THPEA034: DIFFERENCES BETWEEN INTEGRASE STRAND TRANSFER INHIBITORS ON GLUCOSE TOLERANCE: A ROLE FOR MITOCHONDRIAL STRESS

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BACKGROUND

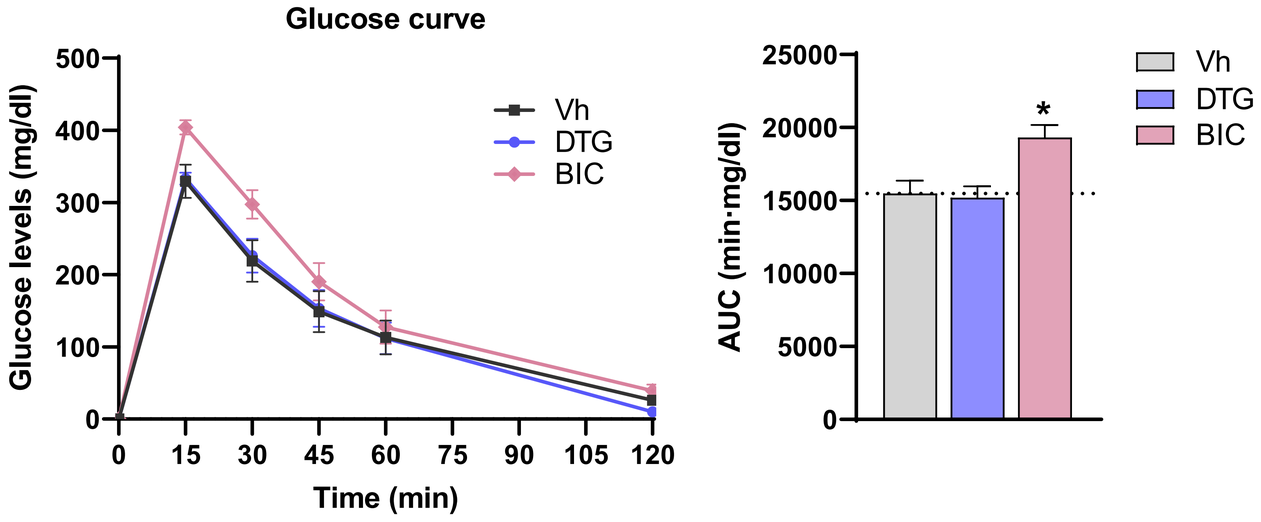
An association between integrase strand transfer inhibitors and metabolic alterations has been suggested. This study assesses the impact of these antiretroviral (ARV) drugs on glucose metabolism *in vitro and in vivo* and evaluates mitochondrial stress as a plausible underlying mechanism.

METHODS

C57BL/6J mice received a 16-week oral treatment, with dolutegravir (DTG, 10mg/kg), bictegravir (BIC, 10mg/kg), or vehicle (Vh) at doses equivalent to those used in humans, followed by glucose tolerance tests (GTT). RNA-sequencing was conducted on liver samples from these mice, and the enrichment analysis was carried out using bioinformatics. *In vitro* studies were conducted with the human hepatoma cell line Hep3B exposed to clinically relevant concentrations (1, 10, 20µM) of DTG or BIC for 48 hours. Assessments included glucose uptake, gene expression of enzymes related to glucose homeostasis (RT-qPCR), cell viability (mitochondrial dehydrogenase activity, acid phosphatase assay), and mitochondrial stress (mitochondrial membrane potential, ROS production; by flow cytometry). Statistical analysis used one-way ANOVA (n≥5).

RESULTS

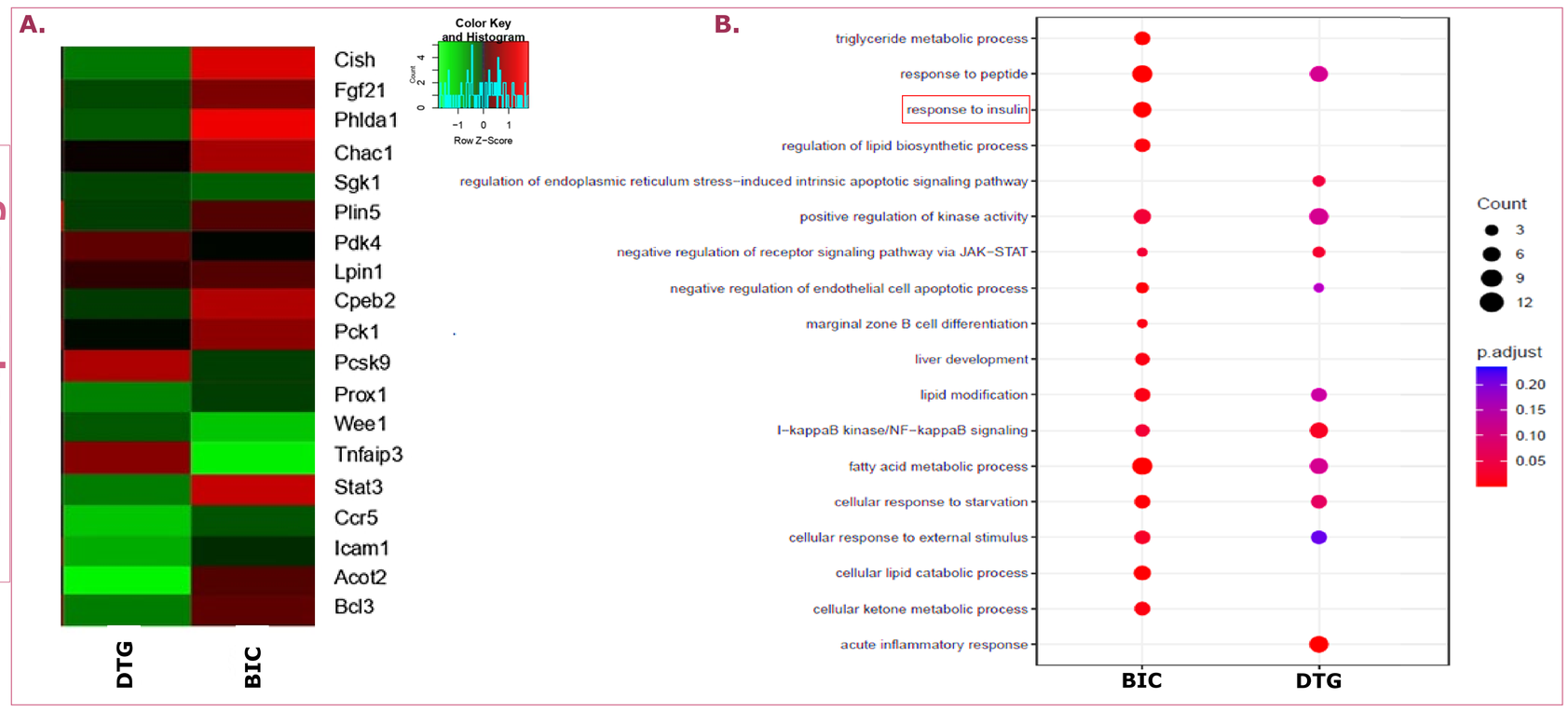
Glucose Tolerance Test (GTT)



Mice treated with BIC exhibited a higher glucose levels and a slower decrease of glucose during GTT.

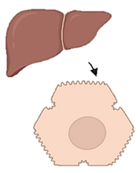
Figure 1. **Results of glucose tolerance test in mice treated with ARVs. A.** Curve of glucose levels after intraperitoneal injection of glucose (data normalized by subtracting the value at time 0; points represent the mean ± SEM). **B.** Area Under Curve (AUC) of the glucose levels. Mean of vehicle dotted line. One way ANOVA * p< 0.05 *versus* vehicle (n=5).

RNA sequencing

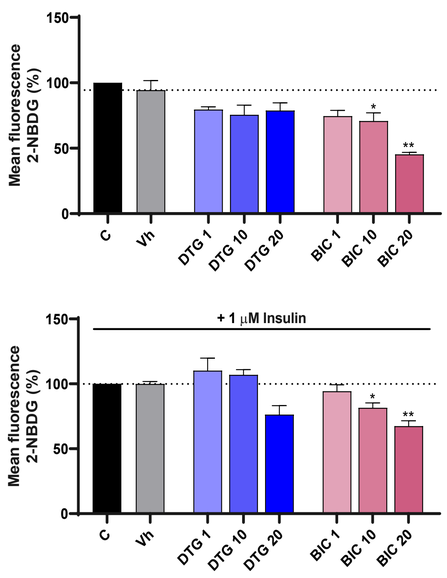


DTG and BIC affected metabolism-related processes. Only BIC altered insulin cellular response, linked to changes in the mRNA expression of *Pdk4, Sgk1, Lpin1, Pck1, Cish, Cpeb2,* and *Pcsk9*.

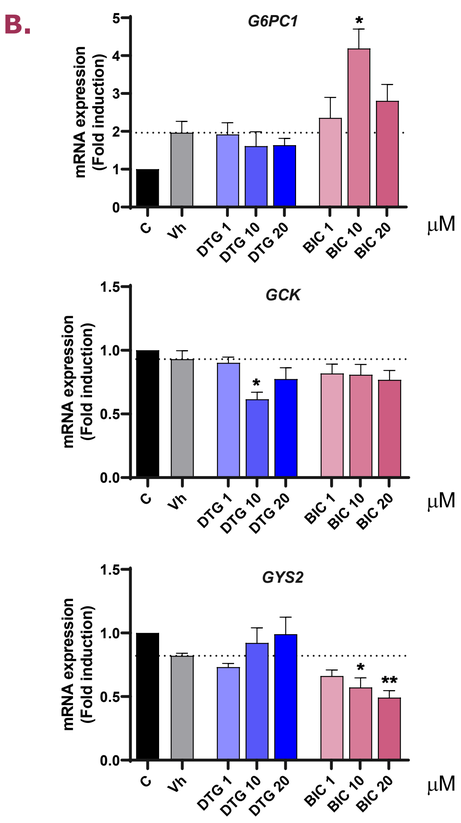
Figure 2. RNA sequencing on mouse liver tissue. A. Heatmap (row Z-Score) differential expression of genes related to metabolism and inflammation. **B.** Enrichment analysis dot plot showing selected key Biological Processes (BP) from Gene Ontology (GO) altered by BIC, associated with cellular stress and metabolism. Color represents the adjusted p-value, and dot size represents the count. Counts represent the number of genes associated with each GO term affected by the drug.



Glucose uptake



Glucose metabolism

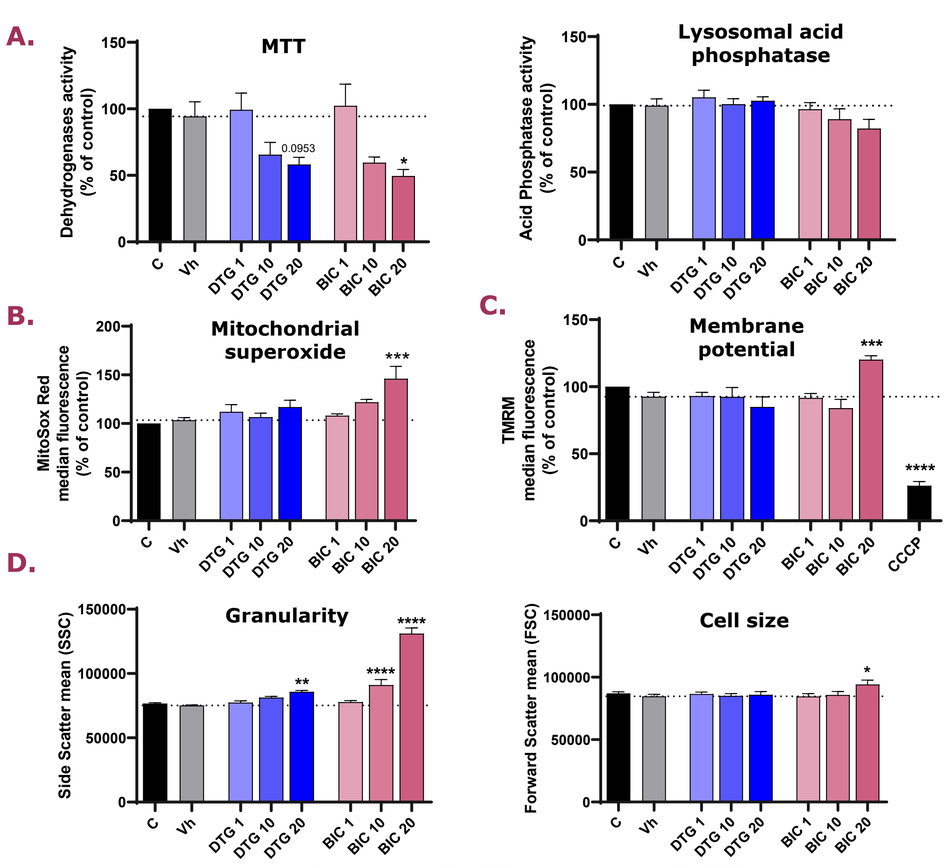


BIC reduced basal and post-insulin glucose uptake in hepatocytes

BIC revealed significant alterations in gene expression of enzymes implicated in glucose synthesis (*G6PC1*), glycolysis (*GKC*), or glycogen storage (*GYS2*).

Figure 3. Glucose uptake and metabolism in ARV-treated Hep3B cells. A. 2-NBDG glucose uptake of Hep3B cells in basal and post-insulin stimulation conditions after 48h-incubation. Data show the mean ± SEM (n=3) and statistical analysis was performed by one-way ANOVA with Tukey *post-hoc*. Mean of vehicle dotted line. *p<0.05 or **p<0.01 *versus* vehicle. **B.** Relative mRNA expression (fold induction *versus* control) of different genes related to glucose. Data show the mean ± SEM (n=5) and *ACTB* was used to normalize the expression data. Mean of vehicle dotted line. Statistically significant differences were evaluated with one-way ANOVA with Tukey *post-hoc* analysis or Kruskal-Wallis with Dunn's *post-hoc* analysis, as appropriate. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 *versus* vehicle.

Cell viability & mitochondrial function



Both drugs reduced mitochondrial dehydrogenase activity, without affecting lysosomal acid phosphatase activity. BIC, but not DTG, increased ROS generation, mitochondrial membrane potential, and cellular granularity, indicating mitochondrial stress.

Figure 4. Cell viability and mitochondrial function in Hep3B cells treated. A. MTT (dehydrogenases activity) assay and lysosomal acid phosphatase assay performed in Hep3B cells treated for 48h. **B.** Mitochondrial superoxide levels assessed in cells stained with MitoSox. **C.** Mitochondrial membrane potential (ΔΨM) state (TMRM). CCCP was used as a negative control. **D.** Cell size (forward scatter, FSC) and granularity (side scatter, SSC). In all graphs, data show the mean ± SEM (n=4-5) and were analyzed by one-way ANOVA with Tukey post-hoc. *p<0.05, **p<0.01, ***p<0.001 or ****p<0.0001 *versus* vehicle. Mean of vehicle dotted line. TMRM: Tetramethylrhodamine methyl ester; CCCP: Carbonyl cyanide 3-chlorophenylhydrazone.

CONCLUSION

Sustained administration of BIC induces gene alterations in the cellular response mechanism to insulin and impairs glucose tolerance in mice. Furthermore, it exerts a more significant influence than DTG on glucose metabolism and mitochondrial function in hepatocytes *in vitro*. These results may help clarify clinical reports associating BIC with changes in glucose metabolism.

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