Article

Studying the Geroprotective Properties of YAP/TAZ Signaling Inhibitors on *Drosophila melanogaster* Model

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Abstract: The transcriptional coactivators YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) are the main downstream effectors of the evolutionary conserved Hippo signaling pathway. YAP/TAZ is implicated in the transcriptional regulation of target genes that are involved in a wide range of key biological processes affecting tissue homeostasis and play dual roles in the aging process depending on cellular and tissue context. The aim of the present study was to investigate whether pharmacological inhibitors of Yap/Taz may increase the lifespan of *Drosophila melanogaster*. qRT-PCR was performed to measure the changes in the expression level of *Yki* (*Yorkie*, the *Drosophila* homolog of YAP/TAZ) target genes. We have revealed a lifespan increasing effect of YAP/TAZ inhibitors that was mostly associated with decreased expression level of *wg* and *E2f1* genes. However further analysis is required to understand how YAP/TAZ pathway is linked with aging.

Keywords: YAP/TAZ; *Drosophila*; Aging; Geroprotector

1. Introduction

With the increase in the average age of the population the problem of preventing premature aging and treating age-related diseases comes to the fore in modern healthcare [1-3]. The promising approach to achieve this goal is to influence the major molecular mechanisms associated with aging which is the main risk factor for age-related diseases, in order to suppress pathological processes and activation of the defense systems of the cell and the body as a whole [4-6].

Recent studies demonstrate that damage to long-lived macromolecules, including extracellular matrix (ECM) proteins, make a significant contribution to the aging process [7,8]. The accumulation of non-enzymatic modifications by glycation, oxidation, and crosslinking of collagen and elastin, the major components of the ECM, occurs during aging [7,9]. The modifications of macromolecules affect the structural and physical properties of the ECM that increase the stiffness of tissues and reduce their viscoelasticity [10-13]. High ECM stiffness promotes activation of YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif), the Hippo pathway effectors [14], that play a key role in the regulation of tissue homeostasis [15]. ECM stiffness has been shown to regulate TAZ/YAP-driven gene transcription independent of the Hippo pathway [16] and make a significant contribution to the deregulation of tissue homeostasis during aging [17]. Due to YAP/TAZ regulates target genes involved in a wide range of key biological processes, such as modulation of nuclear integrity and functions [18], stem cell differentiation [19], cell proliferation [20], regeneration [21], innate immune response

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[22], and tumorigenesis [23], YAP/TAZ activity plays dual roles in the aging process depending on cellular and tissue context[17,18]. For example, reduced expression of connective tissue growth factor (CTGF), an established YAP/TAZ target gene, which is involved in tissue remodeling, has been reported to mediate collagen loss in chronologically-aged human skin [24], whereas persistent activation of CTGF can result in increased deposition of collagen and fibrotic conditions [25].

Despite a large amount of experimental data have demonstrated decrease of YAP/TAZ activity during physiological aging [17,21,22,26], number of studies have shown that, several compounds with proven anti aging properties such as resveratrol [27], rapamycin [28] and metformin [29] inhibit transcriptional activity of YAP/TAZ in cancer cells [17]. However, the effect of geroprotectors on YAP/TAZ activity in normal tissues have not yet been studied.

Drosophila melanogaster is one of the most studied and genetically tractable model organisms for investigating the mechanisms of aging and antiaging interventions [30,31] using evolutionarily conserved aging-related signaling pathways as potential drug targets [32,33]. *Drosophila* Yorkie (Yki) is a homolog of mammalian YAP/TAZ [34] that allows the use of fly models in studies of the geroprotective properties of Yap/Taz inhibitors.

The aim of this study was to investigate whether pharmacological inhibitors of Yki/Yap/Taz may improve the survival of *D. melanogaster*. In this study we investigated the effects of pharmacological inhibitors of Yki/Yap/Taz on the survival of *D. melanogaster*. The following substances with previously established inhibitor effects on Yap/Taz activity were used: Verteporfin [35-37], ML-7 hydrochloride [38], Cytochalasin D [39-41], and AICAR [42].

2. Results

2.1. Expression Levels of YAP/TAZ Target Genes

To study whether the expression level of Yki target genes was changed with aging, the qRT-PCR was performed for male and female flies at the age of 10 and 20 days (Figure 1, Table S2). Two-way ANOVA analysis (gene × age) showed a significant effect of the gene ($p<0.001$), a significant effect of the age ($p<0.05$), and a significant interaction ($p<0.001$) in male and female flies (Figure 1, Table S3). A Duncan post-test of the 10-day-old flies versus 20-day-old ones within each gene revealed a significant ($p<0.05$) age-related decrease in the expression level of *CycE*, *dally*, *Diap1* in males and *CycE*, *dally*, *myc* in females, but age-related increase in the expression level of *wg* gene in females ($p<0.01$).

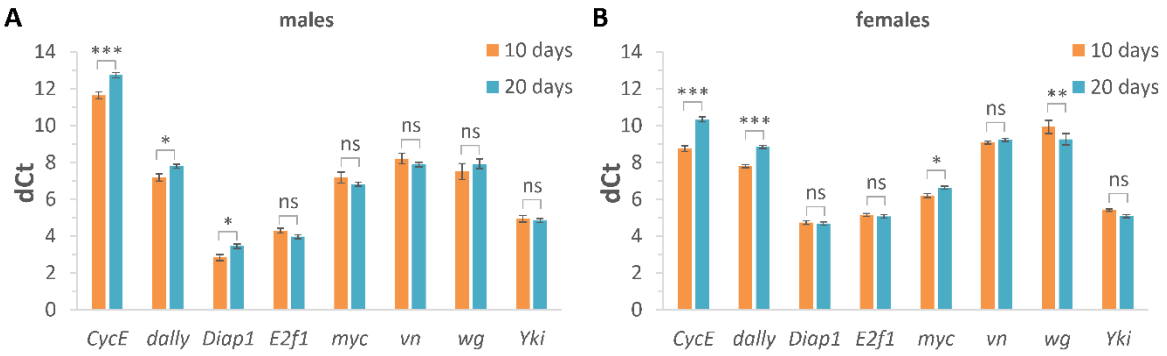

Figure 1. Age-related changes in the expression level of Yki target genes. Estimation of the expression level in males (**A**) and females (**B**) at the age of 10 and 20 days. Two-way ANOVA (age × gene) followed by post hoc Duncan test was used to compare differences in expression levels of genes between 10-day-old and 20-day-old flies, *p<0.05, **p<0.01, ***p<0.001, ns – not significant. The Ct (cycle thresholds) values are inversely proportional to the mRNA transcript levels. The delta Ct (dCt) values were calculated as the differences in Ct values for target genes and reference genes (*β-Tubulin* and *RpL32*). Higher dCt values represent a lower expression level. The error bars show standard errors.

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These results are consistent with previously published data which have demonstrated age-related decrease in transcriptional activity of YAP/TAZ [17,21,26].

To assess whether the treatment with various concentrations of substances was associated with inhibition of YAP/TAZ transcriptional activity, the expression level of Yki target genes was compared between treated and control groups of different ages within each inhibitor.

Two-way ANOVA (gene × concentration) showed that there were significant differences in expression level among different genes ($p<0.001$, source of variation: gene). The ANOVA also revealed significant differences in gene expression between the control animals and animals treated with inhibitors at different concentrations ($p<0.05$, source of variation: concentration), except males and females treated with AI. Additionally, the ANOVA demonstrated that effects of the inhibitor treatment depended on the gene ($p<0.001$, source of variation: interaction), except males treated with CD (Figure 2, Tables S4).

Duncan test of the inhibitor-treated flies versus control ones within each substance in male flies revealed a significant ($p<0.05$) decrease in the expression level of *CycE* (0.01 μM ML7), *Diap1* (1 μM VP and 1 μM CD), *E2f1* (0.1-1 μM CD), *wg* (0.01-10 μM VP; 0.1 μM ML7; 1 μM CD), *Yki* (1 μM CD), but increase ($p<0.05$) in the expression of *CycE* (0.01 μM VP; 0.1 μM AI), *myc* (0.01-10 μM VP), *vn* (0.01-10 μM VP), *wg* (1 μM ML7), *Yki* (0.01 and 10 μM VP) (Figure 2, Tables S5, S6). At the same time the post-hoc pairwise comparison of gene expression level in treated and control females demonstrated decrease ($p<0.05$) in the expression of *CycE* (0.01 μM VP), *vn* (1 μM VP), *wg* (1 μM ML7; 0.1-1 μM CD; 0.1 μM ML7), but increase ($p<0.05$) in the expression of *CycE* (0.01 μM ML7; 0.1 μM CD), *myc* (0.1 μM CD), *vn* (10 μM VP), *wg* (1-10 μM VP), *Yki* (10 μM VP; 0.1-1 μM AI) (Figure 2, Tables S5, S6). All inhibitors have shown the ability to suppress the expression of at least one gene. The most characteristic effect of used inhibitors is the suppression of *wg* gene expression (except for females treated with VP).

The observed differences in the expression level between different genes in individuals of the same sex and between the same genes in males and females are consistent with transcriptional data from FlyAtlas2 database [43], and reflect sex differences in gene expression of most of the studied genes, including Yki target genes.

To determine whether 20 days of treatment is more effective than 10 days of treatment for inhibition of YAP/TAZ transcriptional activity, the expression level of Yki target genes was compared between treated and control groups at the age of 20 days.

Two-way ANOVA (gene × concentration) showed that there was a significant effect of the gene ($p<0.001$), a significant effect of the concentration ($p<0.05$, except males treated with VP, ML7, AI), and a significant interaction ($p<0.05$, except males treated with AI and females treated with ML7, CD, AI) in 20-day-old flies (Figure S1, Tables S7).

Duncan post-test of the 20-day-old flies versus ones within each gene revealed a significant ($p<0.05$) decrease in expression level of *CycE* gene in female flies treated with VP (1 μM) and with CD (0.01 μM), but increased in males treated with ML7 (1 μM). The Duncan's test also demonstrated decrease ($p<0.05$) in expression of *wg* gene in males and females treated with VP (0.01 μM and 1 μM, respectively) and with ML7 (0.01 μM) as well as in females treated with CD (0.1 μM). However, *wg* expression increased in males treated with CD (0.1 μM) and females treated with VP (10 μM) and AI (0.1 μM).

Thus among four Yap/Taz inhibitors the most pronounced effects on gene expression levels were detected in flies at age of 10 days. Based on the obtained results we used concentration which showed the most significant effect on gene expression to determine the effect of substances on *Drosophila melanogaster* lifespan.

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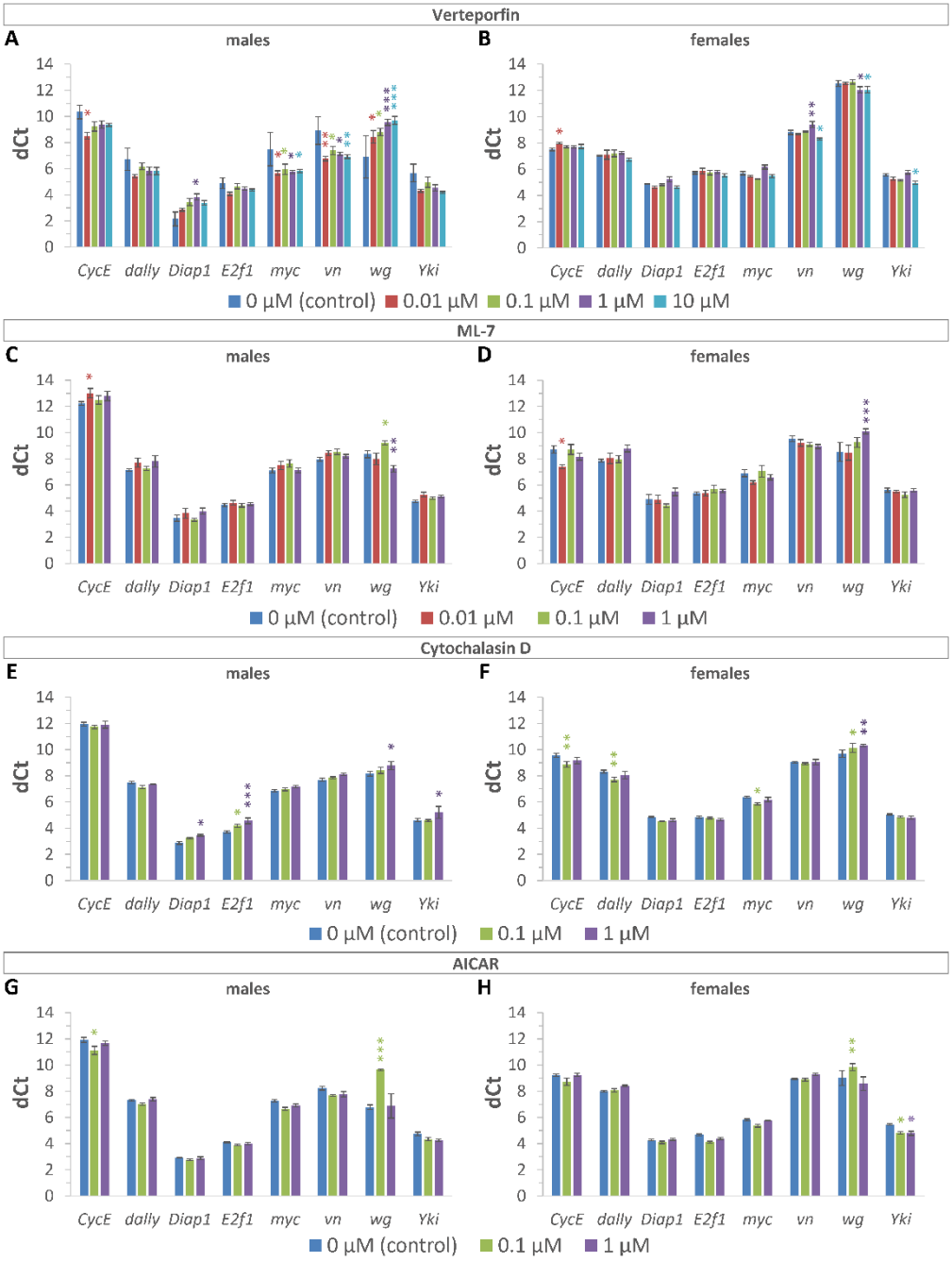

Figure 2. Effects of YAP/TAZ inhibitors on the expression level of Yki target genes. Estimation of the expression level in 10 day-old males and females after treatment with VP (**A**, **B**), ML7 (**C**, **D**), CD (**E**, **F**), AI (**G**, **H**). Two-way ANOVA (age × gene) followed by post hoc Duncan test was used to compare differences in expression levels of genes between 10-day-old and 20-day-old flies, *p<0.05, **p<0.01, ***p<0.001. The Ct (cycle thresholds) values are inversely proportional to the mRNA transcript levels. The delta Ct (dCt) values were calculated as the differences in Ct values for target genes and reference genes (*β-Tubulin* and *RpL32*). Higher dCt values represent a lower expression level. The error bars show standard errors.

2.2. Effects on Survival

According to the results of qRT-PCR analysis, the following concentrations of the YAP/TAZ inhibitors were chosen for the survival analysis: 0.01 µM and 0.1 µM – for VP, 0.1 µM and 1 µM – for ML7, CD, and AI. Two independent replicate experiments were completed for each group (Figure S2 and S3, Table S10).

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To reduce incidental effects, we pooled the results of two replicates (Figure 3, Table S11). Using Fisher's Exact test to compare median and maximum lifespan and Fleming-Harrington test to estimate early and later differences between survival curves of treated and control flies, we revealed lifespan effects of YAP/TAZ inhibitors.

VP at concentrations of 0.01 μM and 0.1 μM increased the maximum lifespan (by 2%, $p<0.001$), as well as reduced the rate of early ($p<0.05$) and late mortality ($p<0.001$) in males, but had no effect ($p>0.05$) on the survival of females (Figure 3A and B, Table S11). ML7 at concentration of 1 μM increased late mortality ($p<0.01$) in males, but at concentrations of 0.1 μM and 1 μM reduced late mortality ($p<0.05$) in females (Figure 3C and D, Table S11). CD at concentrations of 0.1 μM and 1 μM increased median (by 3%, $p<0.05$) and maximum (by3%, $p<0.01$) lifespan in males, respectively. However, CD at concentrations of 0.1 μM increased the rate of early mortality ($p<0.01$) in females (Figure 3E and F, Table S11). AI at concentration of 0.1 μM increased maximum lifespan (by 3%, $p<0.05$), and at concentrations of 0.1 μM and 1 μM reduced the rate of early and late mortality ($p<0.001$) in males. AI at concentrations of 0.1 μM and 1 μM increased median lifespan in females by 3%, $p<0.01$ and by 6%, $p<0.001$, respectively, and at concentrations of 1 μM reduced the rate of early ($p<0.001$) and late mortality ($p<0.001$) in females (Figure 3G and H, Table S11).

Thus, *Drosophila* treatment with YAP/TAZ inhibitors decrease late mortality, which indicates a geroprotective potential of this intervention.

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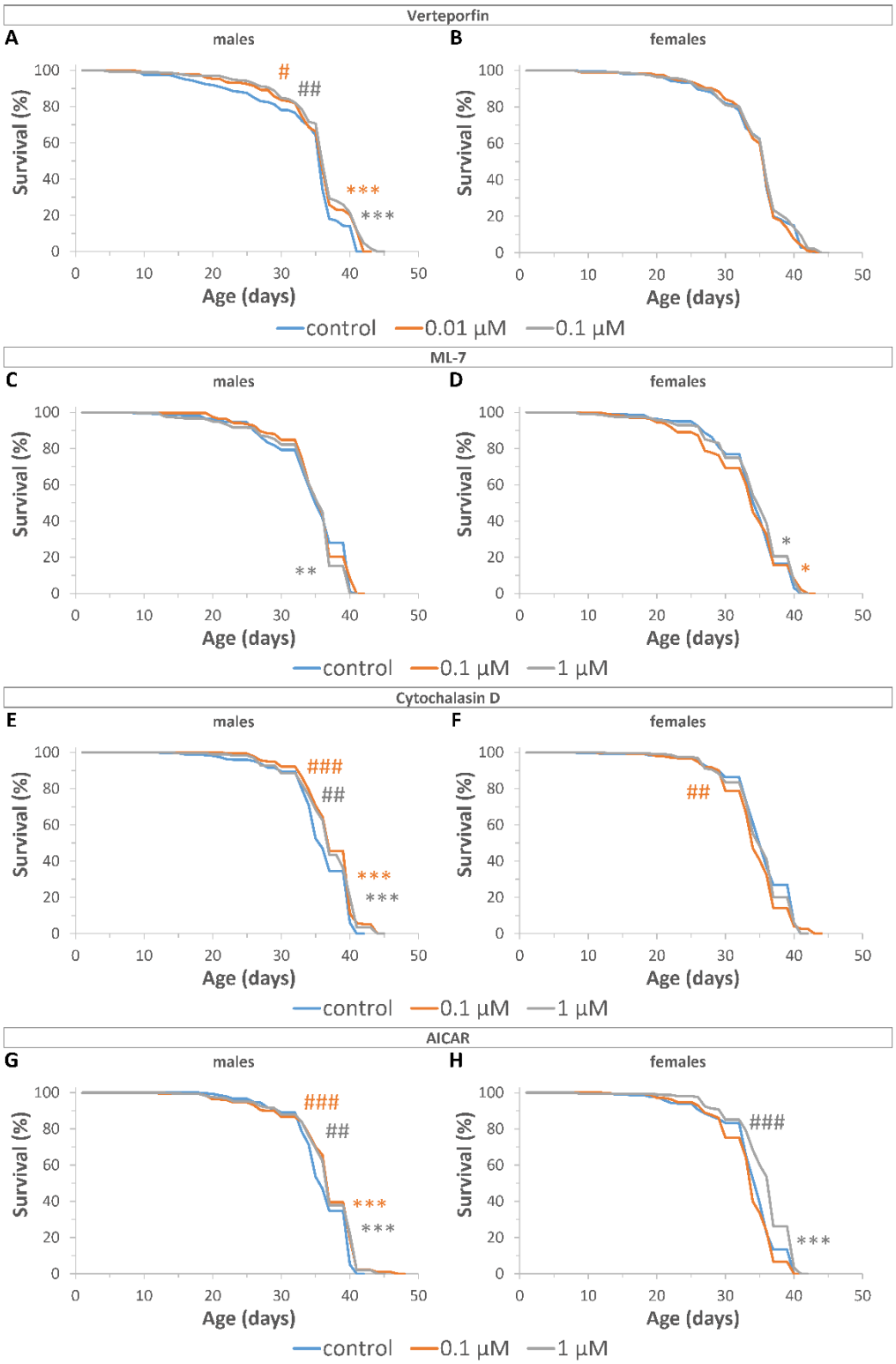

Figure 3. Effects of YAP/TAZ inhibitors on the lifespan of male (**A**, **C**, **E**, **G**) and female (**B**, **D**, **F**, **H**) flies after treatment with VP (**A**, **B**), ML7 (**C**, **D**), CD (**E**, **F**), AI (**G**, **H**). Fleming-Harrington test sensitive against early ($^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$) and later ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$) differences was used to compare survival curves between inhibitor-treated and control flies. Bonferroni correction was used for multiple comparisons.

3. Discussion

Thus, the effects of pharmacological inhibitors of Yki/Yap/Taz on the survival of *D. melanogaster* were investigated in this study. The substances with previously established

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inhibitor effects on Yap/Taz activity, including Verteporfin [35-37] , ML-7 hydrochloride [38], Cytochalasin D [39-41], and AICAR [42] were used.

The inhibitory effect of these compounds on the expression level of Yki/Yap/Taz target genes in *Drosophila* was confirmed using the qRT-PCR test (Figure 2). It should be noted that among the analyzed Yki target genes (*Diap1*, *dally*, *myc*, *wg*, *CycE*, *vn*), not all of them decreased their expression level in response to treatment with inhibitors.

This effect may be associated with tolerance development in response to long-term (10-day) exposure to inhibitors due to possible activation of negative feedback shunts that foster activation of Yki target genes regardless of Yki inhibition. In addition, YAP/TAZ inhibitors may have specificity to *Drosophila* Yki. The decrease in the effect of inhibitors on the expression of target genes after 20 days of treatment also supports the assumption about the development of tolerance to the action of inhibitors.

The set of repressed genes depended on the used inhibitor and on the sex of the fly. Further research is needed to link the change in fly survival to molecular targets. However, according to the obtained results, the increase in survival was most often associated with a decrease in the level of expression of certain genes, namely *wg* and *E2f1*, that may be associated with aging and longevity (Table 1).

For example, the positive effect on survival time in the case of treatment with VP (males: 0.01 μM and 0.1 μM), CD (males: 1 μM), ML7 (females: 1 μM), AI (males: 1 μM) was accompanied by a decrease in the expression level of the *wg* gene. On the contrary, an increase in *wg* expression leads to an increase in mortality in ML7 (1 μM) treated males (Table 1).

The *Drosophila wg* gene is the structural homolog of vertebrate *Wnt* genes [44] , encoding secreted glycoproteins that act as signaling molecules essential for growth, development, and tissue homeostasis [45,46]. Experimental data suggest both a positive and a negative role of the Wnt in cell senescence, aging-associated diseases, and organism longevity [47,48]. For example, the opposing roles of Wnt ligands mom-2/Wnt (pro-aging) and lin-44/Wnt (anti-aging) have been revealed in *Caenorhabditis elegans* [49]. While studies demonstrating increased Wnt signaling in Klotho mouse model of accelerated aging [50] and ameliorated aging-related tissue fibrosis after treatment with Wnt inhibitors [51,52] support the pro-aging function of Wnt signaling. Thus given the dual role of Wnt in aging, the observed mortality-reducing effect may be partly due to its inhibition.

In addition, in CD (0.1 and 1 μM) treated males, a decrease in the level of early and late mortality was accompanied by a decrease in the level of *E2f1* expression (Table 1). RNAi mediated knockdown of *E2f1* was found to increase lifespan in *C. elegans* by FOXO/daf-16 mediated mechanism [53].

The level of transcription of other genes did not show a clear relationship with the survival of the flies (Table 1). In some groups (males and females treated with 1 μM AI, females treated with 1 μM ML), the decrease in mortality was not associated with changes in the expression level of Yki-target genes, suggesting the presence of additional YAP/TAZ-independent activities of these substances.

Indeed, ML7 is widely used as a myosin light-chain kinase (MLCK) inhibitor [54,55]. MLCK is activated by numerous physiological factors and inflammatory or angiogenic mediators, inducing actomyosin contraction and causing endothelial hyperpermeability. Aging is considered as a major risk factor for microvascular dysfunction and hyperpermeability [56] . ML7 has been reported to alleviate advanced glycation end products-induced microvascular hyperpermeability *in vivo* [57] demonstrating the potential to protect against aging. In *Drosophila* suppression of actomyosin contractility via protein kinase A-mediated regulation of MLCK activity has shown to be required for blood-brain barrier integrity [58].

AI is one of the most commonly used pharmacological activators of AMP-activated protein kinase (AMPK) [59]. AMPK is known to control the aging process via an integrated signaling network which affects energy metabolism, autophagic degradation and stress resistance [60]. Metformin-induced AMPK activation in *C. elegans* and mice has been

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demonstrated to increase lifespan by about 20% and 6%, respectively, in comparison with untreated control animals [61]

In addition, aside from Yap/Taz inhibition through preventing YAP/TAZ-TEAD interaction [62] , VP demonstrates antifibrotic effects by reducing the expression of fibrogenic genes in different models [63,64].

Contrary, CD demonstrated pro-aging properties. CD is known as a potent inhibitor of actin cytoskeleton polymerization [65]. Cytoskeletal integrity was found to be closely related with aging and age-associated diseases [66,67] Actin polymerization decreases cell stiffness and lead to cellular aging [68] suggesting possible pro-aging activity of CD.

Considering all data we suggest that Yap/Taz signaling inhibition led to the increasing lifespan of *D. melanogster* mainly through activation of the Wnt *E2f1* signaling pathway. On the other hand we have to emphasize the possible role of the off-target effects of YAP/TAZ inhibitors. Further analysis is therefore required to fully understand how YAP/TAZ pathway is linked with aging. Nonetheless, our results provide an unexpected connection between aging and the well-studied YAP/TAZ signaling pathway and suggest that strategies targeting its activity may provide new approaches to combat aging and prolong lifespan.

Table 1. Overall effects of *Drosophila* treatment with Yap/Taz inhibitors for 10 days.

Inhibitor	C (μM)	Sex	Gene expression								Mortality	
			CycE	*dally*	*Diap1*	*E2f1*	*myc*	*vn*	*wg*	*yki*	Early	Late
VP	**0.01**	♂	↑	0	0	0	↑	↑	↓	↑	↓	↓
	0.1	♂	0	0	0	0	↑	↑	↓	0	0	↓
ML	0.1	♂	0	0	0	0	0	0	↓	0	0	0
	1	♂	0	0	0	0	0	0	↑	0	0	↑
CD	0.1	♂	0	0	0	↓	0	0	0	0	↓	↓
	1	♂	0	0	↓	↓	0	0	↓	↓	↓	↓
AI	0.1	♂	↑	0	0	0	0	0	↓	0	↓	↓
	1	♂	0	0	0	0	0	0	0	0	↓	↓
VP	0.01	♀	↓	0	0	0	0	0	0	0	0	0
	0.1	♀	0	0	0	0	0	0	0	0	0	0
ML	0.1	♀	0	0	0	0	0	0	0	0	0	↓
	1	♀	0	0	0	0	0	0	↓	0	0	↓
CD	0.1	♀	↑	0	0	0	↑	0	↓	0	↑	0
	1	♀	0	0	0	0	0	0	↓	0	0	0
AI	0.1	♀	0	0	0	0	0	0	↓	↑	0	0
	1	♀	0	0	0	0	0	0	0	↑	↓	↓

Effects: 0 – no significant difference, ↑ – increase, ↓ – decrease.

4. Material and Methods

4.1. Drosophila Strain and Experimental Conditions

Drosophila melanogaster wild-type *Canton-S* line was obtained from the *Drosophila* Stock Center at Indiana University (Bloomington, USA). To accelerate the aging, the flies were kept at a temperature of 29° C, which is a common approach in *Drosophila* studies [69]. To maintain constant conditions Binder KT 115 incubator (Binder, Germany) was used. Control end experimental flies were kept on food medium consisting of corn flour – 92 g/L, dry yeast – 32.1 g/L, agar-agar – 5.2 g/L, glucose – 136.9 g/L, 8 ml/L – 10 % solution of Nipagin in ethanol (Merck, USA), and 5 ml/L of propionic acid (Merck, USA).

4.2. Treatment with Yap/Taz Inhibitors

Verteporfin (VP, #SML0534, Merck, USA), ML-7 hydrochloride (ML7, #I2764, Merck, USA), Cytochalasin D (CD, #C8273, Merck, USA), AICAR (AI, #A9978, Merck, USA) were used as pharmacological inhibitors of Yap/Taz activity. Depending on the solubility of the substances, water, ethanol, or Cyrene (dihydrolevoglucosenone, # 807796, Merck, USA)

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[70] were used as solvents to prepare stock solutions. The stock solutions of VP were prepared with Cyrene 10% and water 90%, ML7 – with 50% ethanol and 50% water, CD – with 100% ethanol, AI – with 100% water.

A volume of 30 μL of stock solutions were pipetted onto the medium surface of each experimental vial. In a control vial 30 μL of corresponding solvent were pipetted. The final concentrations of substances in the food media were estimated using Blue food dye (Brilliant Blue FCF, Roha Dyechem Ltd, India) as a tracer of stock solution diffusion [71]. The obtained results demonstrated a 1:30 dilution of the stock solutions and a final concentration of VP – 0.01, 0.1, 1, 10 μM, ML7 – 0.01, 0.1, 1 μM, CD and AI – 0.1, 1 μM.

Treatment with inhibitors was started from the first day of imago life and continued 10 days for analysis of survival throughout the lifetime for qRT-PCR assay.

4.3. RNA Isolation and Quantitative RT-PCR

The expression level of Yki and its target genes [72] [73] , such as *Death-associated inhibitor of apoptosis 1* (*Diap1*), *division abnormally delayed* (*dally*), *Myc* (*myc*), *wingless* (*wg*), *Cyclin E* (*CycE*), *vein* (*vn*), and *E2F transcription factor 1* (*E2f1*) was measured by the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) with a reverse transcription step. RNA was isolated using an Aurum Total RNA Mini kit (Bio-Rad, USA) according to the manufacturer's instructions. RNA concentration was measured using a Quant-iT RNA Assay Kit (Invitrogen, USA) according to the manufacturer's instructions. cDNA was synthesized according to the iScript cDNA Synthesis Kit (Bio-Rad, USA) from the resulting RNA solution. The reaction mixture for the PCR reaction was prepared based on qPCR mix-HS SYBR (Evrogen, Russian Federation) and primers (Table S1). The primer design was performed using the QuantPrime online tool [74]. The polymerase chain reaction was carried out in a CFX96 amplifier (Bio-Rad, USA) using the following program: (1) 95 °C for 30 s, (2) 95 °C for 10 s, (3) 60 °C for 30 s, (4) steps 2–3 were repeated 49 times (5) DNA melting step.

The expression of the studied genes was calculated relative to the expression of the reference genes *β-Tubulin at 56D* (*β-Tubulin*) and *Ribosomal protein L32* (*RpL32*) using threshold cycles (Cts). The delta Ct (dCt) values were calculated as the differences in Ct values for target genes and reference genes. The values of Ct were taken from the CFX Manager 3.1 software (Bio-Rad, USA). For each experimental group, 20 males and 10 females were used. The experiments were carried out in three biological replicates with three analytical replications in each.

4.4. Analysis of Survival

Survival was assessed using the concentrations of substances affecting the expression level of Yki target genes. The emerged imagoes were separated by sex (mated males and non-virgin females) and transferred to the control and experimental vials (30 flies per vial, 5 vials, 150 flies per group) within 24 hours. The flies were maintained at 29 °C. Dead individuals were recorded daily. The median and maximum (age of 90% mortality) lifespan were calculated and survival curves were plotted. The experiments were carried out in 2 biological replicates.

4.5. Statistical Analysis

To determine the statistical significance of differences in the levels of gene expression multi-factor analysis of variance (ANOVA) with post-hoc Duncan's multiple-range test were used [75]. The survival curves were created using Kaplan-Meier method [76] . The statistical significance of differences between survival curves was evaluated using the log-rank test [77]. Fleming-Harrington test was used to estimate differences between control and experimental groups in earlier or later deaths [78] The significance of differences in median maximum lifespan was assessed using Fisher's exact test [79]. Bonferroni correction was used to adjust for multiple comparisons. Statistical data analysis was performed

using the TIBCO Statistica, version 13.3 (TIBCO Software, USA) and the online application for survival analysis OASIS 2 [80].

Supplementary Materials: The following are available online at https://www.mdpi.com/, Figure S1: Effects of YAP/TAZ inhibitors on the expression level of Yki target genes; Figure S2: Effects of YAP/TAZ inhibitors on the lifespan of males; Figure S3: Effects of YAP/TAZ inhibitors on the lifespan of females; Table S1: List of primers; Table S2: Age-related changes in the expression level of Yki target genes; Table S3: Two-way ANOVA of age-related changes in the expression level of Yki target genes in male and female flies; Table S4: Two-way ANOVA of the effect of Yap/Taz inhibitors on the expression level of Yki target genes in 10-day-old male and female flies; Table S5: The expression level of Yki target genes in 10 day-old males after treatment with YAP/TAZ inhibitors; Table S6: The expression level of Yki target genes in 10 day-old females after treatment with YAP/TAZ inhibitors; Table S7: Two-way ANOVA of the effect of Yap/Taz inhibitors on the expression level of Yki target genes in 20-day-old male and female flies; Table S8: The expression level of Yki target genes in 20-day-old males after treatment with YAP/TAZ inhibitors; Table S9: The expression level of Yki target genes in 20-day-old females after treatment with YAP/TAZ inhibitors; Table S10: Effects of treatment with YAP/TAZ inhibitors on the lifespan of male and female flies; Table S11: Effects of treatment with YAP/TAZ inhibitors on the lifespan of male and female flies.

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