Response to Reviewer 2 Comments

Point 1: The changes in the transport and pharmacokinetics of sulfadiazine may be mediated not only by the Pgp, but also by other efflux pumps. This is because: a) rhodamine 123 is apparently a substrate of several multidrug resistance-associated proteins, b) metyrapone induces pleiotropic pharmacological effects.
Please describe in a more detailed fashion the pharmacological effects of the compounds used in the study (metyrapone, ketoconazole, verapamil, Rho123), and their interaction with different transport systems (Pgp, BCRP, other pumps, …). Please revise accordingly the interpretation and the conclusions regarding the relationships of CXR activation or inhibition and the sulfadiazine PK.
Response 1: Thanks for the helpful suggestions. ATP-binding cassette (ABC) transporters are a large superfamily of transmembrane proteins, which utilize hydrolysis of ATP to energize efflux of a broad range of substrates against their concentration gradient. P-gp and BCRP are the important members of the ABC transporter class. In our previous study, we successfully established MDCK cell lines stably expressing functional chicken P-gp or BCRP for screening the substrates of these two transporters. We found enrofloxacin, ciprofloxacin, tilmicosin, ampicillin, and clindamycin were the cosubstrates of P-gp and BCRP. However, sulfadiazine was the substrate of chicken P-gp but not BCRP transport(Zhang et al. 2018). However, this does not exclude the involvement of other transporters or enzymes in the metabolic process of sulfadiazine. Thank you very much for the helpful suggestions. We have revised the interpretation and the conclusions regarding the relationships of CXR activation or inhibition and the sulfadiazine PK and described in a more detailed fashion the pharmacological effects of the compounds used in the study.(Line 25,35-37,69,83,96,213,219)

Point 2: Line 144 (Figure 7 label) – please state that metyrapone was administered 24 hr before sulfadiazine.
Response 2: Thank you for your helpful suggestions. We have changed. (line 161).

Point 3: Line 277 (statistical analyses) and the Figure labels – please state the type of the statistical test/s that were used to analyze each of the specific sets of data.
Response 3: Thank you for your helpful suggestions. We have added. (line 300-302,79,92,127,131,146,164).

Point 4: Line 34 – replace “agents” with “agent”
Response 4: Thank you for your helpful suggestions. We have changed. (line 33).

Point 5: Line 94 - correct the units
Response 5: Thank you for your helpful suggestions. We have changed. (line 107).

Point 6: Line 162 – replace “express” with “expressed”
Response 6: Thank you for your helpful suggestions. We have changed. (line 184).

Zhang YJ, Huang JH, Liu Y, Guo TT, Wang LP (2018) Using the lentiviral vector system to stably express chicken P-gp and BCRP in MDCK cells for screening the substrates and studying the interplay of both transporters. Archives of Toxicology 92(6):2027-2042 doi:10.1007/s00204-018-2209-9