Alpha-Ketoglutarate and 5-HMF

Subjects: Oncology | Biochemical Research Methods

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Clinical and pre-clinical studies of an anti-tumoral solution containing aKG, 5-HMF, N-acetyl-selenomethionine, and N-acetylmethionine for treating tumors showed, on one hand, good therapeutic efficacy during infusion therapy in prostate cancer patients by increasing the PSA doubling time; on the other hand, a reduction of tumoral mass was shown in lung cancer patients.

alpha-ketoglutarate (aKG) | 5-hydroxy-methyl-furfural (5-HMF) | reactive oxygen and nitrogen species (RONS) | leukemia | human fibroblasts (HF-SAR) | proliferation | caspase activity | carbonylated proteins (CP)

1. Introduction

The reduction of oxidatively modified proteins generated by cigarette smoke [1] demonstrated the impressive effects of aKG + 5-HMF as a better potential antioxidative solution compared to vitamin C or its single compounds. aKG itself is not only involved in the energy generation process in humans, but also in several metabolic processes for enzymatic regulation, such as those of hypoxia-inducing factor alpha [2] or 2-oxo-glutarate-dependent dioxygenases in cancer [3] and to suppress tumors in bladder cancer patients [3][4][5].

The compound 5-HMF occurs in honey and apple juice and in even higher rates in dried fruits, caramel products, and coffees [6]. Because there was speculation that 5-HMF is cancerogenic, the National Institute of Environmental Health Sciences demonstrated that no evidence of any carcinogenic activity was found when applying concentrations of 750 mg/kg over 2 years in rats and also provided some evidence in mice. Anti-proliferative and antioxidative activities were found in 5-HMF, suggesting its potential chemoprevention in cancer [7] as well as in melanoma cells [8]. aKG + 5-HMF was demonstrated to increase oxygen saturation during exercise in subjects with normobaric hypoxia [9] because of the antioxidative and anti-sickling effects of 5-HMF and its increased affinity for oxygen [10].

2. Estimation of Different AKG/5-HMF Ratios during Exposure of Cigarette Smoke on FCS Proteins

Figure 1 shows the oxidative modification of FCS protein after 2, 15, 30, and 60 min of exposure of cigarette smoke expressed with carbonylated proteins (nmol/mg protein) using different AKG+5-HMF combination solutions. The best significant reduction of carbonyl proteins was found using the 500 µg/mL + 125 µg/mL 5-HMF solution

compared to control. After 2 min the carbonylated protein was significantly lower (3.06 ± 0.33 vs. 5.96 ± 0.70 nmol/mg; $p < 0.01$), also after 15 min (3.80 ± 0.99 vs. 7.96 ± 0.33 nmol/mg; $p < 0.01$), 30 min (4.49 ± 0.77 vs. 9.89 ± 1.20 nmol/mg; $p < 0.01$), and 60 min (11.51 ± 0.94 vs. 5.21 nmol/mg; $p < 0.01$). At time points 30 and 60 min, this solution (500 µg/mL + 125 µg/mL 5-HMF) also showed a significantly lower carbonylated protein content compared to 500 µg/mL aKG + 62.5 µg/mL 5-HMF (6.68 ± 0.90 nmol/mg, $p < 0.05$ and 7.56 ± 1.20 nmol/mg, $p < 0.01$). The highest used combination 500 µg/mL + 250 µg/mL 5-HMF showed no significant difference compared to 500 µg/mL + 125 µg/mL 5-HMF, but the carbonyl proteins were higher with the highest combination solution. For the following experiments we have used the 500 µg/mL aKG + 166.7 µg/mL 5-HMF solution and its dilutions.

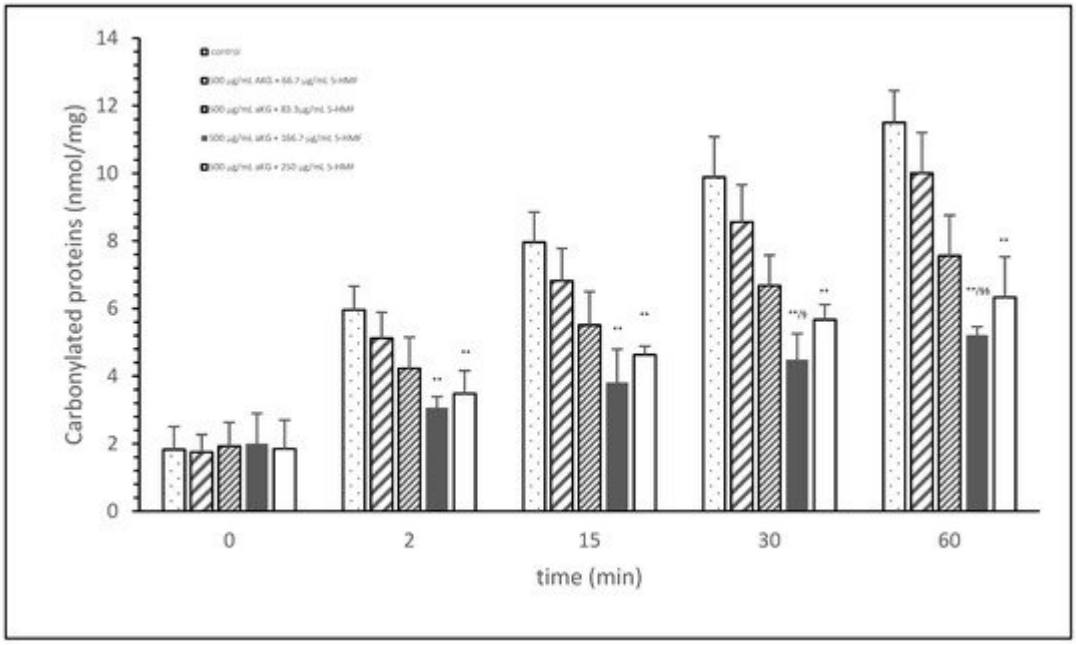

Figure 1. Cigarette smoke oxidatively modified proteins of FCS after cigarette smoke exposure in presence or absence of different AKG and 5-HMF combined solutions expressed with the content of carbonylated proteins (n = 3). ** $p < 0.01$: significance between the control (0 µg/mL aKG + 0 µg/mL 5-HMF) and different combinations of aKG + 5-HMF after 2, 15, 30, and 60 min exposure. § $p < 0.05$: significance between the 500 µg/mL aKG + 83.3 µg/mL 5-HMF and 500 µg/mL aKG + 125 µg/mL 5-HMF. §§ $p < 0.01$: significance between the 500 µg/mL aKG + 83.3 µg/mL 5-HMF and 500 µg/mL aKG + 125 µg/mL 5-HMF.

3. Cell Proliferation Experiments

Figure 2A describes the cell growth with different combinations of aKG + 5-HMF in Jurkat cells over 3 days. After 24 h, only the highest concentration (500 µg/mL aKG and 166.7 µg/mL 5-HMF) showed a significant reduction in cell growth compared to the control at 24 h (22,832 ± 2512 cells vs. 32,537 ± 5231 cells; $p < 0.05$). No significant changes were estimated between the control at 0 h and the Jurkat cells incubated in the presence of 500 µg/mL aKG + 166.7 µg/mL 5-HMF for 24 h. The cell growth at 48 h was significantly reduced compared to the control (52,123 ± 4232 cells, n = 5) by several different concentrations of the combination of aKG + 5-HMF: 125 µg/mL aKG + 41.7 µg/mL 5-HMF (43,511 ± 4209 cells; $p < 0.05$; n = 5), 200 µg/mL aKG + 66.7 µg/mL 5-HMF (36,823 ±

4845 cells; $p < 0.001$; $n = 5$), 250 µg/mL aKG + 83.3 µg 5-HMF (35,098 ± 2150 cells; $p < 0.001$; $n = 5$), 375 µg/mL aKG + 125 µg/mL 5-HMF (31,245 ± 4111 cells; $p < 0.001$; $n = 5$), and 500 µg/mL aKG + 166.7 µg/mL 5-HMF (21,243 ± 55,467 cells; $p < 0.001$; $n = 5$). After 72 h of incubation, the greatest combination, 500 µg/mL aKG + 166.7 µg/mL 5-HMF (23,224 ± 5445 cells; $p < 0.001$; $n = 5$), showed a significant reduction compared to the control after 72 h (82,131 ± 5197 cells; $p < 0.001$; $n = 5$), but did not show a reduction compared to the control cells after 0 or 24 h. A lesser reduction in the cell growth compared to the control after 72 h were obtained with 375 µg/mL aKG + 125 µg/mL 5-HMF (28,433 ± 5247 cells; $p < 0.001$; $n = 5$), 250 µg/mL aKG + 83.3 µg/mL 5-HMF (37,512 ± 5129 cells; $p < 0.001$; $n = 5$), 200 µg/mL aKG + 66.7 µg/mL 5-HMF (44,768 ± 3487 cells; $p < 0.001$; $n = 5$), and 125 µg/mL aKG + 41.7 µg/mL 5-HMF (54,227 ± 3655 cells; $p < 0.05$; $n = 5$).

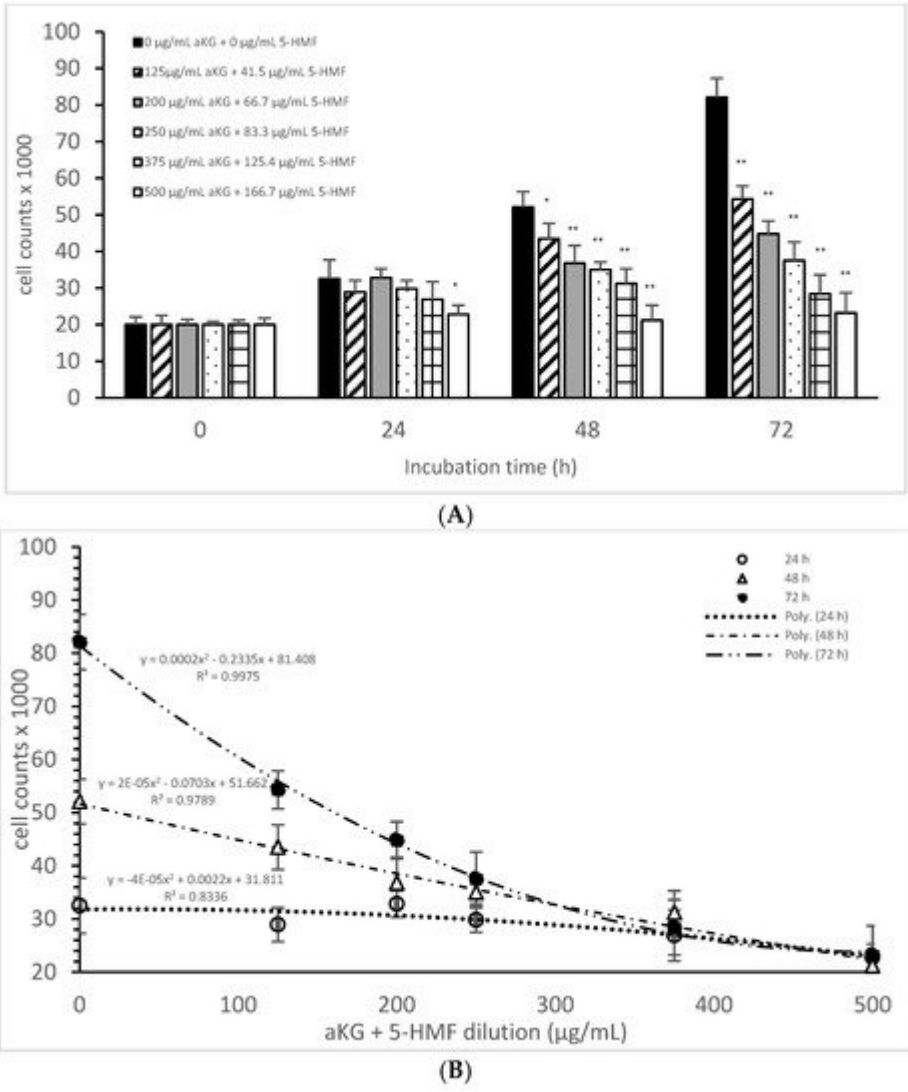

Figure 2. Cell growth of the Jurkat cell line in the absence or presence of different concentrations of the combined aKG + 5-HMF (**A**,**B**) correlation between cell growth and the combined solutions of aKG + 5-HMF after 24, 48, and 72 h of cultivation ($n = 5$). * $p < 0.05$: significance between the control (0 µg/mL aKG + 0 µg/mL 5-HMF) and different combinations of aKG + 5-HMF at the time points after 1, 2, and 3 days of cell culture. ** $p < 0.001$: significance between the control (0 µg/mL aKG + 0 µg/mL 5-HMF) and different combinations of aKG + 5-HMF at the time points after 1, 2, and 3 days of cell culture.

After correlating several concentrations of the combined AKG + 5-HMF solutions with cell growth (**Figure 2**B), all three curves showed a high polynomial correlation. The best correlation was calculated with the cell growth after 72 h of incubation (r = nearly 1; $y = 0.0002x^2 - 0.2335x + 81408$) and the IC50% calculated for the 100 µg/mL AKG + 41.7 µg/mL 5-HMF solution, followed by 48 h of incubation (r = 0.99; $y = 2 \times 10^{-5}x^2 - 0.0703x + 51662$) and the IC50% of the 200 µg/mL aKG + 66.7 µg/mL 5-HMF solution, and finally, the 24-h incubation (r = 0.91; $y = y = -4 \times 10^{-5}x^2 + 0.0022x + 31811$) and the IC50% of around 375 µg/mL aKG + 125 µg/mL 5-HMF. These results showed also that the higher the incubation time with AKG + 5-HMF the lower concentrations are needed to reach the IC 50%. The decline of cell growth after 72 h incubation was higher compared to the 48 h and 24 h incubation.

Table 1 shows the cell proliferations (%) of Jurkat cells and HF-SAR cells after 0, 24, 48, and 72 h incubation in presence or absence of the combined solutions of aKG + 5-HMF. No significant difference was estimated between all used aKG + 5-HMF solutions during all incubations except with the highest concentration 500 µg/mL aKG + 166.7 µg/mL 5-HMF after 72 h incubation ($p < 0.05$) compared to 0, 24, and 48 h incubation.

Table 1. Cell proliferation (%) of the Jurkat cell line and of the HF-SAR after incubation for 0, 24, 48, and 72 h in the absence or presence of different combinations of aKG + 5-HMF solutions ($n = 5$).

Jurkat	**0 h**	**24 h**	**48 h**	**72 h**
Cell growth	%	%	%	%
0 µg/mL aKG + 0 µg/mL 5-HMF	100 ± 7.5	190.5 ± 13.6	286.0 ± 2.1	376.5 ± 5.0
125 µg/mL aKG + 41.7 µg/mL HMF	105 ± 3.5	150.5 ± 14.0	266.0 ± 6.0	331.0 ± 6.3
200 µg/mL aKG + 66.7 µg/mL 5-HMF	101 ± 2.5	168.1 ± 20.2	216.7 ± 5.8	304.5 ± 5.9
250 µg/mL aKG + 83.3 µg/mL 5-HMF	103 ± 9	165.5 ± 6.3	206.4 ± 7.8	290.5 ± 8.8
375 µg/mL aKG + 125 µg/mL 5-HMF	98.7 ± 5.5	179.0 ± 8.7	195.5 ± 4.6	296.5 ± 7.1
500 µg/mL aKG + 166.7 µg/mL 5-HMF	105 ± 3.5	165.5 ± 7.6	222.5 ± 3.4	256.0 ± 9.6
HF-SAR	**0 h**	**24 h**	**48 h**	**72 h**
Cell growth	%	%	%	%
0 µg/mL aKG + 0 µg/mL 5-HMF	101.3 ± 7.5	88.7 ± 9.9	92.3 ± 12.1	126.6 ± 14.5
125 µg/mL aKG + 41.7 µg/mL HMF	100.0 ± 3.1	85.0 ± 7.3	92.8 ± 9.2	121.7 ± 9.2
200 µg/mL aKG + 66.7 µg/mL 5-HMF	104.1 ± 1.5	89.3 ± 8.9	100.6 ± 7.9	121.4 ± 13.1
250 µg/mL aKG + 83.3 µg/mL 5-HMF	99.0 ± 6.1	82.7 ± 4.2	89.3 ± 4.1	137.6 ± 22.1
375 µg/mL aKG + 125 µg/mL 5-HMF	99.9 ± 3.5	93.8 ± 11.0	104.3 ± 11.1	132.6 ± 29.1

4. Cytotoxic Assay

Jurkat	0 h	24 h	48 h	72 h
500 µg/mL aKG + 166.7 µg/mL 5-HMF	102.0 ± 3.1	86.2 ± 4.1	97.9 ± 8.2	140.5 * ± 23

…osence or …ncubation, the highest concentration (500 µg/mL aKG and 166.7 µg/mL 5-HMF (0.112 ± 0.021)) showed a 41% reduction of the mitochondrial activity, which was significant compared to that of the control after 24 h (0.19 ± 0.02; $n = 5$; $p < 0.001$). Using the 375 µg/mL aKG + 125 µg/mL 5-HMF solution (0.136 ± 0.018; $n = 5$; $p < 0.05$) resulted in a 28% reduction compared to the control after 24 h.

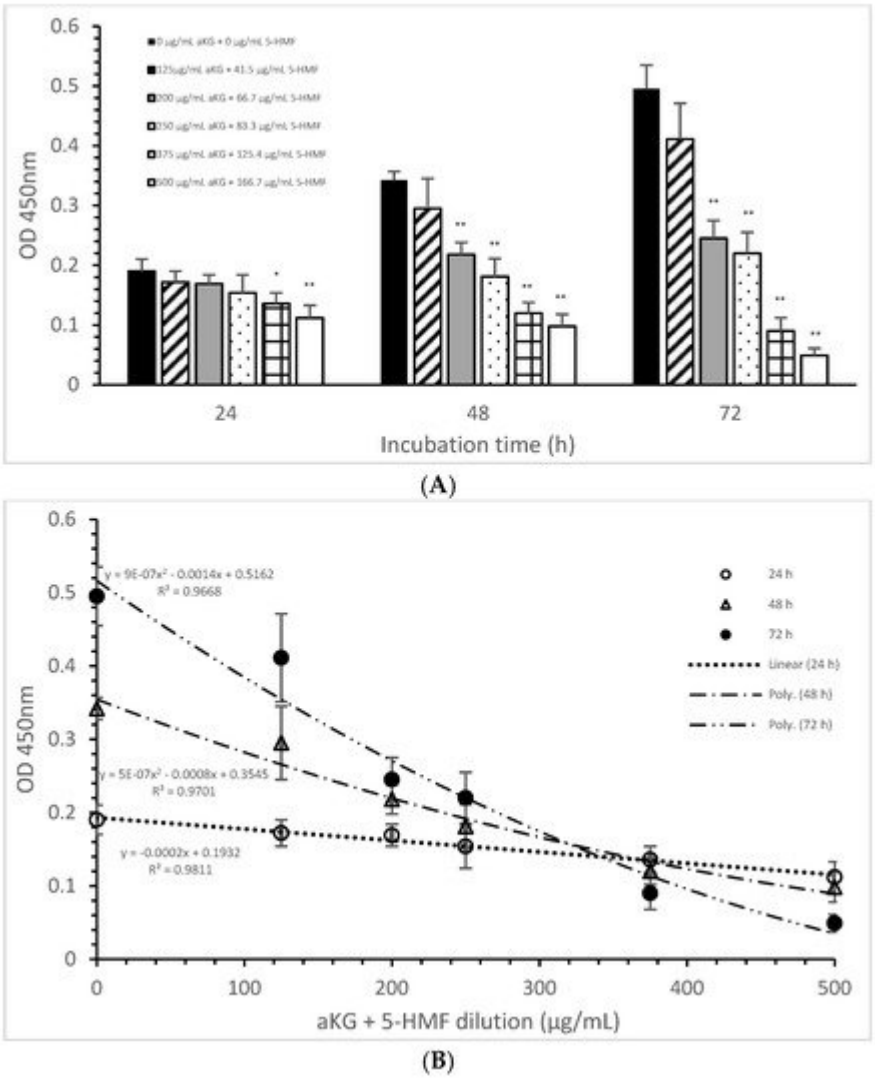

Figure 3. Mitochondrial activity of the Jurkat cell line in the absence or presence of different aKG + 5-HMF concentrations (**A**); (**B**) correlation between mitochondrial activity and the combined aKG + 5-HMF concentration after 24, 48, and 72 h of cultivation ($n = 5$). * $p < 0.01$: significance between the control (0 µg/mL aKG + 0 µg/mL 5-HMF) and different combinations of aKG + 5-HMF at time points after 24, 48, and 72 h of cell culture. ** $p < 0.001$: significance between the control (0 µg/mL aKG + 0 µg/mL 5-HMF) and different combinations of aKG + 5-HMF at time points after 1, 2, and 3 days of cell culture.

The use of 48 h of incubation resulted in a higher reduction (of 36%) of the mitochondrial activity compared to that of the control after 48 h, with the 500 µg/mL aKG and 166.7 µg/mL 5-HMF solution (0.098 ± 0,02; $n = 5$; $p < 0.001$)

showing the greatest reduction, followed by the 375 µg/mL aKG + 125 µg/mL 5-HMF solution (0.120 ± 0.018; n = 5; $p < 0.001$), the 250 µg/mL aKG + 83.3 µg 5-HMF solution (0.181 ± 0.02; n = 5; $p < 0.001$), and the 200 µg/mL aKG + 66.7 µg/mL 5-HMF solution (0.218 ± 0.05; n = 5; $p < 0.001$). The lowest concentration, 125 µg/mL aKG + 41.7 µg/mL 5-HMF (0.295 ± 0.05; n = 5), showed no effects. The same trend could be seen after 72 h of incubation. While no effects compared to the control (0.495 ± 0.04) after 72 h were obtained when using 125 µg/mL aKG + 41.7 µg/mL 5-HMF (0.411 ± 0.06; n = 5), all the other used concentrations showed significant reductions: 200 µg/mL aKG + 66.7 µg/mL 5-HMF (0.245 ± 0.03; n = 5; $p < 0.001$), 250 µg/mL aKG + 83.3 µg 5-HMF (0.222 ± 0.035; n = 5; $p < 0.001$), 375 µg/mL aKG + 125 µg/mL 5-HMF (0.090 ± 0.022; n = 5; $p < 0.001$), and 500 µg/mL aKG and 166.7 µg/mL 5-HMF (0.098 ± 0.02; n = 5; $p < 0.001$). The mitochondrial activity in the Jurkat cells incubated for 72 h in the presence of 500 µg/mL aKG and 166.7 µg/mL 5-HMF was significantly lower than that with 48 and 24 h of incubation when using the same combined concentration ($p < 0.001$). A significant difference was also estimated between incubation for 72 h (0.090 ± 0.022; n = 5; $p < 0.001$) and incubation for 24 h (0.136 ± 0.018) with the use of 375 µg/mL aKG + 125 µg/mL 5-HMF.

Figure 3B shows the correlations between the combined solutions of aKG + 5-HMF and the mitochondrial activity at different incubation times with nearly equal regression terms: r = 0.98 for 72 h with a polynomial function ($y = 9 \times 10^{-7} x^2 - 0.0014x + 0.5162$), r = 0.98 for 48 h with a polynomial function ($y = 5 \times 10^{-7} x^2 - 0.0008x + 0.3545$), and r = 0.99 for 24 h with a linear function ($y = -0.0002x + 0.1932$). The IC50% was calculated for all functions, with nearly the same result of 250 µg/mL + 83.3 µg/mL 5-HMF. The decline of the mitochondrial activity was higher during 72 h incubation followed by 48 h incubation compared to 24 h incubation because of its different functions. The usage of 500 µg/mL aKG and 166.7 µg/mL 5-HMF and 375 µg/mL aKG + 125 µg/mL 5-HMF solutions showed a lower mitochondrial activity in favor of 72 h incubation followed by 48 h incubation compared to 24 h incubation.

Table 2 shows the decrease in the mitochondrial activity in the presence of the combined solutions (aKG + 5-HMF). The longer the incubation time and the higher the concentration of aKG + 5-HMF, the lower the mitochondrial activity was. A reduction of nearly half was obtained by using 200 µg/mL aKG + 66.7 µg/mL 5-HMF after 72 h of incubation or by using 250 µg/mL aKG + 83.3 µg/mL 5-HMF after 48 and 72 h of incubation.

Table 2. Mitochondrial activity (%) of the Jurkat cell line (A) and of the HF-SAR (B) after incubation for 24, 48, and 72 h in the absence or presence of different combinations of aKG + 5-HMF solutions (n = 5). * $p < 0.01$: significance between 24 h of incubation without aKG + 5-HMF and with the combined solutions of aKG + 5-HMF. ** $p < 0.001$: significance between 24 h of incubation without aKG + 5-HMF and with the combined solutions of aKG + 5-HMF. $^{++}$ $p < 0.001$: significance between 48 h of incubation without aKG + 5-HMF and with the combined solutions of aKG + 5-HMF. $^{\$\$}$ $p < 0.001$: significance between 48 h of incubation without aKG + 5-HMF and with the combined solutions of aKG + 5-HMF.

Jurkat	24 h	48 h	72 h
Mitochondrial Activity	%	%	%
0 µg/mL aKG + 0 µg/mL 5-HMF	100 ± 10.5	100 ± 4.4	100 ± 8.1

Jurkat	24 h	48 h	72 h
125 µg/mL aKG + 41.7 µg/mL HMF	90.5 ± 9.5	86.3 ± 14.6	83 ± 12.1
200 µg/mL aKG + 66.7 µg/mL 5-HMF	88.9 ± 7.9	63.7 ± 5.8 $^{++}$	49.5 ± 6.1 $^{\$\$}$
250 µg/mL aKG + 83.3 µg/mL 5-HMF	81.1 ± 15.8	52.9 ± 8.8 $^{++}$	44.4 ± 7.1 $^{\$\$}$
375 µg/mL aKG + 125 µg/mL 5-HMF	71.6 ± 9.5 *	35.1 ± 5.3 $^{++}$	18.2 ± 4.4 $^{\$\$}$
500 µg/mL aKG + 166.7 µg/mL 5-HMF	58.9 ± 11.1 **	28.7 ± 5.8 $^{++}$	9.9 ± 2.4 $^{\$\$}$
HF-SAR	**24 h**	**48 h**	**72 h**
Mitochondrial Activity	%	%	%
0 µg/mL aKG + 0 µg/mL 5-HMF	100 ± 11.4	100 ± 4.1	100 ± 8.4
125 µg/mL aKG + 41.7 µg/mL HMF	86.7 ± 5.3	90.7.3 ± 5.2	83.1 ± 6.2
200 µg/mL aKG + 66.7 µg/mL 5-HMF	90.7 ± 6.9	88.2 ± 8.3	91.1 ± 4.2
250 µg/mL aKG + 83.3 µg/mL 5-HMF	84.3 ± 5.1	85.3 ± 6.8	91.9 ± 5.1
375 µg/mL aKG + 125 µg/mL 5-HMF	90.0 ± 5.5	89.6 ± 6.1	95.8 ± 5.9
500 µg/mL aKG + 166.7 µg/mL 5-HMF	90.8 ± 4.6.1	83.7 ± 11.3	99.1 ± 9.2

L + 125.4
and 20%
with 375
aKG + 5-

5. Caspase-3 Activity Measurements

The loss of mitochondrial activity mostly induces caspase activity (**Figure 4**). Compared to the control (2.9 ± 2.3%), 250 µg/mL aKG + 83.3 µg/mL 5-HMF significantly increased the caspase activity in Jurkat cells after 72 h of incubation (13.5 ± 3.2; n = 3; p < 0.001), but 500 µg/mL aKG + 166.7 µg/mL 5-HMF did so even more (51.6 ± 5.2%; n = 3; p < 0.001). While the activity with 500 µg/mL + 166.7 µg/mL 5-HMF was significantly higher than that of the positive control with 4 µM CPT (43.8 ± 1.8; n = 3; p < 0.01), that of 250 µg/mL aKG + 83.3 µg/mL 5-HMF was lower (13.5 ± 3.2, n = 3; p < 0.001).

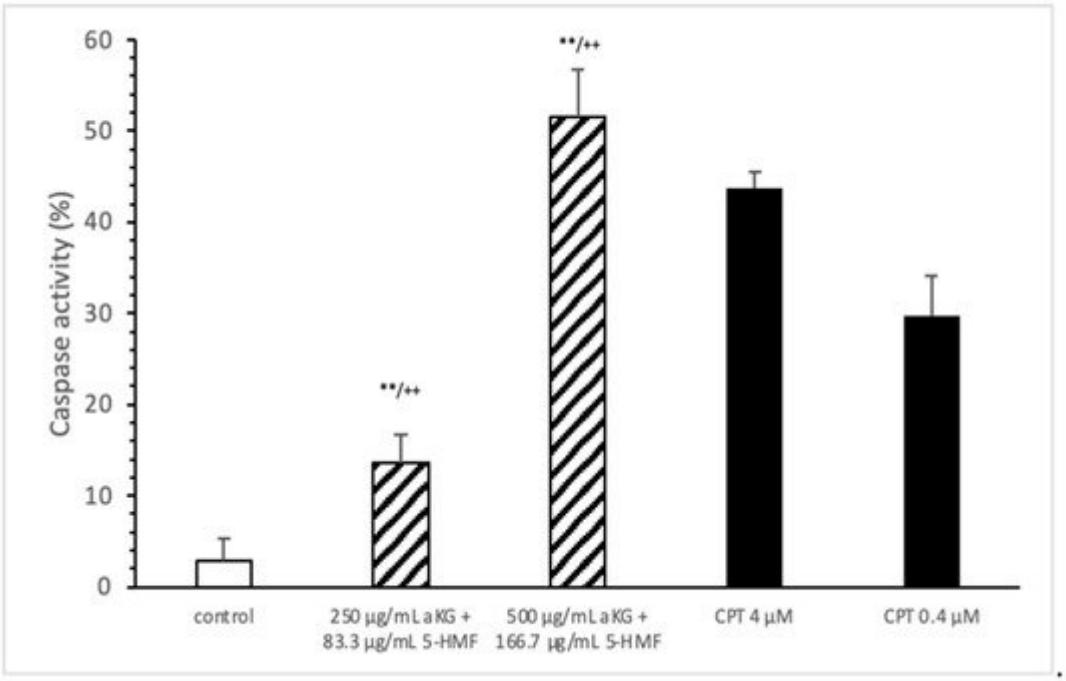

Figure 4. Caspase activity of the Jurkat cell lines after 72 h of cell growth using combined solutions of 250 µg/mL aKG + 83.3 µg/mL 5-HMF solution, 500 µg/mL aKG + 166.7 µg/mL 5-HMF, and 4 or 0.4 µM CPT as positive controls (n = 3). ** $p < 0.01$: significance between the control and combined solutions of aKG + 5-HMF. $^{++}$ $p < 0.001$: significance between the positive control (4 µM CPT) and combined solutions of aKG + 5-HMF.

6. Detection of the Mitochondrial Membrane Potential through Flow Cytometry

The estimation of apoptotic cells (**Figure 5**) was significantly increased with 250 µg/mL aKG + 83.3 µg/mL 5-HMF after 72 h of incubation (31.4 ± 3.2%) compared to the control (14.9 ± 2.2%; n=3; $p < 0.001$), but was significantly decreased compared to 4 µM CPT (50.7 ± 5.6%). The 500 µg/mL aKG + 166.7 µM 5-HMF combination showed a higher significance (63.2 ± 5.6%; n = 3; $p < 0.001$) compared to the control and to 4 µM CPT (n = 3; $p < 0.05$).

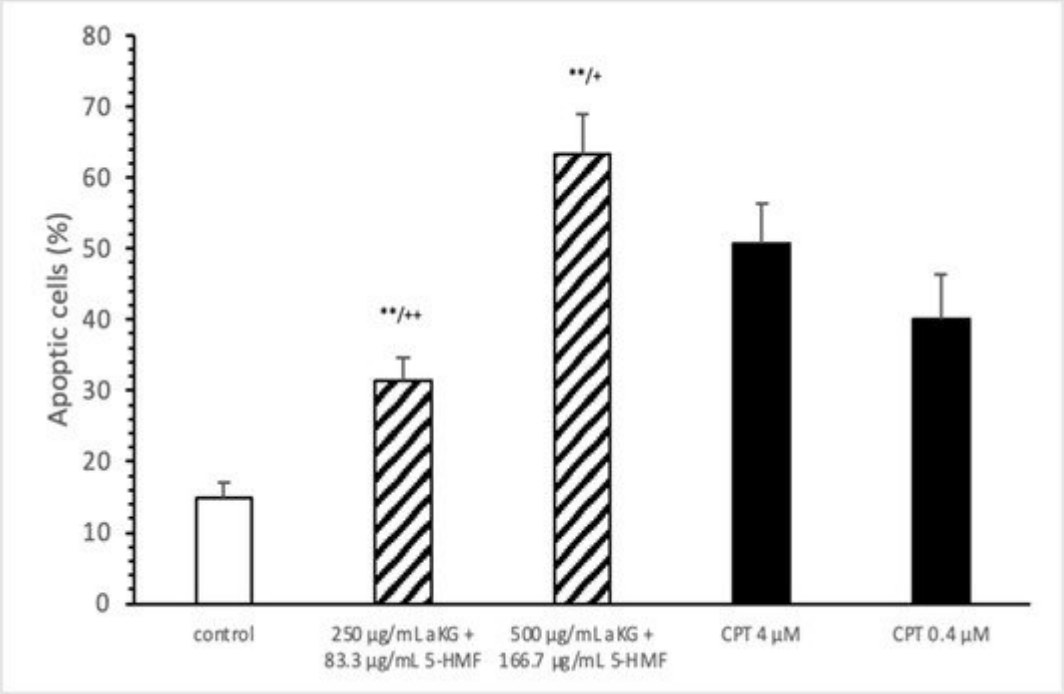

Figure 5. Estimation of apoptotic cells (JC-1) of the Jurkat cell lines after 72 h of cell growth using a combined solutions of 250 µg/mL aKG + 83.3 µg/mL 5-HMF, 500 µg/mL aKG + 166.7 µg/mL 5-HMF, and 4 µM or 0.4 µM CPT as positive controls (n = 3). ** p < 0.001: significance between the control and combined solutions of aKG + 5-HMF. $^{+}$ p < 0.01: significance between the positive control (4 µM CPT) and combined solutions of aKG + 5-HMF. $^{++}$ p < 0.001: significance between the positive control (4 µM CPT) and combined solutions of aKG + 5-HMF.

7. Estimation of Carbonylated Proteins in Jurkat and HF-Sar Cells

After 72 h incubation with 250 µg/mL aKG + 83.3 µg/mL 5-HMF the carbonylated protein content of isolated membrane proteins of Jurkat cell line was significantly lower compared to 0 h (11.6 ± 0.67 vs. 7.44 ± 0.93 nmol/mg; p < 0.01), but not of HF-SAR as presented in **Table 3**. Using 500 µg/mL aKG + 166.7 µg/mL 5-HMF the carbonylated protein level showed a significant reduction (10.6 ± 0.37 vs. 5.55 ± 1.22; p < 0.01) in Jurkat cells and also in HF-SAR (2.5 ± 0.6 vs. 1.73 ± 0.52 nmol/mg; p < 0.05). Furthermore, the carbonylated protein content of Jurkat lysates showed a significantly higher content (11.1 ± 0.70 nmol/mg) compared to HF-SAR lysate (2.30 ± 0.66 nmol/mg; p < 0.01) before incubation with 250 µg/mL aKG + 83.3 µg/mL 5-HMF or 500 µg/mL aKG + 166.7 µg/mL 5-HMF.

Table 3. Carbonylated proteins of Jurkat and HF-SAR lysates after 0 and 72 h incubation in absence or presence of 500 µg/mL aKG + 125 µg/mL 5-HMF and 500 µg/mL aKG + 62.5 µg/mL 5-HMF (n = 5). * p < 0.05: significance between 0 h and 72 h incubated aKG + 5-HMF solutions. ** p < 0.01: significance between 0 h and 72 h incubated aKG + 5-HMF solutions. §§ p < 0.01 = significance between Jurkat cell and HF-SAR lysate.

	Carbonylated Proteins (nmol/mg Protein)			
	Jurkat Lysate		HF-SAR Lysate	
	0 h	72 h	0 h	72 h
250 µg/mL aKG + 83.3 µg/mL 5-HMF	11.6 §§ ± 0.67	7.44 ± 0.93 **	2.10 ± 0.72	1.91 ± 0.82
500 µg/mL aKG + 166.7 µg/mL 5-HMF	10.6 §§ ± 0.37	5.55 ± 1.22 **	2.5 ± 0.6	1.73 ± 0.52 *

1. Greilberger, J.F.; Wintersteiger, R.; Astrid, O.; Greilberger, M.; Herwig, R. Combination of 2-oxoglutarate/ascorbic acid/5-hydroxy-methyl-furfur-aldehyde/carnosine inhibits protein oxidation during radical exposure of cigarette smoke. Proteins 2018, 5, 10–12.
2. Kaelin, W.G. Cancer and altered metabolism: Potential importance of hypoxia-inducible factor and 2-oxoglutarate-dependent dioxygenases. Cold Spring Harb. Symp. Quant. Biol. 2011, 76, 335–345.
3. Losman, J.A.; Koivunen, P.; Kaelin, W.G. 2020. 2-Oxoglutarate-dependent dioxygenases in cancer. Nat. Rev. Cancer 2020, 20, 710–726.
4. Yi, W.; Yu, Y.; Li, Y.; Yang, J.; Gao, S.; Xu, L. The tumor-suppressive effects of alpha-ketoglutarate-dependent dioxygenase FTO via N6-methyladenosine RNA methylation on bladder cancer patients. Bioengineered 2021, 12, 5323–5333.
5. Ward, P.S.; Patel, J.; Wise, D.R.; Abdel-Wahab, O.; Bennett, B.D.; Coller, H.A.; Cross, J.R.; Fantin, V.R.; Hedvat, C.V.; Perl, A.E.; et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell 2010, 17, 225–234.
6. Shapla, U.M.; Solayman, M.; Alam, N.; Khalil, M.I.; Gan, S.H. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health. Chem. Cent. J. 2018, 12, 35.
7. Zhao, L.; Su, J.; Li, L.; Chen, J.; Hu, S.; Zhang, X. Mechanistic elucidation of apoptosis and cell cycle arrest induced by 5-hydroxymethylfurfural, the important role of ROS-mediated signaling pathways. Food Res. 2014, 66, 186–196.
8. Bito, T.; Koseki, K.; Asano, R.; Ueda, N. 5-hydroxymethyl-2-furaldehyde purified from Japanese pear (Pyrus pyrifolia Nakai cv. Nijisseiki) juice concentrate inhibits melanogenesis in B16 mouse melanoma. Bioscience 2020, 4, 2374–2384.
9. Kössler, F.; Mair, L.; Burtscher, M. 5-Hydroxymethylfurfural and α-ketoglutaric acid supplementation increases oxygen saturation during prolonged exercise in normobaric hypoxia. Int. J. 2019, 91, 63–68.
10. Kassa, T.; Wood, F.; Strader, M.B.; Alayash, A.I. Antisickling Drugs Targeting βCys93 Reduce Iron Oxidation and Oxidative Changes in Sickle Cell Hemoglobin. Front. Physiol. 2019, 10, 931.