Immunosuppressive drugs (6-MP, MPA, Rapa) act as modulators of metabolic reprogramming in Jurkat T cell line

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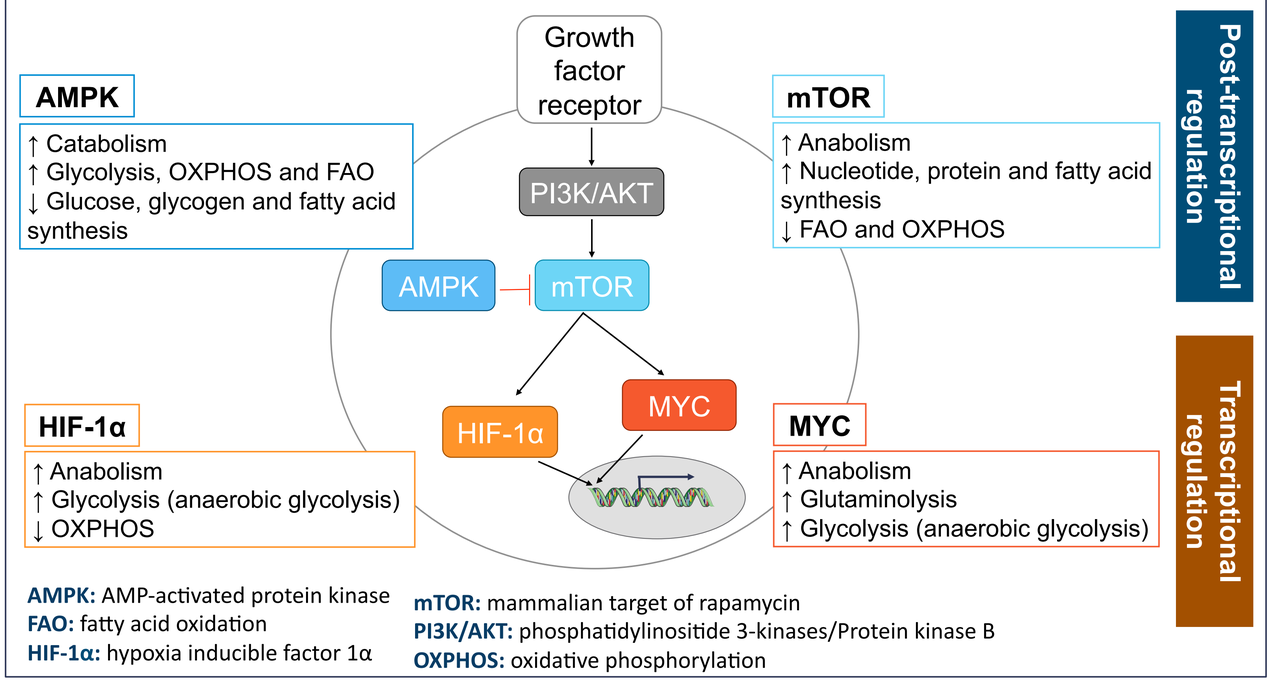
Introduction and Objectives

Metabolic reprogramming is critical for T cell fate and polarization. Naïve T cells rely more into oxidative phosphorylation but proliferative T cells use aerobic glycolysis because it supports rapid cell proliferation and growth [1,2]. This metabolic shift is regulated through different metabolic checkpoints, including Myc, HIF-1α, AMPK and mTOR [3]. Thus, pharmacological inhibition of mTORC1 pathway by rapamycin (Rapa), an immunosuppressive drug, decreases the glycolytic metabolism in T cells and the critical role of mTORC1 in T cell differentiation is now well established. Our objective was to determine the effects of the immunosuppressive drugs 6-mercaptopurine (6-MP), mycophenolic acid (MPA) and Rapa on the metabolism of proliferating T cells.

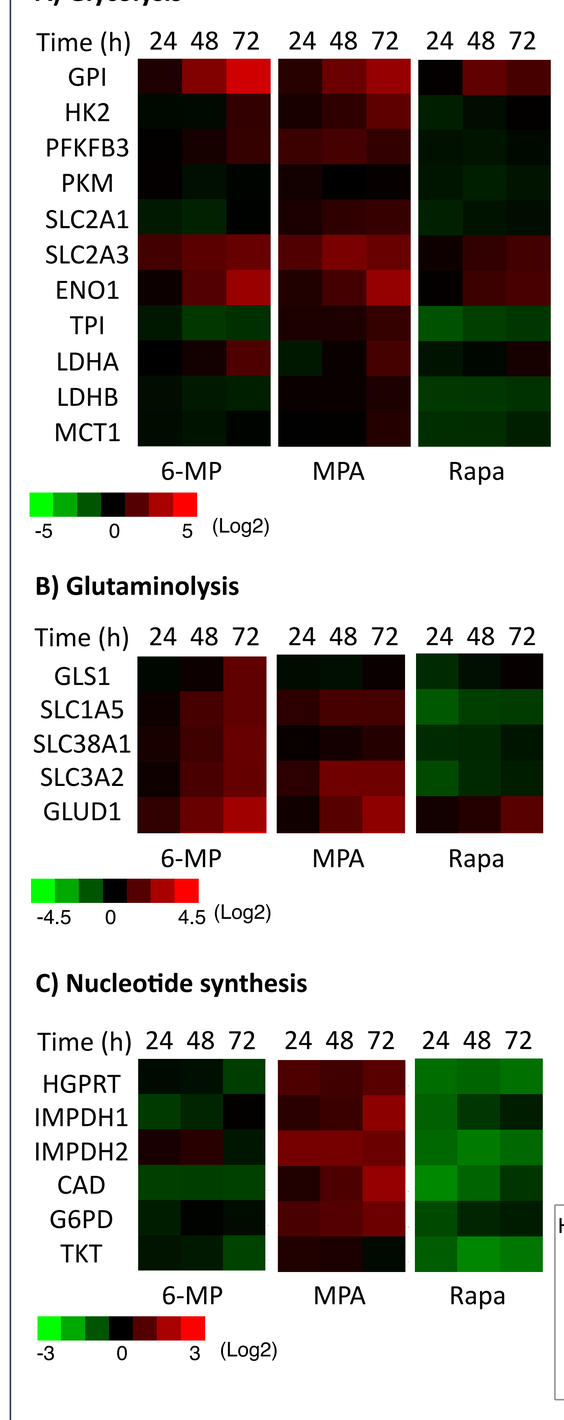


AMPK: AMP-activated protein kinase
FAO: fatty acid oxidation
HIF-1α: hypoxia inducible factor 1α
mTOR: mammalian target of rapamycin
PI3K/AKT: phosphatidylinositide 3-kinases/Protein kinase B
OXPHOS: oxidative phosphorylation

Methods

In vitro experiments were performed on the Jurkat T cell line incubated with 6-MP, MPA and Rapa from 24 to 72 hours. We used RT-PCR, Western Blot, glucose uptake, glycolytic and glutaminolytic flux experiments and lactate and ATP dosage.

Results and Conclusions



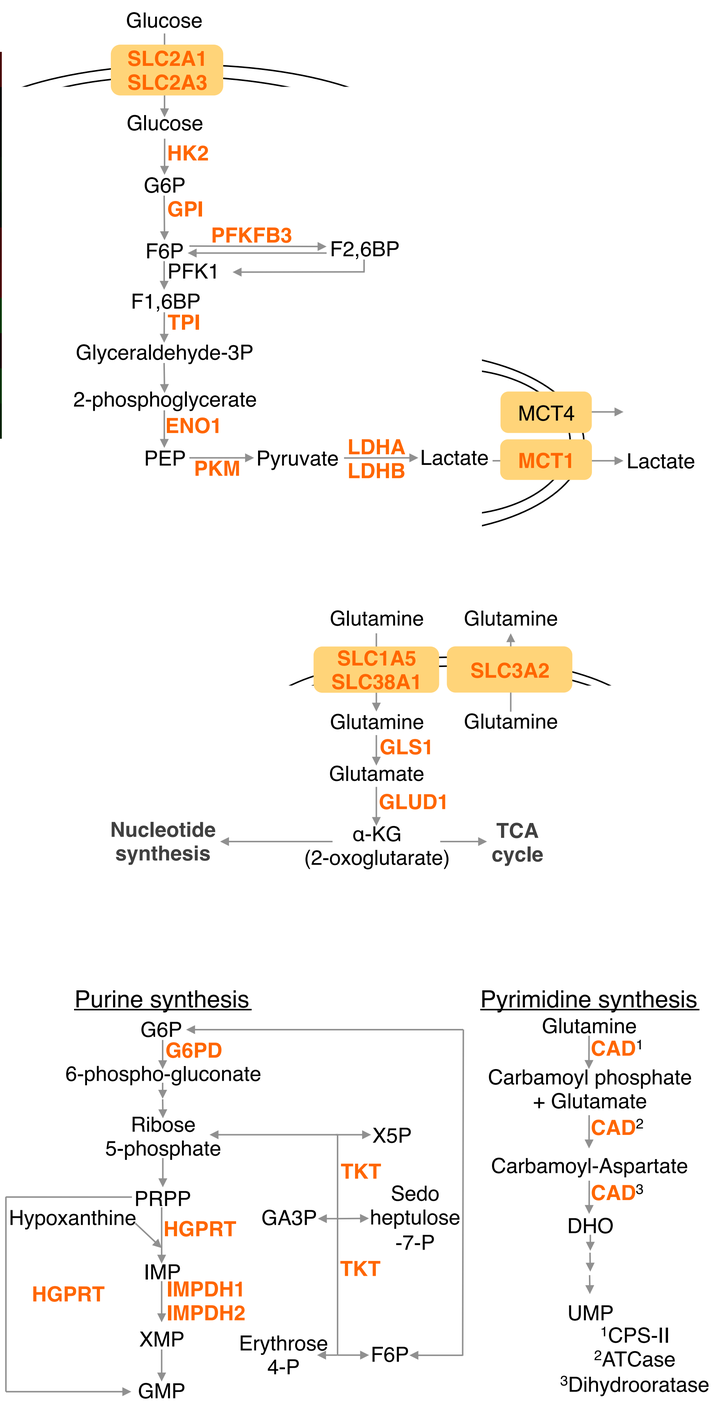


Fig. 1. 6-MP, MPA and Rapa modify the expression of genes implicated in glycolysis, glutaminolysis and nucleotide synthesis. (Left) Heat map representation of a transcriptomic profile of genes implicated in **A)** glycolysis; **B)** glutaminolysis; and **C)** nucleotide synthesis after 24, 48 or 72 h of incubation with 50µM 6-MP, 0.5µM MPA or 5µM Rapa on Jurkat T cell line. (Right) Schematic representation of genes implicated in glycolysis, glutaminolysis and nucleotide synthesis. Data represent three independent experiments.

ATCase: aspartate carbamoyltransferase; CAD: carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase; CPS-II: carbamoyl phosphate synthetase II; DHO: dihydroorotate; ENO1: enolase 1; F1,6BP: fructose1,6-biphosphate; F6P: fructose-6-phosphate; G6P: glucose-6-phosphate; G6PD: glucose-6-phosphate dehydrogenase: GA3P: glyceraldehyde 3-phosphate; GLS1: glutaminase 1; GLUD1: glutamate dehydrogenase 1; GMP: guanosine monophosphate; GPI: glucose-6-phosphate isomerase; HGPRT: hypoxanthine-guanine phosphoribosyltransferase; HK2: hexokinase II; IMP: inosine monophosphate; IMPDH1: inosine 5'-monophosphate dehydrogenase 1; IMPDH2: inosine 5'-monophosphate dehydrogenase 2; LDHA: lactate dehydrogenase A; LDHB: lactate dehydrogenase B; MCT1: monocarboxylate transporter 1; MCT4: monocarboxylate transporter 4; PEP: phosphoenolpyruvate; PKFKB3: 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PKM: pyruvate kinase muscle; PRPP: phosphoribosyl pyrophosphate; SLC1A5: solute carrier family 1, member 5; SLC2A1: solute carrier family 2, member 1; SLC2A3: solute carrier family 2, member 3; SLC38A1: solute carrier family 38, member 1; SLC3A2: solute carrier family 3, member 2; TCA cycle: tricarboxylic acid; TKT: transketolase; TPI: triosephosphate isomerase; UMP: uridine monophosphate; X5P: xylulose-5-phosphate.

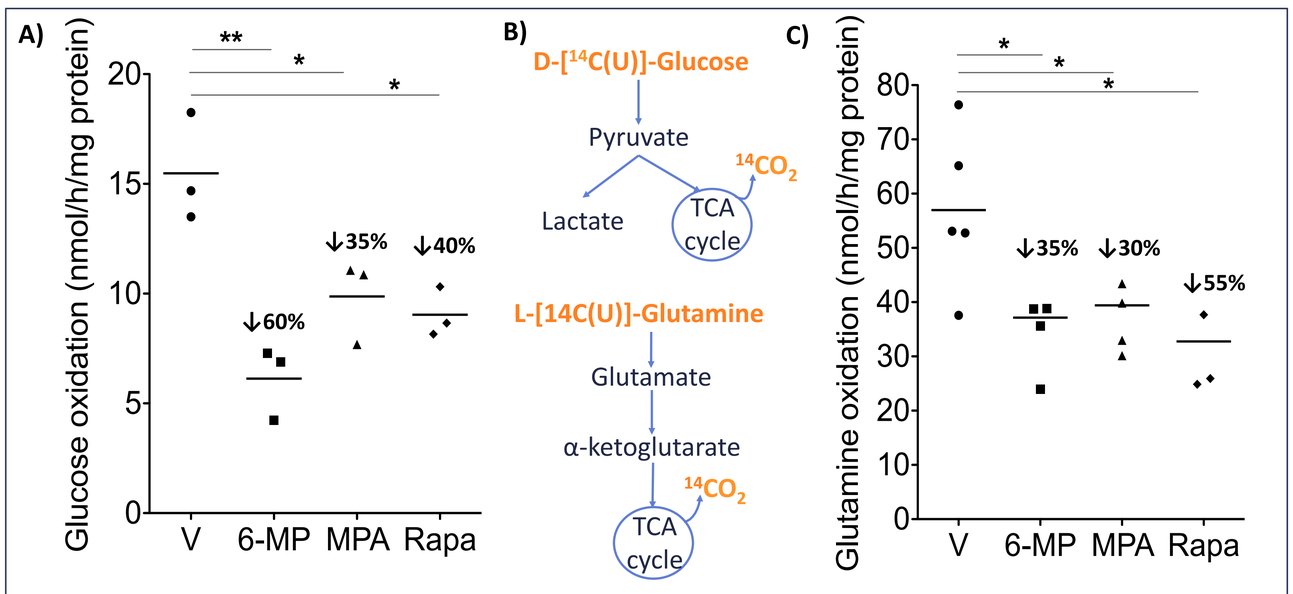


Fig. 2. 6-MP, MPA and Rapa decrease glycolytic and glutaminolytic flux. **A)** Glycolytic; and **C)** glutaminolytic flux after 48 hours of treatment by 50 µM 6-MP, 0.5 µM MPA, 5 µM Rapa or vehicle (V) in a Jurkat T cell line. The generation of $^{14}CO_2$ from ^{14}C-glucose (**A**) or from ^{14}C-glutamine (**C**) was analysed. **B)** Up, schematic representation of the procedure for glucose oxidation and, down, for glutamine oxidation. Data represent four independent experiments. *p<0.05, **p<0.001, Mann-Whitney test.

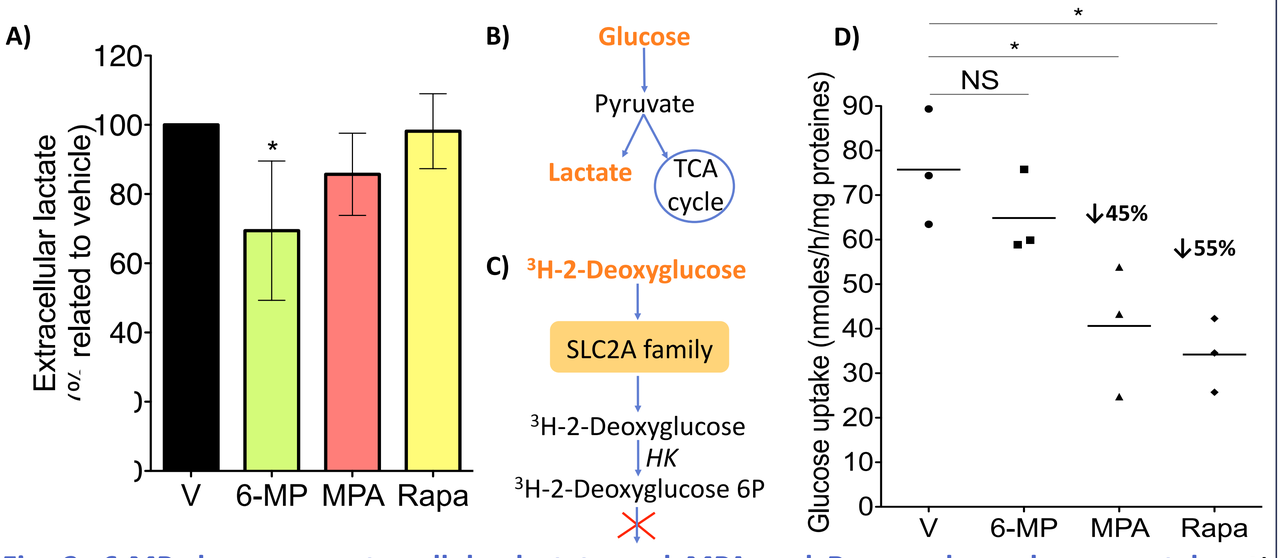


Fig. 3. 6-MP decreases extracellular lactate and MPA and Rapa reduce glucose uptake. **A)** Extracellular lactate production after incubation for 48 hours with 50 µM 6-MP, 0.5 µM MPA, 5 µM Rapa or vehicle (V) in Jurkat T cell line. **B)** Schematic representation of the protocol. Data represent four independent experiments. *p<0.05, Mann-Whitney test. **C)** Schematic representation of glucose uptake protocol. **D)** Glucose uptake after incubation for 48 hours with 50 µM 6-MP, 0.5 µM MPA, 5 µM Rapa or vehicle (V). Data represent three independent experiments. *p<0.05, Mann-Whitney test.

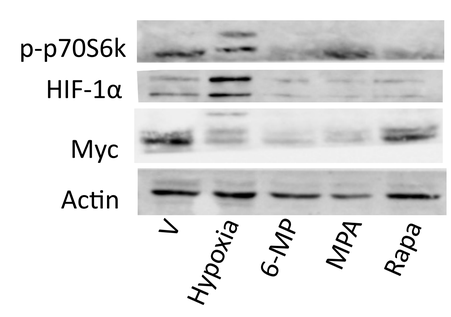


Fig. 4. Modulation of the metabolic checkpoints (mTOR, HIF-1α and Myc) by 6-MP, MPA or Rapa. Immunoblots representing phospho P70S6K, HIF-1α, Myc and actin at the protein level in Jurkat human T-cell line incubated for 48 hours with 50 µM 6-MP, 0.5 µM MPA, 5 µM Rapa or vehicle (V). Hypoxia is used as a control for HIF-1α.

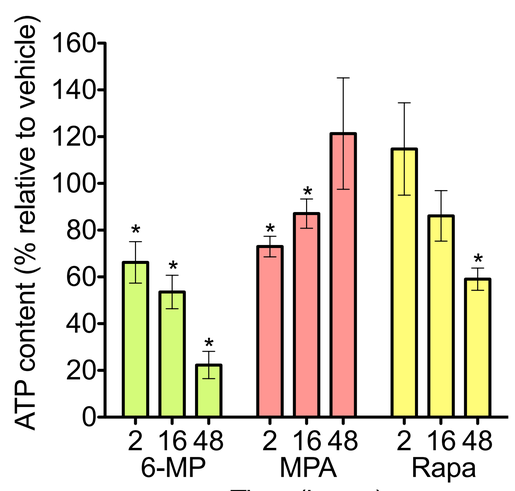
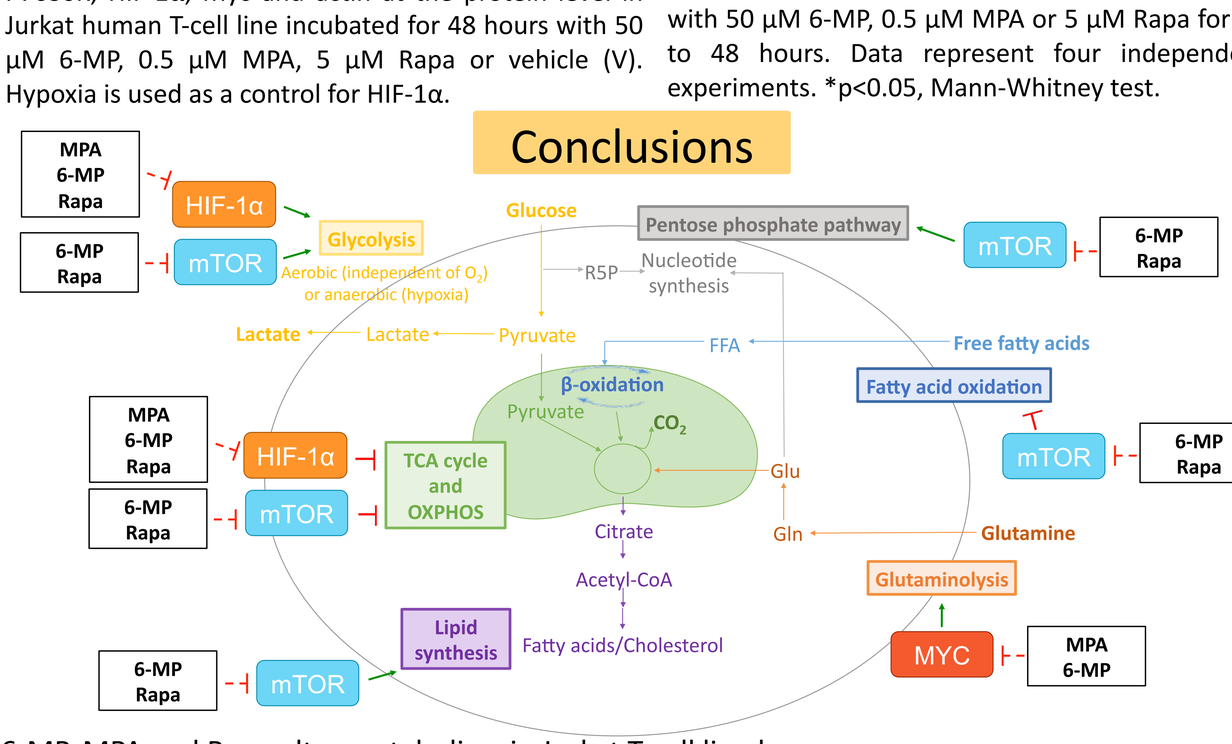


Fig. 5. 6-MP, MPA or Rapa induce ATP depletion. Percentage of ATP in cells incubated with 50 µM 6-MP, 0.5 µM MPA or 5 µM Rapa for up to 48 hours. Data represent four independent experiments. *p<0.05, Mann-Whitney test.

Conclusions



6-MP, MPA and Rapa alter metabolism in Jurkat T-cell line by:

- Modifying transcriptional expression of genes implicated in glycolysis, glutaminolysis and nucleotide synthesis
- Decreasing extracellular lactate and glycolytic and glutaminolytic flux
- Diminishing the metabolic checkpoints mTOR, HIF-1α and Myc at the protein level

6-MP, MPA et Rapa profoundly alter the metabolism of proliferating cells. Their efficacy or side effects could be, in part, consequence of modifications in the metabolism of T cells

References

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