Monoamine oxidase (MAO) inhibitory effects of candidate MAO inhibitors found in cigarette smoke

Prakshit Niraula[1] , Barry Palmer[1] , Penelope Truman[1] , Rachel Page[1]

[1] School of Health Sciences, Massey University, Wellington

Abstract

There is strong evidence that tobacco smoke inhibits both MAO A and MAO B isoforms in the body. However, which components of cigarette smoke are responsible for MAO inhibition is not clear yet. Our group has identified six previously unidentified candidate MAO inhibitors from the tobacco smoke. The MAO inhibitory effects of these candidate inhibitors were compared with that of nicotine and TPM (Tobacco Particulate Matter). An SH-SY5Y cell line was exposed to different regimens of ethanol (control), nicotine, TPM and the cocktail of candidate inhibitors. A final concentration 0.2 µM nicotine was used and the concentration of each candidate inhibitor was relative to that originally found in TPM. We found that nicotine did not have any significant MAO inhibitory effect compared to the control. TPM inhibited overall MAO activity by 39%, while the MAO inhibition by cocktail of candidate inhibitors was 47%. The results suggest that the candidate inhibitors identified by our group are the major contributors to the total MAO inhibitory activity depicted by cigarette smoke and potentially unlocks the mystery behind the components responsible for MAO inhibition by cigarette smoke in smokers

Keywords: Smoking, Monoamine oxidase, MAO inhibitors, MAO A, MAO B

Materials and Methods

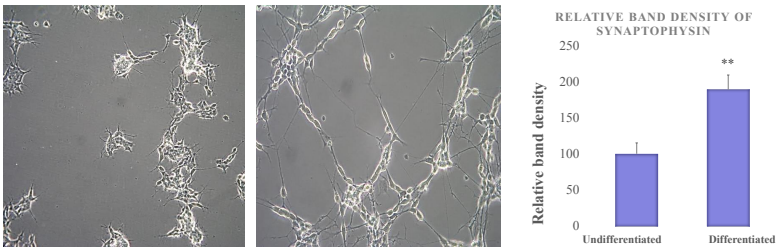
Cell culture and differentiation: Human neuroblastoma cell sub-line SH-SY5Y was cultured in RPMI 1640 medium with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin and maintained at 37˚C in a humidified atmosphere of 5% carbon dioxide (CO2). The cells were differentiated using 10 µM retinoic acid in RPMI Medium containing 3% FBS and 1% Penicillin-Streptomycin for a period of 7 days. The media was replaced with fresh media every other day. The confirmation of differentiation was done using cell images showing neurites and western blot to check the level of synaptophysin, which is a differentiation marker

Exposure to treatment groups: Cultured and differentiated SH-SY5Y cells were exposed to 4 different groups treatment (Ethanol, Nicotine, TPM and Compound cocktail) for 1, 3, 5 and 7 days. 3 replicates for each treatment group for each of 1-, 3-, 5- and 7-days time period were taken. The control group contained 0.05% ethanol, nicotine group contained 0.2 µM nicotine as it was physiologically relevant, TPM group contained concentration of TPM matched such that final solution contained 0.2 µM nicotine and the Compound cocktail group contained candidate inhibitors with concentration of each candidate inhibitor relative to that originally found in TPM. Cell lysates were collected after the end of exposure regimen to perform MAO assay and Western blot

MAO Assay: 25 µL of lysates from different exposure groups was added in the wells of 96 well plate. 25 µL aliquots of samples were taken in an Eppendorf tube that contained 217.5 µL of sodium phosphate buffer (50 mM sodium phosphate, 150 mM sodium chloride, pH 7.4). 7.5 µL of 3mM kynuramine hydrobromide was then added to each of the reaction tubes and incubated for 30 minutes at 37°C. 75µL of 0.4 M perchloric acid was then added to terminate the reaction and the tubes were centrifuged at 13,000 x g for 30 sec at 4°C in an benchtop centrifuge. 100 µL aliquots of supernatant was then pipetted in triplicates to the wells of black polystyrene 96 well plates and 50 µL of 2 M NaOH was added. Fluorescence was measured immediately using spectrophotometer at 318 nm excitation and 380 nm emission wavelength.

Western blot: Western blot was performed to check protein levels for MAO A and MAO B in different treatment groups using HRP conjugated Anti-Monoamine oxidase A and Anti-Monoamine oxidase B respectively primary antibodies respectively.

Results



1a 1b 1c

Fig 1. Confirmation of Cell differentiation: The confirmation of differentiation was done using cell images of undifferentiated (1a) and differentiated cells (1b) and western blot (1c) to confirm the protein levels of synaptophysin.

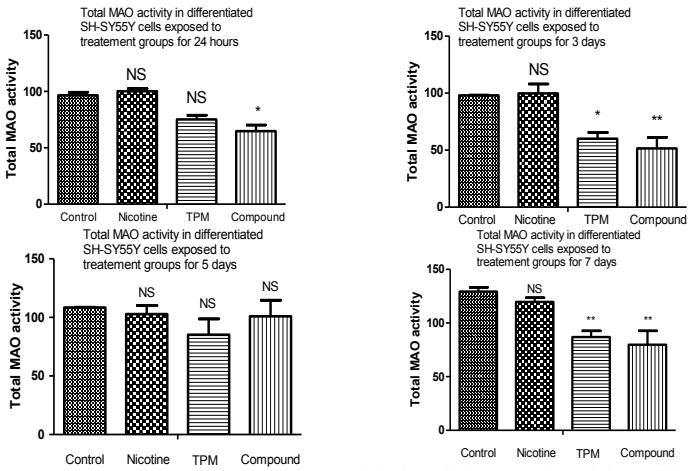


Fig 2. Total MAO activity: Total MAO activity in cell lysates collected from differentiated cells exposed to Ethanol, Nicotine, TPM and Compounds for a period of 1, 3, 5 and 7 days. 2

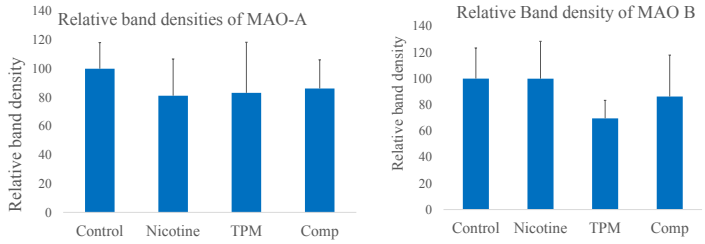


Fig 3. Western blot: MAO-A and MAO-B protein levels in cell lysates collected from differentiated cells exposed to Ethanol, Nicotine, TPM and compound for 3 days.

Discussion

The SH-SY5Y cell line is a commonly used neuronal cell, as it exerts several biochemical and functional characteristics of a neuron. Differentiation process enables SH-SY5Y cells to reduce proliferation and transform to neuronal phenotype that have morphological and biochemical properties similar to the neurons in the living brain. We have confirmed differentiation using cell images showing extended neurite in differentiated cells compared to undifferentiated cells and using western blot showing significant increase in neuronal marker synaptophysin in differentiated cells compared to undifferentiated cells. The results of MAO assay showed a tendency of decrease in MAO activity in TPM and Compound group from day 1 but only the later was statistically significant. There is significant decrease in total MAO activity in TPM group (39%) and compound group (47%) when exposed for 3 days. There was decrease in activity in the TPM and Compound treatment group in day 7 but was no significant decrease in day 5. Since, the maximal inhibition was found with 3 days of exposure after which the cells started growing on the top of each other, 3 days exposure was selected as optimal time period for further assays. The western blot to determine protein levels of MAO-A and MAO-B using lysates from cells exposed to different treatment groups however did not show significant differences.

Conclusion

The candidate inhibitors identified by our group significantly decreases the MAO activity to a comparable level as shown by TPM and hence, can be considered as major components responsible for MAO inhibition by cigarette smoke. Further research is required to completely elucidate the mechanism behind the MAO inhibition by the candidate MAO inhibitors, which might be helpful in better understanding smoking

Monoamine oxidase (MAO) inhibitory effects of candidate MAO inhibitors found in cigarette smoke.

Niraula, P

2021-11-23