

Article

Oxidative stress favours herpes virus infection in vertebrates: a meta-analysis

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Abstract

Herpes viruses are responsible for a variety of pathological effects in humans and in both wild and domestic animals. One mechanism that has been proposed to facilitate replication and activity of herpes viruses is oxidative stress (OS). We used meta-analytical techniques to test the hypotheses that (1) herpes virus infection causes OS and (2) supplementation of antioxidants reduces virus load, indicating that replication is favoured by a state of OS. Results based on studies on mammals, including humans, and birds show that (1) OS is indeed increased by herpes virus infection across multiple tissues and species, (2) biomarkers of OS may change differently between tissues, and (3) the effect size does not differ among different virus strains. In addition, the increase of oxidative damage in blood (tissue commonly available in ecological studies) was similar to that in the tissues most sensitive to the herpes virus. Our results also show that administration of antioxidants reduces virus yield, indicating that a condition of OS is favorable for the viral replication. In addition, some antioxidants may be more efficient than others in reducing herpes virus yield. Our results point to a potential mechanism linking herpes virus infection to individual health status.

Key words: antioxidants, herpes virus infection, immune response, interaction host–pathogen, oxidative stress.

An organism's physiological equilibrium is critically reliant on its immune system, which provides protection against parasites, pathogens, tumor cells, and allows recovery from injuries (Aparicio-Burgos et al. 2015; Staley and Bonneaud 2015). There are, however, circumstances under which the immune system is suppressed, making the organism particularly sensitive to infections. For example, host exposure to environmental stressors, such as limited food supply, increased ambient temperature, or exposure to contaminants, can facilitate the spread, persistence, or emergence of infectious and parasitic diseases (Apanius 1998; Rohr et al. 2008; Martin et al. 2010). Understanding the patho-physiological mechanisms associated with pathogens and the response to them is therefore of central importance because it enables us to characterize the mode of action

of the pathogen, to infer on the way an individual responds to the pathogen and to develop strategies to mitigate the activity of the pathogen in the organism.

Herpes viruses are one of the most common infectious diseases in humans and in both wild and domestic animals. Latent, non-productive, herpes virus infections occur for prolonged times in many species (Kaleta 1990). Activation to persistent, productive infection, and to disease generally includes depression of normal activity, respiratory distress, extremity paralysis, head-shaking, hemorrhagic lesions, twisting of the neck, and sudden mortality (Kaleta 1990). The appearance of clinical symptoms likely indicates that individuals have been exposed to a chronic stressor. Vertebrates translate stressful stimuli into secretions of hormones (glucocorticoids), which

activate the physiological stress response (Sapolsky et al. 2000; Romero 2004). The action of glucocorticoids involves a high diversity of physiological and behavioral effects, including immunosuppression (Scheinman et al. 1995; Webster et al. 2002; Bailey et al. 2003; Padgett and Glaser 2003). Antiviral T-cell immune responses are, for example, compromised by glucocorticoids that are either stress induced or pharmacologically administered (Elftman et al. 2010; Hunzeker et al. 2011). Chronic exposure of vertebrates to glucocorticoids is also known to induce oxidative stress (OS) through the increase in production of free radicals and oxidative damage (Costantini et al. 2011). OS is a complex multifaceted biochemical condition of cells, which occurs when there is an increase in oxidative damage to biomolecules and oxidation of nonprotein and protein thiols that regulate the cell oxidative balance (Halliwell and Gutteridge 2007). It has been shown that herpes virus infection may be facilitated by a cell state of OS, while increased intake of antioxidants might prevent replication of the virus (Nucci et al. 2000; Valyi-Nagy et al. 2000; Palamara et al. 2004; Kavouras et al. 2007). For instance, reactive oxygen species (ROS) inhibit NF- κ B (Nuclear Factor kappa-light-chain-enhancer of activated B cells), whose function is to avoid Kaposi's sarcoma-associated herpesvirus (KSHV) reactivation and lytic gene expression (Li et al. 2011). However, it is unclear whether OS is a patho-physiological mechanism promoting herpes virus infection.

The main goal of this study was to assess the role of OS as a mechanism underlying herpes virus infection. To this end, we used meta-analytic techniques to address the following two hypotheses: (1) herpes virus infection causes OS and (2) supplementation of antioxidants reduces virus infection, indicating that infection is favored by a state of OS. To test the first hypothesis, a meta-analysis was carried out using experimental infectious studies and looked at the effects on reactive species production, oxidative damage, and both nonenzymatic and enzymatic antioxidants. To test the second hypothesis, a meta-analysis was carried out including studies that assessed the effects of antioxidants on experimental herpes virus infections. Both meta-analyses were performed while taking into account some moderators (e.g., which biomarkers were measured, in which tissue and at which stage of infection; vertebrate species; virus strain), which might help to explain the diverse results obtained in previous studies. For instance, it has been shown that enzymatic activity during herpes virus infections may differ consistently among different cells (Gargouri et al. 2009), and that oxidative damage (MDA, malondialdehyde) can either slightly increase or decrease among different tissues (Lassoued et al. 2008).

Materials and Methods

Article collection

The articles included in both meta-analyses (MAs) are the result of an extensive research on the Web of Knowledge and on PubMed, in all databases for all years. The key words “herpesvirus” or “herpes virus” were combined with “antioxidant”, “lipid peroxidation”, “oxidative damage”, or “reactive oxygen species”. Articles were then searched on the specific journal website or online libraries. When articles were not available, authors were contacted by e-mail. This search resulted in the screening of more than 2,000 articles. Additional studies were identified by screening references quoted in the selected articles.

Article selection and eligibility criteria

Articles were included in one or both MAs when they tested (1) the effect of herpes virus infection on OS metrics or (2) the effects of antioxidants on the infection. Studies were excluded if they met the following criteria: (i) they did not provide baseline values of metrics of oxidative damage or antioxidant status (i.e., before herpes virus infection); (ii) they combined administration of antioxidants with other compounds, which makes impossible to discriminate among the effects of different compounds on the virus yield; (iii) it was impossible to extrapolate data from graphs; (iv) they did not provide standard error nor standard deviation. We have included both experimental studies and clinical trials, for a total of 26 articles. The scheme illustrating the steps of article search and selection according to PRISMA guidelines (Liberati et al. 2009), is shown in the supplementary information (Supplementary Figure S1).

Since there was variation among studies in experimental settings (e.g., OS measured as oxidative damage or antioxidant defences); evaluation performed during an acute (e.g., few hours) or chronic (e.g., days) infection, we also collected data on study species, biomarker, tissue, herpes virus strain, and time elapsed from the detection of the basal level until the subsequent measurements (during the viral infection). These data were used as moderators in MAs. Most of the articles unfortunately lacked information about gender and age, and hence, these factors could not be included in the statistical analyses.

Data collection

From each article, we collected statistical outcomes of (1) effects of herpes virus infection on OS metrics and (2) effects of antioxidants on infection. These outcomes were used to calculate the effect size (Supplementary Table S1). When statistical outcomes were not available, data (mean, standard error, or standard deviation) were collected from graphs and these were used to calculate the effect size. A detailed explanation of the effect size calculation is given in the following section.

For meta-analysis testing hypothesis 1 (OS-MA), data were collected for the following metrics of OS:

- Oxidative damage to DNA (comet assay), to protein (protein carbonyls), to lipids (lipid peroxidation, specifically hydroxynonenal HNE and hydroxyguanosine OHG, malondialdehyde MDA, F2 and F4 isoprostanes, thiobarbituric acid reactive substances—TBARS).
- Nonenzymatic antioxidant levels (GSH, S-GSH, Thiols, GSSG, GSH:GSSG ratio), including nonenzymatic antioxidant capacity (TAS assays).
- Antioxidant enzyme activity (catalase, superoxide dismutase, glutathione peroxidase)
- Reactive oxygen species (ROS), reactive nitrogen species (NOS), reactive species synthase enzymes, oxidative stress index (OSI), and total oxidant status (TOS).

For meta-analysis testing hypothesis 2 (ANTIOX-MA), data were collected for the following variables:

- Virus yield before and after inoculation of antioxidants (from which we calculated the percentage of change in virus yield).

We further divided the data between *in vivo* and *in vitro* experiments (30 and 61, respectively, Supplementary Table S2 and S3). *In vivo* experiments included a large heterogeneity in the way of administering antioxidants, so we could not include these data in the

analyses. In contrast, for all *in vitro* experiments (61), we could calculate the percentage of the virus yield change.

Effect size calculation

For both MAs, according to previous work (Gontard-Danek and Moller 1999; Nakagawa and Cuthill 2007; Simons et al. 2012), Cohen's *d* effect size was calculated and was subsequently transformed to Fisher's *z* using *des* function in Compute.es package in R (Del Re 2013). According to the significance of their associated *r*-values (Bartz 1999), effect size *z* can be considered strong when the range is between 0.69 and 1.07, and very high when it exceeds 1.10.

Overall, we had 198 estimates of effect size for OS-MA (Supplementary Table S1) and 61 estimates of virus change for ANTIOX-MA from *in vitro* studies, respectively. The sign of effect sizes for OS-MA, according to previous MAs (Costantini and Moller 2009; Costantini et al. 2011), was considered to be positive when oxidative damage and reactive species were increased, and when enzymatic and nonenzymatic antioxidants were decreased, respectively. On the other hand, the effect size was considered to be negative when oxidative damage and reactive species were decreased and when enzymatic and nonenzymatic antioxidants were increased, respectively (Costantini and Moller 2009; Costantini et al. 2011). When there was no difference between pre- and post-infection values, effect size was considered to be 0.

Effect size estimates were not weighted for sample size (Rosenthal 1991, pp. 27–28) because some of the articles included in the first MA did not provide the sample size. However, a comparison of unweighted *z*-values with the mean weighted *z* was not significant (Wilcoxon: $W=72$, $P=1$), indicating that in our case the sample size did not affect the effect size estimates.

Meta-analytic technique

Preliminary tests (e.g., Funnel test) showed that there was no publication bias; for further information we refer to the Supplementary Material and Supplementary Figure S2.

OS-MA

To assess how moderators affected the effect size, we performed a linear mixed model including biomarker, tissue, virus strain, and time of measurement as fixed effects, and species and study as random effects. Each single mixed model was repeated twice, having the time of measurement as a fixed covariate or as a fixed factor, respectively. In order to include it in the models as a fixed factor, we distinguished between short term (measurements taken within 4 days from the measurement of the basal level of OS biomarkers) and long term (measurements taken after 4 days from the measurement of the basal level of OS biomarkers). This distinction between short- and long-term was done to test whether the effect of the virus on OS changed after the activation of the specific immune response, which is known to occur after around 4 days from the detection of pathogens and viruses (Murphy 2012). Outcomes of models having time as a fixed factor or covariate were similar, hence we presented only outcomes of models with time as a covariate. In each model, we also included the interaction between tissue and biomarker; further interactions among the other main factors could not be included because no data were available for some combinations of the factors. Post-hoc analyses were performed using lmerTest package in R (Kuznetsova et al. 2013).

ANTIOX-MA

To assess how moderators affected the percentage of virus change after administration of antioxidants, we performed a linear mixed model including concentration and type of antioxidant administered, virus strain, tissue (cellular line), and time elapsed since the emergence of the infection as fixed factors, while study was included as a random factor. Unfortunately, we could not include two antioxidant types in the analysis (extract of rosemary and extract of *Zatria multiflora*), since it was impossible to express concentrations in millimol in order to make them comparable to those of other antioxidants. Post-hoc analyses were carried out using lmerTest package in R (Kuznetsova et al. 2013) to compare antioxidant treatments and to evaluate which antioxidant was the most efficient in reducing herpes virus yield according to its concentration. Finally, since the administration of ebselen, vaticaffinol, and piperitenone oxide was tested in one study only, we focused on the comparison among antioxidants measured in more than one study, in order to increase the statistical power of this comparison, and to test the efficiency of glutathione as compared to other antioxidants, since glutathione is one of the most important cell antioxidants (Halliwell and Gutteridge 2007).

Results

OS-MA

The best-fit linear mixed model showed a significant interaction between biomarker and tissue ($F=2.16$, $df=8$, $P=0.038$; Table 1). Post-hoc analyses showed that there was a significant increase of (1) oxidative damage in blood, kidney, and lung, (2) of ROS production in dermal tissue, and (3) of nonenzymatic antioxidants in dermal tissue and kidney (Figure 1). A further comparison of effect size among tissue for each single biomarker showed that the production of ROS and the reduction of nonenzymatic antioxidants were similar between tissues, while oxidative damage and enzymatic antioxidants changed differently between tissues (Table 2). The effect size of herpes virus was already high a few hours after the measurement of the basal levels of a given OS biomarker and did not show any significant decrease over time (Table 1, Figure 2).

ANTIOX-MA

The best-fit linear mixed model showed that the concentration and the kind of antioxidant influenced significantly the change in virus yield (Table 3). There was a significant reduction of herpes virus for most of the treatments, with the exception of the administration of *N*-butanoyl glutathione (GSH-C4), and *S*-acetylglutathione (S-

Table 1. Full model and best fit model for OS-MA.

	Sum of squares	Mean of squares	<i>F</i> value	<i>P</i> -value
Full model for OS-MA				
Biomarker	2.79	0.93	0.99	0.42
Time	1.06	1.06	1.12	0.29
Strain	0.38	0.38	0.41	0.53
Tissue	27.90	5.58	5.93	0.0002
Biomarker × Tissue	13.19	2.64	2.80	0.024
Best fit model for OS-MA				
Biomarker	0.59	0.20	0.22	0.88
Tissue	33.72	5.62	6.20	< 0.0001
Biomarker × Tissue	15.72	1.96	2.17	0.038

Significant *P*-values are shown in bold.

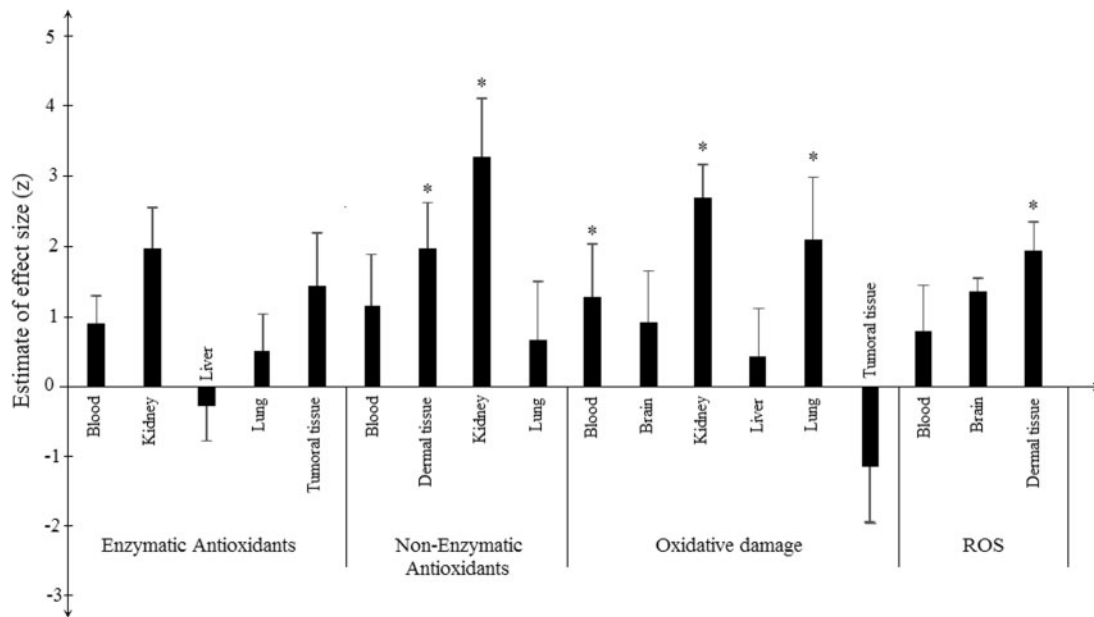


Figure 1. Effect of herpes virus infection on each single biomarker of OS for each tissue. Data are shown as mean + SE. Asterisk indicates a $P < 0.05$.

Table 2. Significant post-hoc comparisons showed that oxidative damage and enzymatic antioxidants changed differently between tissues

Biomarker	Tissues	Estimate (SE)	<i>t</i> -value	<i>P</i> -value
Oxidative damage	Blood–Kidney	–1.4 (0.36)	–3.82	0.0003
Oxidative damage	Blood–Liver	0.80 (0.38)	2.21	0.03
Oxidative damage	Blood–Tumoral tissue	2.40 (0.65)	3.74	0.0004
Oxidative damage	Brain–Kidney	–1.70 (0.73)	–2.35	0.05
Oxidative damage	Brain–Tumoral tissue	2.10 (0.92)	2.29	0.03
Oxidative damage	Kidney–Liver	2.20 (0.44)	5.11	<0.0001
Oxidative damage	Kidney–Tumoral tissue	3.80 (0.74)	5.17	<0.0001
Oxidative damage	Liver–Lung	–1.70 (0.75)	–2.2	0.03
Oxidative damage	Liver–Tumoral tissue	1.60 (0.75)	2.12	0.04
Oxidative damage	Lung–Tumoral tissue	3.20 (0.77)	4.22	0.0001
Enzymatic antioxidants	Blood–Liver	1.20 (0.60)	2.01	0.049
Enzymatic antioxidants	Kidney–Liver	2.30 (0.72)	3.17	0.002
Enzymatic antioxidants	Liver–Tumoral tissue	–1.80 (0.66)	–2.68	0.009

GSH), and glutathione (GSH), respectively (Table 4). Piperitenone oxide caused the highest reduction relatively to the concentration administered, followed by vaticaffinol, resveratrol, and ebselen. The comparison among these antioxidants revealed nonsignificant differences in reducing herpes virus yield (Table 4). Finally, the comparison between resveratrol and glutathione revealed that resveratrol had a stronger effect than glutathione (Table 4). In fact, with an average administration dose of 0.1445 mM, resveratrol caused an average reduction of 89.32% on the virus yield, while an average administration dose of 8.92 mM of GSH was needed to cause a reduction of 78.93% (Figure 3).

Discussion

The results from our first MA support our first hypothesis that herpes virus infection caused increased OS across diverse vertebrate species, but this effect was contingent on the tissue and biomarker of OS. Our second MA supports our second hypothesis that an increased intake of antioxidants reduced the virus load, indicating that the viral replication may be favored by a status of OS. The

positive effects of antioxidant administration were dependent on the concentration and the kind of antioxidant.

Herpes virus infection resulted in OS in most tissues analyzed, with the exception of brain, liver, and tumoral tissue. Our results show that kidney and dermal tissue were the most affected tissues, with a significant decrease in nonenzymatic antioxidants in both tissues, and a significant increase in oxidative damage in kidney and ROS level in dermal tissue, respectively. OS can affect some key regulators of kidney homeostasis and controls a number of signaling pathways that are relevant to kidney disease (Yang et al. 2014). The oxidative balance of dermal tissue was also significantly affected by the infection. We found a significant increase in ROS production and a significant decrease of nonenzymatic antioxidants, respectively. This is not surprising since the dermal tissue is one of the major candidates of OS because it is rich in potential biological targets for such reactions, especially lipids (Kohen and Gati 2000). Moreover, the fact that herpes virus reactivation is associated with skin lesions (Blauvelt 2001; de Thoisy et al. 2009), suggests that skin is particularly sensitive to the virus spread. On the other hand, it was, however, surprising to see that the oxidative balance of brain

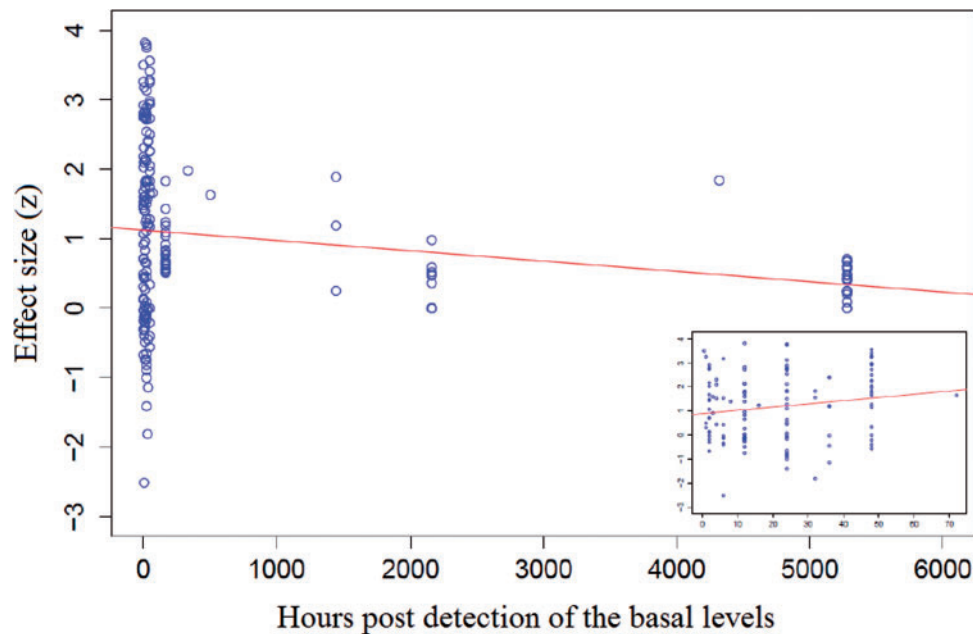


Figure 2. Effect size of herpes virus infection on OS in relation to the time elapsed from the detection of the basal levels up to 250 days, and within 4 days from the detection of the basal level (smaller figure in the bottom right). Trends are shown by lines.

Table 3. Full model and best fit model for ANTIOX-MA

	Sum of squares	Mean of squares	F value	P-value
Full model for ANTIOX-MA				
Concentration	26 077	26 076	14.09	<0.001
Strain	26	13	0.01	0.99
Treatment	36 371	7274	3.93	0.006
Cellular line	2902	967	0.52	0.67
Detection after	2040	510	0.27	0.89
Best fit model for ANTIOX-MA				
Concentration	28 751	28 751	17.89	0.0001
Treatment	67 049	11 175	6.95	<0.0001

Significant differences are shown in bold.

tissue was not affected by the infection. Brain cells have high metabolic intensities, low antioxidant defences, and high contents of polyunsaturated fatty acids, which make them an important target of OS (Barja 2004). These results are interesting especially if we consider that brain is the primary target of encephalitis caused by different strains of herpes viruses. Since results are based on the comparison of the effect sizes among different tissues, the nonsignificant increase of OD and ROS generation in the brain during herpes virus infection is probably due to a higher effect size measured in other tissues for the same biomarker. However, the effect size in brain tissue was still very high, and further studies will be needed to clarify why the effects of herpes virus on the oxidative balance differ between other tissues and brain tissue and to clarify if OS biomarkers predict clinical signs.

Tumoral tissue is characterized by an acceleration of the cell cycle, increased cell mobility, metabolic rate, and production of ROS (Baba and Cătoi 2007), and a decreased activity of antioxidant enzymes (Gargouri et al. 2009). For this reason, we decided to include this tissue to be able to assess the effect of herpes virus on OS in a highly proliferative tissue. Our data show that infection had a stimulating effect of enzymatic antioxidants, resulting in a reduction of oxidative damage. It would be interesting to assess whether this

Table 4. Percentage of virus yield reduction after administration of antioxidants

Treatment	Estimate (SE)	t-value	P-value	Comparison
Ebselen	95.80 (23.84)	4.02	<0.001	a, b
GSH	29.29 (16.16)	1.81	0.076	c
GSH-C4	-23.19 (18.47)	-1.26	0.22	d
Piperitenone oxide	120.63 (23.79)	5.07	<0.0001	a
Resveratrol	112.37 (9.69)	11.59	<0.0001	a
S-GSH	20.48 (29.72)	0.69	0.49	b, c, d
Vaticaffinol	115.85 (20.84)	5.56	<0.0001	a

Significant P-values are shown in bold. Antioxidants who share the same letter in the “Comparison” column showed nonsignificant differences.

increased antioxidant protection favors or not the persistence and the development of tumoral cells.

The liver was the least affected tissue. Although earlier studies have shown that the administration of glucocorticoids and other chemicals can strongly increase OS in liver (Costantini et al. 2011; Zlatković et al. 2014; Buha et al. 2015), the high regenerative capacity and molecular turnover that occur in this particular tissue might make its oxidative balance weakly sensitive to herpes infection (Michalopoulos and DeFrances 1997; Taub 2004; Michalopoulos and Khan 2005; Fausto et al. 2006).

Finally, we found that lung and blood showed a significant increase in the effect size of oxidative damage, but not in the effect size of other OS biomarkers. Interestingly, the change in OS was similar between blood and other tissues. This is indeed important because this result suggests that blood levels of OS reflect the general oxidative status of the whole organism during a viral infection. However, since herpes viruses are highly host and tissue specific, our results should be taken carefully. Indeed, given the high tissue tropism of some viruses, it might be possible that a specific herpes virus strain causes OS in a specific cell, while it does not cause any patho-physiological changes in the oxidative status of other cell lines in the same tissue. It is therefore important to consider that even if our study underlined a

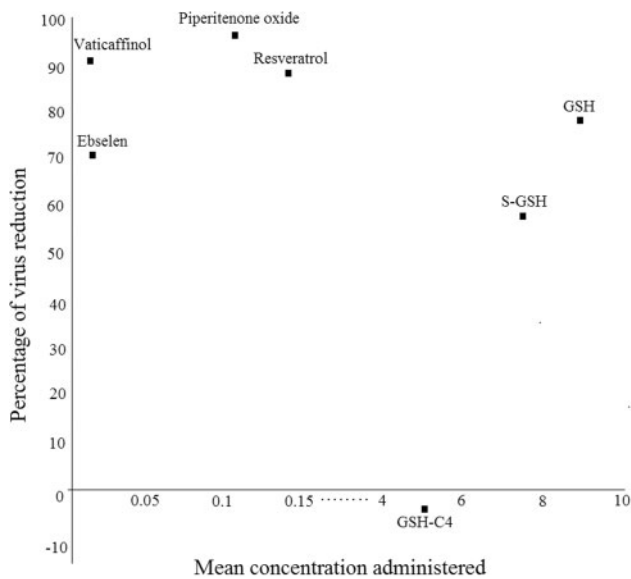


Figure 3. Relationship between concentration of antioxidants administered and percentage of virus reduction. The y axis represent the average percentage of virus reduction, while the x axis represent the average concentration of a given compound expressed as millimolar. Please note rescaling on the x axis.

significant increase of OS at a tissue level, it is not certain that this effect can be generalized to all the cell lines in a same tissue.

The effect size of herpes virus infection on tissue oxidative balance was also significantly dependent on the biomarker of OS. Our first MA clearly shows that the effect of infection on oxidative damage and enzymatic antioxidants differed among tissues, while there was a similar decrease of nonenzymatic antioxidants and increase in ROS production across all tissues. For instance, as compared to brain, liver, and tumor tissue, we found a higher increase in oxidative damage in blood, kidney, and lung. These results suggest that blood, kidney, and lung may be considered the most sensitive tissues in terms of capacity of detecting oxidative damage induced by herpes virus infection. It would also be interesting in future studies to assess whether among tissue differences in OS may also be related to cycle of the virus, i.e., replication stage and site, and excretion from cells.

The effects of virus infection were not dependent on the time elapsed since the measurement of the basal levels of a given OS biomarker. A visual examination of Figure 2 suggests that OS is rapidly increased in the first hours following the measurement of the basal levels (bottom right of Figure 2), but then the effect size decreases slightly, but not significantly, with time. The effect size was actually still high at over 200 days post-infection, indicating that herpes virus may alter the baseline oxidative status in the long term. These results suggest that long-term alterations of the oxidative status may be responsible for the immunosenescence and telomere shrinking that have been attributed to chronic herpes virus infection (Effros 2011). However, since there was heterogeneity on the time at which measurement of the basal levels of a given OS biomarker was performed (e.g., some studies started to count time after the inoculation of the virus, while some others calculated the time from the induction of the lytic cycle), caution must be used to interpret the results of how OS changes over time.

Our second MA (ANTIOX-MA) supports our hypothesis that administration of different antioxidants, especially piperitenone

oxide, vaticaffinol, ebselen, and resveratrol, may reduce infection. This suggests that herpes viruses are favoured by oxidative conditions, and that a disruption of these conditions can reduce herpes infection, as suggested by previous studies (Palamara et al. 1995; Docherty et al. 2005; Vogel et al. 2005; Kavouras et al. 2007). In this line of thoughts, it would be interesting to examine whether individuals with higher level of pre-existing oxidative damage are more susceptible to herpes virus than individuals with lower levels of oxidative damage. Although our results showed that the effect of a given type of antioxidant was dependent on its concentration, the best-fitted model showed that the type of antioxidant was much more significant than its concentration. As a result, the nonsignificant effect of glutathione in reducing herpes virus yield is probably due to its concentration, which had to be much higher than the other antioxidants to cause the same reduction. This result indicates that some antioxidants may be more efficient than others in reducing infection. However, this does not seem to explain why the concentration of glutathione needed to reduce virus yield was around 70 times higher than that of resveratrol. This is surprising because glutathione is one of the most important cell antioxidants (Halliwell and Gutteridge 2007) that was found to inhibit (1) the replication of HSV-1 by interfering with very late stages of the virus life cycle, and (2) HSV-1 glycoprotein B expression, which is considered essential for the production of enveloped infectious virus particles (Palamara et al. 1995). One reason might lie with glutathione having a short life in blood, making it necessary to administer it in large amounts (Fraternali et al. 2009). Moreover, glutathione cannot cross the cell membrane, but it needs to be broken down into amino acids and then resynthesized in the cell by the consecutive actions of γ -glutamylcysteine and GSH synthetases (Fraternali et al. 2009). The strongest effect of resveratrol administration on virus yield may also be due to its indirect effects on other antioxidants, hence indirectly helping the cell to maintain a balanced oxidative status. For example, resveratrol can increase synthesis of glutathione (Kode et al. 2008) and induce upregulation of the antioxidant enzymes catalase and glutathione peroxidase (Sadi et al. 2014). Moreover, resveratrol appears to negatively alter a host factor, NF- κ B, resulting in inhibition of virus replication (Faith et al. 2006). There was, however, heterogeneity in the experimental settings of studies that tested the effects of glutathione or resveratrol on herpes virus infection. It would therefore be of great importance to test the effects of both these antioxidants under similar experimental conditions.

Regarding the *in vivo* experiments, we could not compare results from different studies because there was high variation in the experimental conditions (administration of antioxidants or dermal application of cream-based antioxidants; days of administration and number of administrations per day; results showed as number of survivals or percentage of virus reduction). Despite this strong heterogeneity between experiments, these studies suggest that administration of antioxidants may also reduce the virus yield in the whole organism (Docherty et al. 1999; Nucci et al. 2000; Vogel et al. 2005), indicating that availability of antioxidants may constrain individual's capability of controlling viral infection.

In conclusion, our MAs showed that herpes virus infection (1) increases OS; (2) causes different levels of OS among tissues, with kidney and dermal tissue being the most susceptible tissues to OS; and (3) decreases after the administration of antioxidants, whose effect is type and concentration dependent. Moreover, the increase of oxidative damage in blood (tissue commonly available in studies on free-ranging organisms) was similar to that in the tissues most

sensitive to herpes virus. All these evidences support the hypothesis that a condition of OS promotes the activity of the herpes virus.

The significant levels of residual heterogeneity in OS-MA suggest there might be other factors affecting the variation in effect sizes, for example sex or age (excluded since the majority of studies did not provide data on both these factors), which require investigation in future studies. Such heterogeneity does not appear to depend on the virus strain because effect size was similar across different herpes virus strains. The effect size was also similar across species. However, our sample includes a limited number of species, mostly rodents and humans tested under controlled conditions, indicating the need of replication of similar studies in wild vertebrates. Our results provide novel insight into how OS may be a mechanism implicated as a pathogenic factor in herpes virus infections.

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Supplementary Material

Supplementary material can be found at <http://www.cz.oxfordjournals.org/>

References

- Apanius V, 1998. Stress and immune defense. *Adv Study Behav* 27:133–153.
- Aparicio-Burgos JE, Zepeda-Escobar JA, de Oca-Jimenez RM, Estrada-Franco JG, Barbabosa-Pliego A et al., 2015. Immune protection against *Trypanosoma cruzi* induced by TcVac4 in a canine model. *PLoS Negl Trop Dis* 94. doi:10.1371/journal.pntd.0003625.
- Arican O, Ozturk P, Kurutas EB, Unsal V, 2013. Status of oxidative stress on lesional skin surface of plantar warts. *J Eur Acad Dermatol Venereol* 27:365–369.
- Athar M, 2002. Oxidative stress and experimental carcinogenesis. *Indian J Exp Biol* 40:656–667.
- Baba AI, Cătoi C, 2007. *Comparative Oncology*. Bucharest: The Publishing House of the Romanian Academy.
- Bailey M, Engler H, Hunzeker J, Sheridan JF, 2003. The hypothalamic-pituitary-adrenal axis and viral infection. *Viral Immunol* 16:141–157.
- Barja G, 2004. Free radicals and aging. *Trends Neurosci* 27:595–600.
- Bartz AE, 1999. *Basic Statistical Concepts*. 4th edn. Upper Saddle River, NJ: Prentice Hall.
- Blauvelt A, 2001. Skin diseases associated with human herpesvirus 6, 7, and 8 infection. *J Invest Dermatol Symp Proc* 6:197–202.
- Buha A, Antonijević B, Milovanović V, Janković S, Bulat Z et al., 2015. Polychlorinated biphenyls as oxidative stress inducers in liver of subcutaneously exposed rats: implication for dose-dependence toxicity and benchmark dose concept. *Environ Res* 136:309–317.
- Costantini D, Møller AP, 2009. Does immune response cause oxidative stress in birds? A meta-analysis. *Comp Biochem Physiol Part A* 153:339–344.
- Costantini D, Marasco V, Møller AP, 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J Comp Physiol B Biochem Syst Environ Physiol* 181:447–56.
- Del Re AC, 2013. *compute.es: Compute Effect Sizes*. R package. Available from: <http://CRAN.R-project.org/package=compute.es>.
- de Thoisy B, Lavergne A, Semelin J, Pouliquen J-F, Blanchard F et al., 2009. Outbreaks of disease possibly due to a natural avian herpesvirus infection in a colony of young Magnificent Frigatebirds *Fregata magnificens* in French Guiana. *J Wildl Dis* 45:802–807.
- Docherty JJ, Fu MM, Hah JM, Sweet TJ, Faith SA et al., 2005. Effect of resveratrol on herpes simplex virus vaginal infection in the mouse. *Antiviral Res* 67:155–62.
- Docherty JJ, Fu MM, Stiffler BS, Limperos RJ, Pokabla CM et al., 1999. Resveratrol inhibition of herpes simplex virus replication. *Antiviral Res* 43:145–55.
- Effros RB, 2011. Telomere/telomerase dynamics within the human immune system: effect of chronic infection and stress. *Exp Gerontol* 46:135–140.
- Elftman MD, Hunzeker JT, Mellinger JC, Bonneau RH, Norbury CC et al., 2010. Stress-induced glucocorticoids at the earliest stages of herpes simplex virus-1 infection suppress subsequent antiviral immunity, implicating impaired dendritic cell function. *J Immunol* 184:1867–1875.
- Faith SA., Sweet TJ, Bailey E, Booth T, Docherty JJ, 2006. Resveratrol suppresses nuclear factor-B in herpes simplex virus infected cells. *Antiviral Res* 72:242–51.
- Fausto N, Campbell JS, Riehle KJ, 2006. Liver regeneration. *Hepatology* 43:45–53.
- Fraternali A, Paoletti MF, Casabianca A, Nencioni L, Garaci E et al., 2009. GSH and analogs in antiviral therapy. *Mol Aspects Med Elsevier Ltd* 30:99–110.
- Gargouri B, Van Pelt J, El Feki AEF, Attia H, Lassoued S, 2009. Induction of Epstein-Barr virus (EBV) lytic cycle *in vitro* causes oxidative stress in lymphoblastoid B cell lines. *Mol Cell Biochem* 324:55–63.
- Gontard-Danek MC, Moller AP, 1994. The strength of sexual selection: a meta-analysis of bird studies. *Behav Ecol* 10:476–86.
- Halliwell BH, Gutteridge JMC, 2007. *Free Radical in Biology and Medicine*. 4th edn. Oxford: Clarendon Press/Oxford University Press, 160–165.
- Hunzeker JT, Elftman MD, Mellinger JC, Princiotta MF, Bonneau RH et al., 2011. A marked reduction in priming of cytotoxic CD8+ T cells mediated by References 265 stress-induced glucocorticoids involves multiple deficiencies in cross-presentation by dendritic cells. *J Immunol* 186:183–194.
- Kaleta EF, 1990. Herpesviruses of birds: a review. *Avian Pathol* 19:193–211.
- Kavouras JH, Prandovszky E, Valyi-Nagy K, Kovacs SK, Tiwari V et al., 2007. Herpes simplex virus type 1 infection induces oxidative stress and the release of bioactive lipid peroxidation by-products in mouse P19N neural cell cultures. *J Neurovirol* 13:416–25.
- Kode A, Rajendrasozhan S, Caito S, Yang SR, Megson IL, 2008. Rahman I. Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 294:478–488.
- Kohen R, Gati I, 2000. Skin low molecular weight antioxidants and their role in aging and in oxidative stress. *Toxicology* 148:149–157.
- Kuznetsova A, Brockhoff PB, Christensen RHB, 2013. lmerTest: tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R package. Available from: <http://CRAN.R-project.org/package=lmerTest>.
- Lassoued S, Ameer RB, Ayadi W, Gargouri B, Mansour RB et al., 2008. Epstein-Barr virus induces an oxidative stress during the early stages of infection in B lymphocytes, epithelial, and lymphoblastoid cell lines. *Mol Cell Biochem* 313:179–186.
- Li X, Feng J, Sun R, 2011. Oxidative stress induces reactivation of Kaposi's sarcoma-associated herpesvirus and death of primary effusion lymphoma cells. *J Virol* 85:715–24.
- Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC et al., 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med*. 339:b2700.
- Martin LB, Hopkins WA, Mydlarz LD, Rohr JR, 2010. The effects of anthropogenic global changes on immune functions and disease resistance. *Ann NY Acad Sci* 1195:129–48.

- Michalopoulos GK, DeFrances MC, 1997. Liver regeneration. *Science* 276:60–66.
- Michalopoulos GK, Khan Z, 2005. Liver regeneration, growth factors, and amphiregulin. *Gastroenterology* 128:503–506.
- Murphy K, 2012. *Janeway's Immuno Biology*. 8th edn. London/New York: Garland Science.
- Nakagawa S, Cuthill IC, 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol Rev* 82:591–605.
- Nucci C, Palamara AT, Ciriolo MR, Nencioni L, Savini P et al., 2000. Imbalance in corneal redox state during herpes simplex virus 1-induced keratitis in rabbits: effectiveness of exogenous glutathione supply. *Exp Eye Res* 70:215–20.
- Padgett DA, Glaser R, 2003. How stress influences the immune response. *Trends Immunol* 24:444–448.
- Palamara AT, Perno CF, Ciriolo MR, Dini L, Balestra E et al., 1995. Evidence for antiviral activity of glutathione: *in vitro* inhibition of herpes simplex virus type 1 replication. *Antiviral Res* 27:237–53.
- Palamara AT, Brandi G, Rossi L, Millo E, Benatti U et al., 2004. New synthetic glutathione derivatives with increased antiviral activities. *Antivir Chem Chemother* 15:83–91.
- Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N et al., 2008. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* 455:1235–1239.
- Romero LM, 2004. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol* 19:249–255.
- Rosenthal R, 1991. *Meta Analytic Procedures for Social Research*. New York: Sage Publications.
- Sadi G, Bozan D, Yildiz HB, 2014. Redox regulation of antioxidant enzymes: post-translational modulation of catalase and glutathione peroxidase activity by resveratrol in diabetic rat liver. *Mol Cell Biochem* 393:111–122.
- Sapolsky RM, Romero LM, Munck AU, 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21:55–89.
- Scheinman RI, Gualtebro A, Jewell CM, Cidowski JA, 1995. Characterization of mechanism involved in transpression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol* 15:943–953.
- Simons MJP, Cohen AA, Verhulst S, 2012. What does carotenoid-dependent coloration tell? Plasma carotenoid level signals immunocompetence and oxidative stress state in birds: a meta-analysis. *PLoS ONE* 7(8):e43088.
- Staley M, Bonneaud C, 2015. Immune responses of wild birds to emerging infectious diseases. *Parasite Immunol* 37:242–254.
- Surai P, 2002. *Natural Antioxidants in Avian Nutrition and Reproduction*. Nottingham: Nottingham University Press.
- Taub R, 2004. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 5:836–847.
- Valyi-Nagy T, Olson SJ, Valyi-Nagy K, Montine TJ, Dermody TS, 2000. Herpes simplex virus type 1 latency in the murine nervous system is associated with oxidative damage to neurons. *Virology* 278:309–21.
- Vogel JU, Cinatl J, Dauletbaev N, Buxbaum S, Treusch G et al., 2005. Effects of S-acetylglutathione in cell and animal model of herpes simplex virus type 1 infection. *Med Microbiol Immunol* 194:55–59.
- Webster JI, Tonelli L, Sternberg EM, 2002. Neuroendocrine regulation of immunity. *Annu Rev Immunol* 20:125–163.
- Yang SK, Xiao, Liu F, Sun L, 2014. Oxidative stress, a common molecular pathway for kidney disease: role of the redox enzyme p66Shc. *Ren Fail* 36:313–320.
- Zlatković J, Todorović N, Tomanović N, Bošković M, Djordjević S et al., 2014. Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. *Eur J Pharm Sci* 59:20–30.