCount NXT Microplate Counter technology. HPLC radio-chromatograms were reconstructed using MicroSoft<sup>®</sup> Excel software. The prominent metabolites generated by EM and PM subjects were characterized or identified by LC/MS and/or NMR (for nebivolol glucuronides), as well as enzyme deconjugation.

## PM

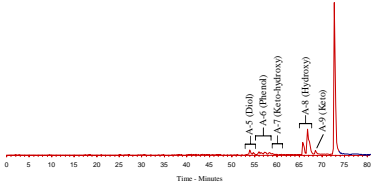
### A (Plasma)



### B (Urine)



### C (Feces)

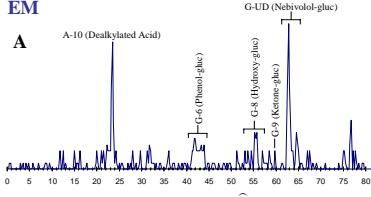


**Figure 1.** HPLC radio-chromatograms of pooled 0-96 h plasma extract (A) 0-168 h urine sample (B), and 0-168 h fecal extracts (C) from three 2D6 poor metabolizers (PM).

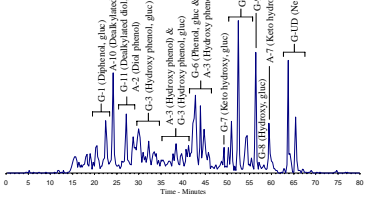
## Results and Discussion

## EM

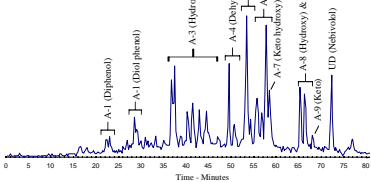
### A



### B



### C



**Figure 2.** HPLC radio-chromatograms of pooled 0-96 h plasma extract (A) 0-168 h urine sample (B), and 0-168 h fecal extracts (C) from three 2D6 extensive metabolizers (EM).

**1** Nebivolol was rapidly absorbed and extensively metabolized in humans following a single oral administration. A total of 43.57% and 38.36% of doses were recovered in EM feces and urine. Corresponding values for PM were 13.06% and 66.49%.

**2** Radio-profiling revealed significant difference in metabolite profiles between EM and PM subjects. Nebivolol undergoes extensive oxidative metabolism on the alicyclic, aromatic, or both rings to form mono-, di-, and tri-hydroxylated metabolites. The hydroxylation was mostly confined to the alicyclic ring for PM, but occurred on alicyclic, aromatic, or both rings for EM. High glucuronidation was observed, notably of nebivolol for PM and mono- and di-hydroxy metabolites for EM. N-dealkylation resulting in hydroxy carboxylic acid or dihydroxylated cleavage products plus direct glucuronidation of unchanged drug was also observed for both EM and PM subjects.

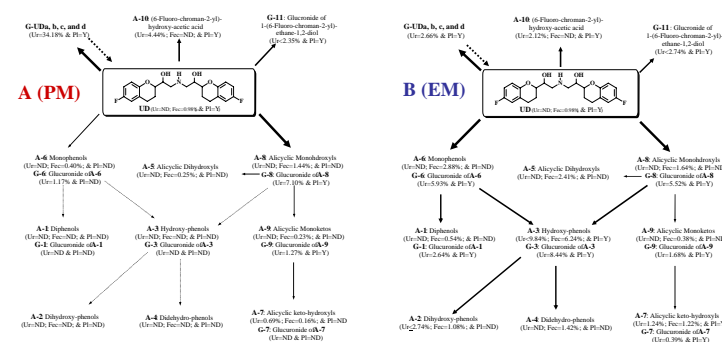
**3** The *d*-nebivolol (i.e., SRRR) isomer appeared more susceptible to glucuronidation than *l*-nebivolol (i.e., RSSS). Nebivolol was more prone to metabolism in EM subjects than in PM subjects. Metabolic profiles in PM subjects consisted mostly of nebivolol and/or nebivolol glucuronides.

## Conclusion

The apparent differences in the excretion and metabolite profiles between EM and PM suggests that the metabolism of nebivolol was strongly dependent on CYP2D6.

## Acknowledgement

Special thanks to Dr. Dawei Zhou, John E. Ryan, Dr. Dave D. W. Liu, Dr. Manik Desai, Dr. Wei-Zao Luo, and Hao Feng from XenoBiotic Laboratories, Inc.



**Figure 3.** Proposed metabolic pathways of nebivolol following a single oral dose in 2D6 poor metabolizers (A) and extensive metabolizers (B); \*No conjugated metabolites were observed in fecal samples; numbers in brackets are % of administered dose values; Fec: feces; PE: plasma; ND: non-detectable; U: urine; and Y: yes, observed.

## References

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2. Cockcroft, J.R. et al. (1995): Nebivolol vasodilates human forearm vasculature: evidence for an L-arginine-NO-dependent mechanism. *J. Pharmacol. Exp. Ther.* 274(3): 1067-1071.