

Young plasma transfer enhances antioxidant defense and preserves structural integrity in aged lung tissue

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Abstract

Background: Heterochronic plasma exchange is widely used to investigate systemic aging; however, its pulmonary consequences particularly regarding oxidative stress, antioxidant defense, and tissue architecture have not been systematically examined.

Methods: Twenty-four-month-old male Sprague–Dawley rats received 0.5 mL of young plasma intravenously daily for 30 days, while 8-week-old rats received 0.25 mL of aged plasma. After treatment, lung tissues were analyzed histologically, biochemically, and molecularly.

Results: Quantitative PCR showed that young plasma markedly upregulated antioxidant defenses, with SOD and CAT expression increasing by ~2.5-fold and 1.8-fold, respectively ($p < 0.01$), accompanied by higher SOD and GPX enzyme activities ($p < 0.05$). Additional antioxidant genes (GR, GST, TXN/TXNR) were also significantly upregulated, confirming a broad activation of the antioxidant network. In contrast, aged plasma suppressed antioxidant responses, reducing CAT activity by ~35% ($p < 0.01$) and similarly decreasing other enzymes. Histological analyses revealed preserved alveolar structure, thinner septa, and reduced inflammation in old + young plasma rats, while young + old plasma transfer caused structural deterioration. Immunohistochemistry confirmed increased GPX, SOD, and CAT expression in aged rats receiving young plasma, consistent with transcriptional and protein-level activation. Moreover, heterochronic plasma exchange attenuated collagen accumulation, suggesting reduced fibrillar matrix deposition, and restored the balance between alveolar epithelial Type I (AT1) and Type II (AT2) cells, indicating improved epithelial homeostasis. Toluidine Blue staining showed decreased mast-cell density after young plasma treatment ($p < 0.05$), reinforcing its anti-inflammatory effect.

Conclusions: Young plasma exerts regenerative and anti-inflammatory actions in the aged lung, highlighting it as a key target of systemic rejuvenation.

Keywords: Plasma exchange, Lung tissue, Antioxidant, Oxidative stress, Sprague-Dawley

Introduction

Aging involves not just a gradual decline in function but also a comprehensive process driven by circulating molecular signals, which results in the breakdown of homeostatic control mechanisms throughout various organ systems. This process involves numerous molecular events, including mitochondrial dysfunction,¹ accumulation of DNA damage,² impairment of epigenetic regulatory mechanisms,³ and the chronic activation of inflammatory signaling pathways.⁴ These molecular alterations manifest in various tissues and organ systems through

biologically active circulating molecules, contributing to the systemic phenotype of aging. In this context, heterochronic plasma transfer models have emerged as a prominent experimental strategy for investigating the effects of systemic factors on the pathophysiology of aging.^{5,6}

In studies of heterochronic plasma transfer, it has been demonstrated that plasma derived from young individuals and administered to aged organisms can decelerate neurodegenerative processes⁷ and enhance hematopoietic and hepatic regenerative capacity.⁸ Conversely, it has been reported that plasma

obtained from elderly individuals suppresses cellular functions and adversely affects regenerative processes in young organisms.⁹ Notably, antioxidant defense systems are central to the redox imbalances associated with aging; the reduced expression or activity of genes encoding enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), thioredoxin (TXN2), thioredoxin reductase (TXNRD2), and glutathione reductase (GR) leads to the accumulation of free radicals, loss of organelle function, and acceleration of inflammatory processes.^{10,11} It has been reported that the administration of young plasma can reverse these aging-related dysfunctions; for instance, it reduces oxidative damage in the central nervous system (eg, hippocampus, cortex) by regulating mitochondrial function¹² and, in peripheral organs (eg, liver, intestine), lowers oxidative stress markers and strengthens the anti-inflammatory response.⁸ In contrast, the transfer of old plasma has been reported to increase oxidative stress load in young tissues and decrease the expression of antioxidant response genes.⁹

These general findings from the literature are corroborated by heterochronic plasma studies conducted by our team. For instance, it has been demonstrated that young plasma enhances the morphological integrity of aged intestinal tissue, resulting in significant improvements in histomorphometric parameters such as villus height and crypt depth¹³; it restores DNA methylation and PCNA expression in aged testes to youthful levels¹⁴; and it significantly suppresses NLRP3 inflammasome and necroptotic signaling in the liver.¹⁵ These data indicate that young plasma establishes a consistent biological signature at both the systemic and multi-organ levels, contributing to the reduction of oxidative stress and the reinforcing of regenerative capacity.

Despite this accumulating evidence, the effects of young plasma on lung tissue particularly concerning oxidative stress, antioxidant defense, inflammation, and histological integrity have been insufficiently explored in the literature. To our knowledge, no previous studies have directly examined the structural, oxidative, or inflammatory responses of pulmonary tissue following heterochronic plasma exchange or parabiosis. The lung is among the organs most sensitive to alterations in redox homeostasis due to its continuous exposure to high oxygen pressure, extensive capillary network, and susceptibility to immune cell infiltration.¹⁶ Therefore, in the present study, the effects of heterochronic plasma exchange conducted over 30 days between young and old rats were comprehensively evaluated in terms of gene expression and enzyme activities of antioxidant defense system components in lung tissue, as well as histological architecture and integrity. We hypothesized that young plasma transfer would preserve pulmonary architecture and reduce age-associated degeneration by reinforcing antioxidant defenses, whereas old plasma transfer would exacerbate oxidative burden and promote inflammatory remodeling in young lung tissue.

Methods

Animal studies

In this investigation, Male Sprague-Dawley rats were employed as the model organism. Four groups were established: Old Control (OCnt), Old+Young Plasma (O+YPls), Young Control (YCnt), and Young+Old Plasma (Y+OPls). Each group included seven rats ($n = 7$); however, for gene expression and enzyme

assays, five biological samples per group were analyzed in triplicate to confirm data consistency. The O+YPls group (24 months old) received 0.5 ml/day plasma from YCnt donors (8 weeks old) for 30 days via tail vein injection, while the Y+OPls group (8 weeks old) received 0.25 ml/day plasma from OCnt donors (24 months old) for 30 days.¹⁷ The number of animals utilized for plasma collection was determined based on the volume of plasma obtainable from each animal. On average, a 24-month-old rat contains 10 ml of blood, from which approximately 5 ml can be extracted as plasma. Similarly, a 5-week-old rat contains an average of 5 ml of blood, with about 2.5 ml being plasma. The volume of plasma transferred was calculated to be approximately 1/10 of the recipient animal's blood plasma volume.⁷

The young and aged rats used in the study were sourced from a facility proficient in the production and handling of experimental animals. Each group was housed separately in individual cages, and animals housed together remained in the same group throughout the experiment. The animals were maintained in transparent plexiglass cages (6 rats per cage), under a 12-hour light/12-hour dark cycle, at a constant temperature of 21°C, with unrestricted access to food and water. There was no loss of animals during the course of the experiment. On the day following the completion of the treatment process, the animals in both the experimental and control groups were sacrificed under ether anesthesia. Lung tissues were collected, immediately shock-frozen on dry ice, and stored at -80°C until analyses were conducted. Our study was carried out with the approval of the Ethics Committee (approval number: 2021/03) from the Saki Yenilli Experimental Animal Production and Practice Laboratory.

Plasma collection

Pooled rat plasma was obtained via terminal cardiac puncture during euthanasia. Blood samples were collected into tubes containing EDTA and subsequently centrifuged at 1000g to isolate plasma. To inactivate plasma proteins, the collected plasma was subjected to heating at 95°C for 2-3 minutes, followed by brief centrifugation at 1000g. All plasma aliquots were stored at -80°C until required for use. Prior to utilization, dialysis was conducted in PBS using 3.5 kDa D-tube dialyzers (EMD Millipore) to remove EDTA from the plasma.¹³

Enzyme activity assays

The tissues were homogenized in a 50 mM potassium phosphate buffer (pH 7.4) utilizing a TissueLyser LT (Qiagen) homogenizer operating at 50 Hz, in two sessions of 3 minutes each (totaling 6 minutes). The resultant homogenates were subjected to centrifugation at 10,000 rpm for 1 hour, and the clear supernatants obtained were employed for the assessment of SOD and CAT enzyme activities. CAT activity was quantified at a wavelength of 240 nm at 15-second intervals over a duration of 1 minute.¹⁸ SOD activity was determined at a fixed wavelength of 560 nm.¹⁹ For the evaluation of GR, GPx, and GST activities, the tissues were re-homogenized in a 50 mM Tris-HCl buffer (pH 7.6), supplemented with 1 mM DTT (dithiothreitol), 1 mM EDTA (ethylenediaminetetraacetic acid), and 1 mM PMSF (phenylmethylsulfonyl fluoride). The lysates were centrifuged at 13,000×g for 1 hour to obtain supernatants, which were subsequently used in the spectrophotometric measurements of GR, GPx, and GST activities.

GR activity was measured at 340 nm for 3 minutes following the method of Carlberg and Mannervik.²⁰ GPX activity was determined in accordance with the Sigma Aldrich assay protocol (Cat. No: SPGLUT02). As per the protocol, the reaction mixture was incubated for 10 minutes to stabilize absorbance changes; subsequently, following the addition of H₂O₂, GPX activity was kinetically measured at 340 nm for 5 minutes. GST activity was assessed at 340 nm for 3 minutes according to the method of Habig et al.²¹ Specific activities were expressed as EU/mg protein, normalized to protein concentration (Bradford method).²²

RNA isolation and cDNA synthesis

Total RNA was isolated from tissue samples utilizing the Gene-All Hybrid-R kit (Cat. No. 305-101, Korea) in strict accordance with the manufacturer's guidelines. The integrity and concentration of the isolated RNA were assessed using a 96-well spectrophotometer (Multiskan GO, Thermo Scientific, Waltham, MA, USA) equipped with NanoDrop QC SkanIt software 4.1. Subsequent to RNA isolation, cDNA synthesis was performed using the ProtoScript First Strand cDNA Synthesis Kit (SuScript cDNA Synthesis Kit, Cat. No. RT01A025, Turkey) following the manufacturer's protocol. The procedure entailed a 5-minute pre-denaturation at 70°C, a 1-hour incubation at 42°C, and a 5-minute enzyme inactivation at 80°C. These thermal cycling processes were executed using the Sensoquest Thermocycler Labcycler (Thermoblock 96 Gold Plated Silver 012-103, Germany). The synthesized cDNA samples were stored at -20°C to preserve their integrity for subsequent genetic analyses.²³

Primers and gene expression analysis

The gene-specific primers employed in this study were designed utilizing Primer3 software (<http://primer3.ut.ee/>). Real-time quantitative PCR (qPCR) analyses were conducted on the Rotor-Gene Q system (QIAGEN, Hilden, Germany) using the SuScript 1-Step SYBR qPCR Kit (Catalog No: RT01A046, 200Rxn, Ankara, Turkey) with a final reaction volume of 10 µL in 0.1 mL strip tubes. The thermal cycling protocol comprised an initial incubation at 50°C for 2 minutes, a pre-denaturation step at 95°C for 1 minute, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds. The GAPDH gene served as the internal reference for normalization. While the 2^{-ΔΔCT} method,²⁴ which is extensively utilized in the literature, was considered, a modified normalization strategy was implemented in this study to facilitate statistical comparisons between individual biological replicates. Accordingly, for each sample, the ΔCT value was calculated using the formula CT_{target}—CT_{reference}; to maintain intra-group variance and ensure suitability for statistical analyses, the obtained ΔCT values were normalized by dividing them by the mean ΔCT value of the relevant control group. This approach particularly prevents the masking of biologically meaningful variations by ΔΔCT calculations based on group means in studies with small sample sizes.²⁵ Five biological replicates were used in each group, and each biological replicate was analyzed in three technical repeats. All statistical evaluations were conducted using the normalized ΔCT values. The primer sequences used in the study are presented in Table S1 (see online supplementary material).

Histological and histomorphometric examination

At the conclusion of the experimental period, lung tissues were collected, fixed in 10% neutral-buffered formalin, and

processed into 5-µm paraffin sections. Routine hematoxylin and eosin (H&E) and Masson's trichrome (MT) stainings were conducted for general histological assessment and evaluation of the extracellular matrix, respectively.²⁶ Morphometric analyses were performed using ImageJ software (Fiji, NIH, Bethesda, MD, USA) at 20× magnification. Quantitative parameters included the area of lymphocytic infiltration (%), collagen fiber area (%) in MT-stained sections, vessel wall thickness (µm), bronchiolar diameter (µm), and interalveolar septal thickness (µm). Measurements were obtained from five randomly selected sections per animal, with 10-30 fields analyzed per section.²⁷

Mast cells were identified using toluidine blue (TB) staining, following the protocol of Vidal and Mello (2019). Briefly, a 0.1% TB stock solution was prepared in 70% ethanol with 1% sodium chloride, and the pH was adjusted to 2.0-2.5 using acetic acid. Sections (5 µm) were deparaffinized, stained for 3-10 minutes, rinsed, and dehydrated through graded alcohols before xylene clearing. Due to their metachromatic properties, mast cell granules appeared as deep violet structures under light microscopy.²⁸

Immunohistochemical analysis

The lung tissues underwent standard post-fixation protocols, and 5 µm thick sections were sliced with a microtome and mounted on polylysine-coated slides. After deparaffinization and rehydration, endogenous peroxidase activity was inhibited using 3% hydrogen peroxide. Antigen retrieval was conducted utilizing citrate buffer (pH 6.0). All slides were incubated with designated primary antibodies for glutathione peroxidase (GPx-1/2, Santa Cruz (H-151), sc30147, dilution: 1:200), catalase (CAT, Santa Cruz (H-300), sc50508, dilution: 1:200), and superoxide dismutase-1 (SOD-1, Santa Cruz (FL-154), sc11407, dilution: 1:200), followed by a biotinylated secondary antibody and a streptavidin-peroxidase system (TP-125-BN, Thermo Fisher Scientific, US). Labeling was performed using DAB chromogen solution (Thermo Fisher Scientific, US) and counterstained with Mayer's haematoxylin. The sections were evaluated using a light microscope following dehydration and mounting. The expression levels of GPx, CAT, and SOD, regarding immunoreactivities, were assessed as a percentage of area utilizing ImageJ (Fiji) software (National Institutes of Health, NIH).²⁶

Data collection from databases and verification of obtained data

Gene Expression Profiling Interactive Analysis (GEPIA), Genotype-Tissue Expression (<https://www.gtexportal.org/>), and the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN) (<https://ualcan.path.uab.edu/>) are interactive web servers that provide data on normal and tumor samples through their databases. Lung adenocarcinoma (LUAD) is a prevalent subtype of lung cancer globally, and alterations in oxidative stress and antioxidant defense mechanisms significantly contribute to the disease's progression. The relationship between the human antioxidant system and LUAD was evaluated using the GEPIA²⁹ and UALCAN³⁰ databases.

Initially, the gene expression profiles of antioxidant system components in lung tissue from LUAD cases were obtained from <http://gepia.cancer-pku.cn/>, and changes in protein levels were acquired from <https://ualcan.path.uab.edu>. Based on the

information obtained from the databases, gene and protein expressions present in LUAD were compared, and the findings from our current study were interpreted in an integrated manner.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism version 10.0.5 (GraphPad Software, San Diego, CA, USA). Data are presented as mean $\pm$ standard error of the mean (SEM). Comparisons between two groups were performed using an unpaired two-tailed Student's *t*-test. A *p*-value of < 0.05 was considered statistically significant, with levels of significance denoted as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

Results

Regulatory effects of heterochronic plasma transfer on antioxidant enzyme gene expression

Comprehensive quantitative PCR analyses revealed that heterochronic plasma exchange significantly altered the transcriptional profile of antioxidant defense genes in lung tissue (Figure 1A₁-A₇, B₁-B₇). In aged rats receiving young plasma (O+YPls), the expression of all examined genes was significantly elevated compared to aged controls (OCnt). Specifically, GST ($p = 0.0188$), GPX ($p = 0.0019$), and GR ($p = 0.0007$) were upregulated, indicating a restoration of the glutathione redox cycle. Similarly, CAT ($p = 0.0386$) and SOD ($p < 0.0001$) transcripts increased, reflecting a recovery of hydrogen peroxide and superoxide detoxification pathways. TXN2 ($p < 0.0001$) and TXNRD2 ($p = 0.0048$) were also significantly elevated, suggesting an enhancement of thioredoxin-mediated redox regulation.

Conversely, in young rats administered old plasma (Y+OPls), a consistent decline in antioxidant gene expression was observed. GST ($p < 0.0001$), GPX ($p = 0.0295$), CAT ($p < 0.0001$), SOD ($p = 0.0051$), TXN2 ($p = 0.0386$), and TXNRD2 ($p = 0.0095$) transcripts were significantly reduced relative to young controls (YCnt), while GR remained unaffected ($p = 0.3470$). Collectively, these findings indicate that young plasma promotes broad transcriptional activation of antioxidant defense systems in aged lungs, whereas old plasma exerts a suppressive effect on multiple components of the redox network in young lungs.

Antioxidant enzyme activity modulation following heterochronic plasma exchange

Biochemical analyses have elucidated significant bidirectional effects of plasma exchange on enzymatic antioxidant defenses within lung tissue (Figure 2A₁-A₅, B₁-B₅). In aged rats treated with young plasma (O+YPls), all assessed enzymes exhibited substantial activity increases compared to aged controls (OCnt). Notably, GST activity was significantly elevated ($p < 0.0001$), indicating an enhanced detoxification capacity for xenobiotic and oxidative intermediates. GPX activity also showed an increase ($p = 0.0039$), suggesting improved hydroperoxide elimination, while GR activity rose significantly ($p = 0.0011$), supporting the efficient regeneration of reduced glutathione. Catalase activity demonstrated a robust enhancement ($p < 0.0001$), consistent with strengthened hydrogen peroxide clearance, and SOD activity was substantially augmented ($p < 0.0001$), underscoring restored superoxide detoxification potential in the aged lung.

Conversely, young rats receiving old plasma (Y+OPls) exhibited consistent reductions across the same enzymatic panel compared

to young controls (YCnt). GST ($p = 0.0133$), GPX ($p = 0.0331$), GR ($p = 0.0117$), CAT ($p = 0.0117$), and SOD ($p = 0.0117$) activities all declined significantly, suggesting that aged systemic factors impair multiple components of the enzymatic antioxidant defense network in young lungs. Collectively, these findings demonstrate that young plasma restores enzymatic antioxidant capacity across glutathione-dependent, catalase, and superoxide dismutase pathways in aged organisms, whereas old plasma diminishes these critical defense systems in young recipients.

Histopathological assessment of pulmonary structure following heterochronic plasma exchange

Hematoxylin and eosin (H&E) staining revealed significant age- and treatment-dependent variations in pulmonary structure and inflammatory cell infiltration (Figure 3A₁-A₄). In the aged control group (OCnt), there was extensive perivascular and peribronchiolar lymphocytic infiltration, accompanied by thickened vessel walls, increased interalveolar septal thickness, and disorganized bronchiolar architecture. Conversely, aged rats treated with young plasma (O+YPls) exhibited a markedly preserved pulmonary architecture, characterized by reduced lymphocytic infiltration, decreased septal thickening, and near-normal vascular and airway dimensions.

In the OCnt group, alveolar septa lined by Type I alveolar epithelial cells (black arrows) exhibited marked thickening, irregularity, and focal disruption, accompanied by dense interstitial infiltration of inflammatory cells (Figure S1—see online supplementary material for a color version of this figure). A pronounced increase in Type II pneumocytes (red arrowheads) with reactive hyperplasia was also evident, indicating advanced age-related alveolar remodeling. In contrast, the O+YPls group showed a striking restoration of alveolar architecture, with septa appearing thinner, more uniform, and structurally continuous. The reduced density of Type II pneumocytes suggested that young plasma mitigated age-associated epithelial activation and structural deterioration. In the YCnt group, alveolar septa remained thin and intact, with Type I cells displaying normal morphology and Type II cells distributed at physiological levels, consistent with preserved pulmonary homeostasis (Figure S1—see online supplementary material for a color version of this figure). Conversely, in the Y+OPls group, mild septal thickening and a moderate increase in Type II pneumocytes were observed relative to young controls, suggesting that aged plasma induced an early stress-repair response in otherwise healthy lung tissue (Figure S1—see online supplementary material for a color version of this figure).

Quantitative histomorphometry corroborated these observations. Compared to OCnt, young plasma treatment significantly reduced lymphocytic infiltration (Figure 3B₁, $p < 0.01$), vessel wall thickness (Figure 3B₂, $p < 0.0001$), bronchiolar width (Figure 3B₃, $p < 0.0001$), and interalveolar septal thickness (Figure 3B₄, $p < 0.01$). These findings suggest that circulating factors from young donors exert a protective effect on age-associated pulmonary inflammation and remodeling.

In contrast, young rats receiving old plasma (Y+OPls) exhibited structural changes reminiscent of senescent lung pathology. Histological sections showed dense lymphocytic accumulation, vascular hypertrophy, bronchiolar dilatation, and widespread septal thickening compared to age-matched controls (YCnt). Morphometric analyses confirmed these pathological changes, revealing significant increases in lymphocytic infiltration (Figure 3C₁, $p < 0.0001$), vessel wall thickness (Figure 3C₂, $p < 0.001$), bronchiolar

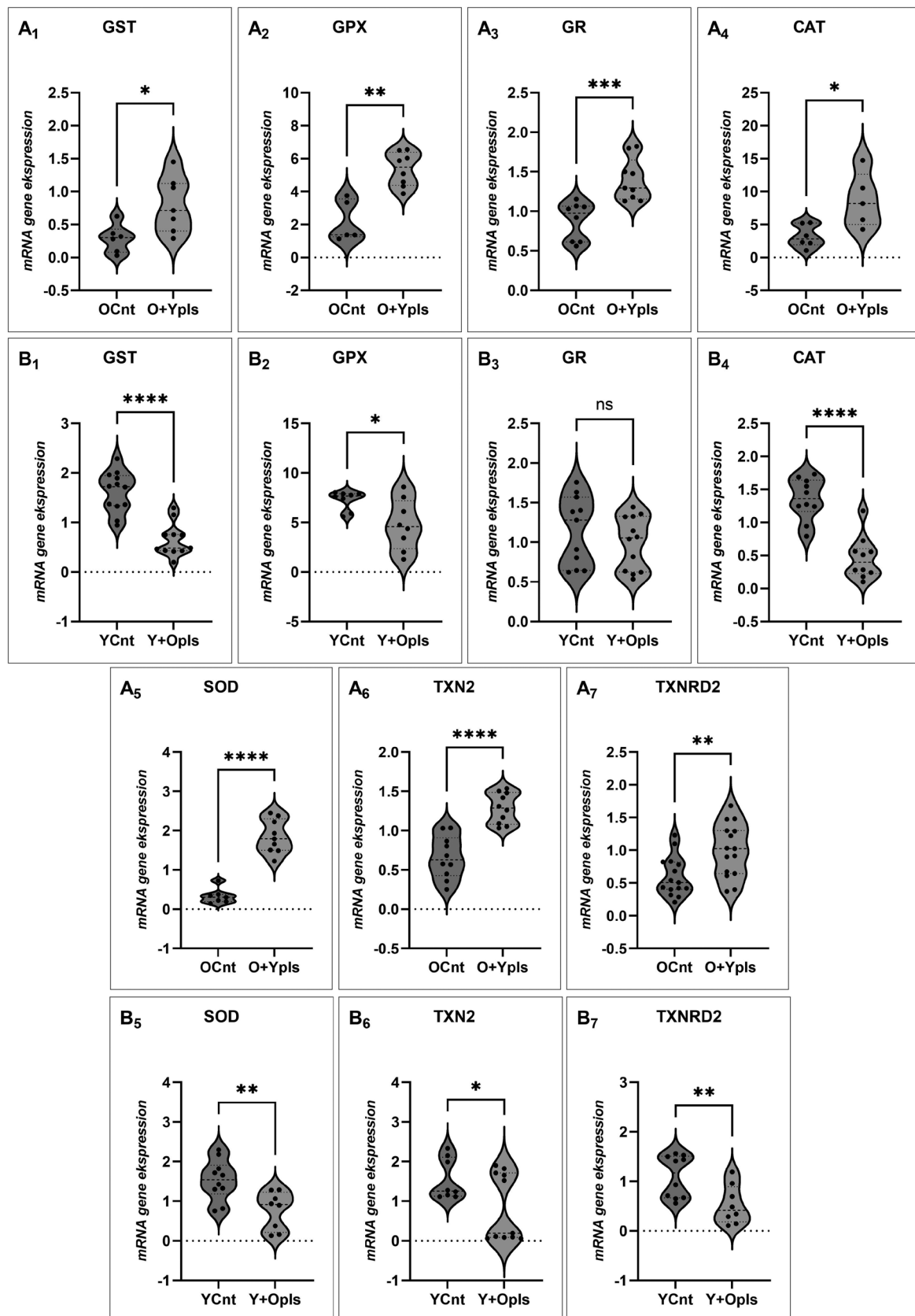


Figure 1. Effects of heterochronic plasma transfer on the mRNA expression of antioxidant defense genes in rat lung tissue. Panels A₁-A₇ show comparisons between the aged control group (OCnt) and aged rats receiving young plasma (O+Ypls), while panels B₁-B₇ depict comparisons between the young control group (YCnt) and young rats receiving old plasma (Y+Opls). Genes analyzed include glutathione S-transferase (GST, A₁, B₁), glutathione peroxidase (GPX, A₂, B₂), glutathione reductase (GR, A₃, B₃), catalase (CAT, A₄, B₄), superoxide dismutase (SOD, A₅, B₅), thioredoxin (TXN2, A₆, B₆), and thioredoxin reductase (TXNRD2, A₇, B₇). Data are presented as violin plots with individual biological replicates ($n = 5$) and three technical replicates per sample. Statistical analysis was performed using unpaired two-tailed t -tests. Significance levels: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). ns, not significant.

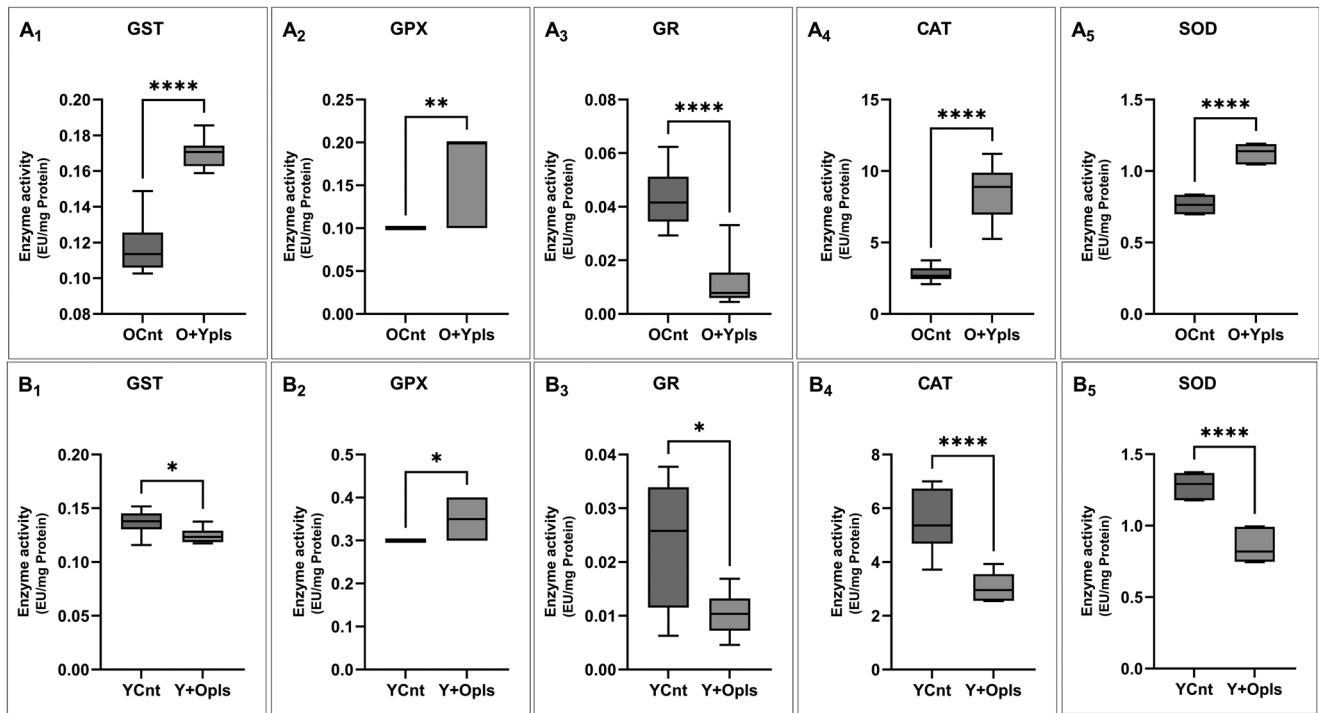


Figure 2. Effects of heterochronic plasma transfer on antioxidant enzyme activities in rat lung tissue. Panels A₁-A₅ show comparisons between the aged control group (OCnt) and aged rats receiving young plasma (O+Ypls), while panels B₁-B₅ depict comparisons between the young control group (YCnt) and young rats receiving old plasma (Y+Opls). Enzymes analyzed include glutathione S-transferase (GST, A₁, B₁), glutathione peroxidase (GPX, A₂, B₂), glutathione reductase (GR, A₃, B₃), catalase (CAT, A₄, B₄), and superoxide dismutase (SOD, A₅, B₅). Data are presented as box-and-whisker plots showing individual biological replicates ($n = 5$) with three technical replicates per sample. Statistical analysis was performed using unpaired two-tailed t -tests. Significance levels: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****).

width (Figure 3C₃, $p < 0.001$), and septal thickness (Figure 3C₄, $p < 0.001$). Collectively, these results indicate that heterochronic young plasma transfer ameliorates age-associated pulmonary degeneration by suppressing inflammatory remodeling and preserving structural integrity, whereas old plasma exerts deleterious effects on the otherwise intact young lung.

Collagen evaluation of collagen deposition and mast cell infiltration in lung tissue

Masson's trichrome (MT) staining revealed distinct age- and treatment-dependent alterations in extracellular matrix remodeling (Figure 4A₁-A₄). In aged control rats (OCnt), dense blue-stained collagen fibers were observed in perivascular and peribronchiolar regions, indicative of fibrotic remodeling. In contrast, aged rats treated with young plasma (O+Ypls) displayed markedly reduced collagen deposition and preserved parenchymal architecture. Young control lungs (YCnt) exhibited minimal collagen accumulation, whereas young rats administered old plasma (Y+Opls) demonstrated evident peribronchiolar and interstitial collagen deposition. Quantitative morphometry confirmed these patterns (Figure 4A₅), showing significantly decreased collagen density in O+Ypls compared with OCnt ($p < 0.01$) and significantly increased density in Y+Opls compared with YCnt ($p < 0.01$). These results highlight the anti-fibrotic influence of young plasma and the pro-fibrotic effects of aged plasma.

Toluidine blue (TB) staining was employed to evaluate mast cell infiltration (Figure 4B₁-B₄). In OCnt lungs, mast cells were abundant within the alveolar interstitium, particularly surrounding vascular and airway structures. Administration of young plasma substantially reduced mast cell density in aged rats,

whereas aged plasma transfer increased mast cell infiltration in young lungs. Morphometric analysis (Figure 4B₅) revealed a significant reduction in mast cell density in O+Ypls compared with OCnt ($p < 0.05$), and a significant increase in Y+Opls relative to YCnt ($p < 0.05$). Taken together, these findings demonstrate that young plasma ameliorates age-associated pulmonary fibrosis and mast cell-mediated inflammatory remodeling, whereas old plasma promotes both fibrotic deposition and immune cell accumulation, even in structurally intact young lungs.

Immunohistochemical validation of antioxidant enzyme expression

Immunohistochemical analyses for GPX, CAT, and SOD revealed distinct staining patterns consistent with the transcriptional and enzymatic findings. Aged rats that received young plasma (O+Ypls) exhibited markedly stronger immunoreactivity for all three enzymes compared with age-matched controls (OCnt), whereas young rats transfused with old plasma (Y+Opls) showed a notable reduction in staining intensity relative to young controls (YCnt). Quantitative image analysis confirmed these differences ($p < 0.0001$ for all comparisons). These data corroborate that heterochronic plasma exchange modulates antioxidant enzyme expression in the lung at both the transcriptional and protein levels (Figure 5).

Determining alterations in the antioxidant system associated with LUAD

The lung adenocarcinoma (LUAD) dataset was examined utilizing the GEPIA and UALCAN databases to ascertain the mRNA and protein expression profiles of key antioxidant genes

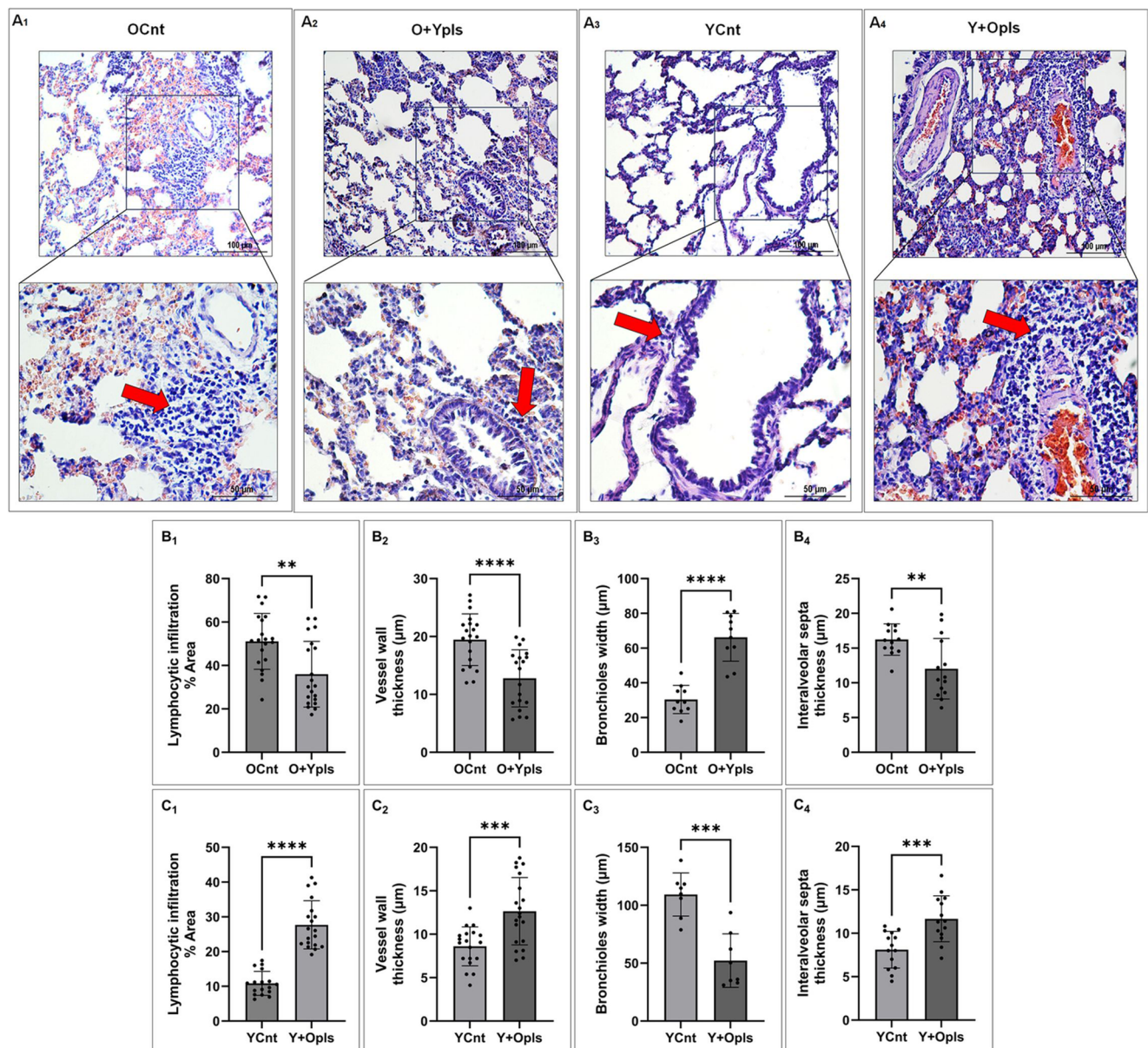


Figure 3. Histopathological and morphometric evaluation of lung tissue following heterochronic plasma administration. (A₁–A₄) Representative H&E-stained lung sections from old control (OCnt), old rats receiving young plasma (O+Ypls), young control (YCnt), and young rats receiving old plasma (Y+Opls) groups. The old control group exhibited pronounced lymphocytic infiltration and structural distortion (red arrows), which were markedly alleviated by young plasma treatment, as evidenced by reduced inflammatory foci and preservation of alveolar integrity. Conversely, old plasma transfer to young rats induced prominent inflammatory cell accumulation and architectural disruption relative to young controls. (B₁–B₄, C₁–C₄) Quantitative morphometric analyses. In aged rats, young plasma administration significantly decreased lymphocytic infiltration (** $p < 0.01$), vessel wall thickness (**** $p < 0.0001$), bronchiolar width (**** $p < 0.0001$), and interalveolar septal thickness (** $p < 0.01$), thereby restoring near-youthful morphology. In contrast, aged plasma infusion into young rats provoked a significant increase in vessel wall thickness (** $p < 0.01$), bronchiolar width (*** $p < 0.001$), and septal thickness (*** $p < 0.001$), consistent with an accelerated aging-like phenotype. Data are presented as mean $\pm$ SEM; statistical significance determined by unpaired t -test. ns, not significant. H&E stain; scale bars: 100 μ m (upper panels), 50 μ m (lower panels).

in both normal lung tissue and LUAD samples. Analyses conducted via GEPIA indicated an increase in the mRNA levels of certain antioxidant genes (eg, GR, SOD, TXN), whereas others (eg, GST, GPX, CAT) exhibited a decrease. The UALCAN CPTAC protein level analyses revealed an overall reduction in the abundance of antioxidant enzymes in LUAD, suggesting discrepancies between mRNA and protein levels. This observation, where some genes display elevated transcript levels but reduced protein levels, may imply disruptions in post-transcriptional or translational regulation (Figure S2—see

online supplementary material for a color version of this figure).

Protein expression analysis by age group in LUAD

To evaluate age-related variations in antioxidant proteins, the UALCAN CPTAC dataset was categorized into age ranges of 21–40, 41–60, 61–80, and 81–100 years. The analyses demonstrated a general decline in all antioxidant proteins studied (GST, GPX, GR, CAT, SOD, TXN) in LUAD samples compared to normal controls. Notably, the further reductions in

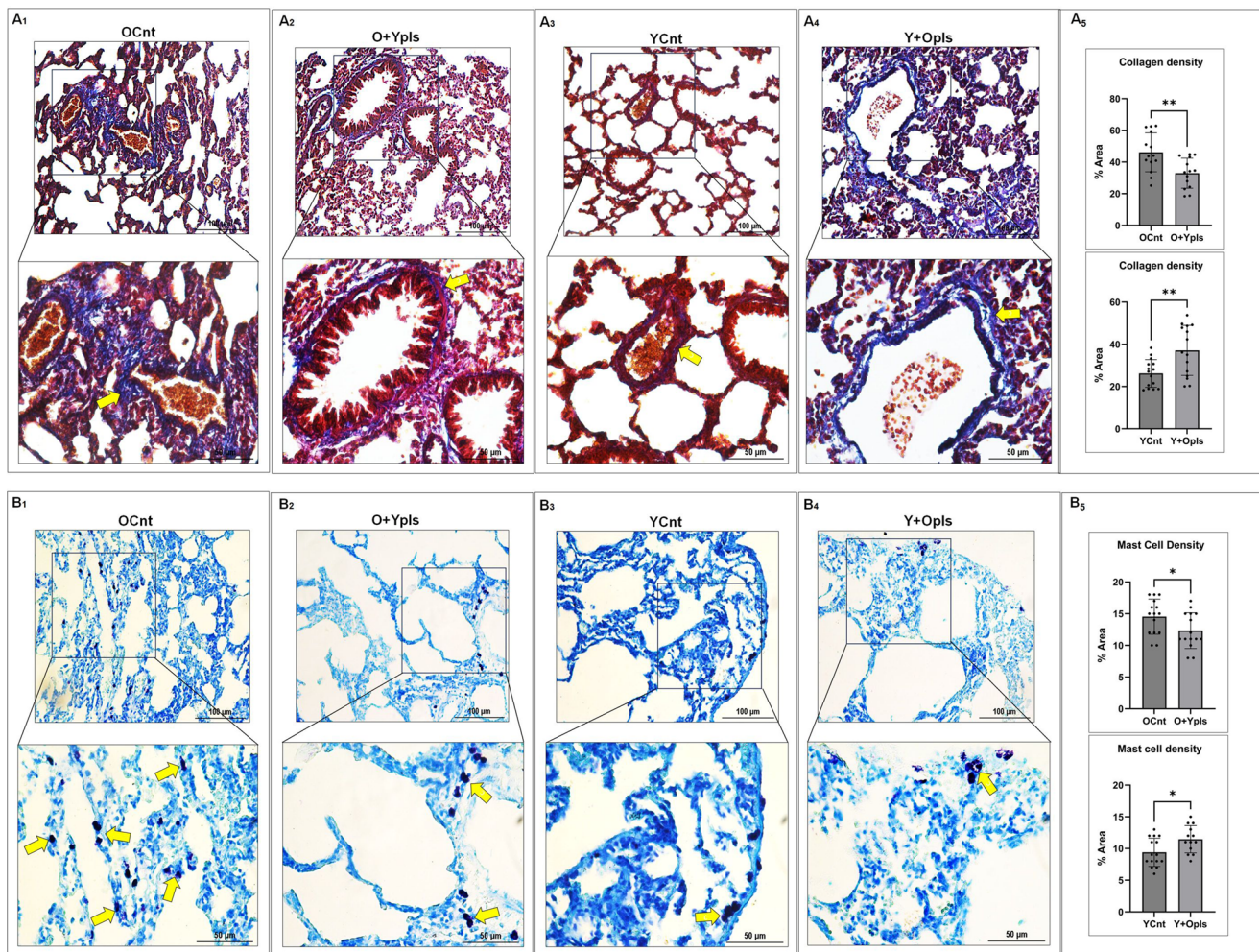


Figure 4. Collagen deposition and mast cell infiltration in lung tissue following heterochronic plasma transfer. (A₁–A₄) Representative Masson's Trichrome–stained lung sections from old control (OCnt), old rats receiving young plasma (O+Ypls), young control (YCnt), and young rats receiving old plasma (Y+Opls). Collagen-rich regions are visualized as blue areas (yellow arrows), whereas parenchymal tissue is stained red/pink. Old controls exhibited dense peribronchiolar and perivascular collagen deposition, indicative of fibrotic remodeling. Young plasma treatment markedly attenuated these fibrotic changes in aged lungs, whereas old plasma infusion into young rats provoked increased collagen accumulation relative to young controls. (A₅) Quantitative morphometry confirmed these observations: collagen density (% area) was significantly reduced in O+Ypls lungs (** $p < 0.01$), while Y+Opls rats displayed significantly elevated collagen deposition compared with YCnt (** $p < 0.01$). (B₁–B₄) Toluidine blue–stained sections highlighting mast cell distribution (yellow arrows). In aged control lungs, mast cells were abundant within alveolar septa and peribronchiolar regions. Young plasma transfer markedly decreased mast cell infiltration in old lungs, whereas aged plasma infusion increased mast cell density in young lungs compared to controls. (B₅) Quantitative analysis corroborated these findings: mast cell density was significantly reduced in O+Ypls relative to OCnt (* $p < 0.05$) and significantly elevated in Y+Opls relative to YCnt (* $p < 0.05$). All data are presented as mean $\pm$ SEM, and statistical significance was determined using unpaired student's *t*-tests. Scale bars: 100 μ m (upper panels), 50 μ m (lower panels).

GST, CAT, and SOD protein levels in older age groups suggest that age may exacerbate antioxidant system insufficiency in LUAD (Figure S3—see online supplementary material for a color version of this figure). The findings derived from the analyses of the GEPIA and UALCAN databases were employed as a reference in interpreting our current experimental results. Consequently, a direct comparison between the structural changes observed in lung tissue in our study and human LUAD datasets was conducted. These parallel trends of dysregulation in antioxidant genes underscore the translational value of the findings from our animal model to human lung pathology.

Discussion

This study contributes to the body of literature demonstrating that aging is not merely a degenerative process occurring at the

organ level, but rather a complex, multidimensional phenomenon that encompasses immunometabolic reprogramming at the systemic level.³¹ Among the organs particularly vulnerable to these processes, the lung represents a critical yet often underappreciated target, where age-associated structural changes including alveolar simplification, septal thickening, and extracellular matrix remodeling have been consistently documented in both human and murine models.^{32–34} The present study provides compelling evidence that heterochronic plasma transfer between young and aged Sprague-Dawley rats directly modulates these pathological hallmarks, thereby positioning the lung as a novel and important readout of systemic rejuvenation.

Our findings demonstrated that young plasma administration to aged rats significantly upregulated antioxidant defenses, with SOD and CAT expression rising by ~ 2.5 - and 1.8-fold, respectively, accompanied by elevated SOD and GPX activities.

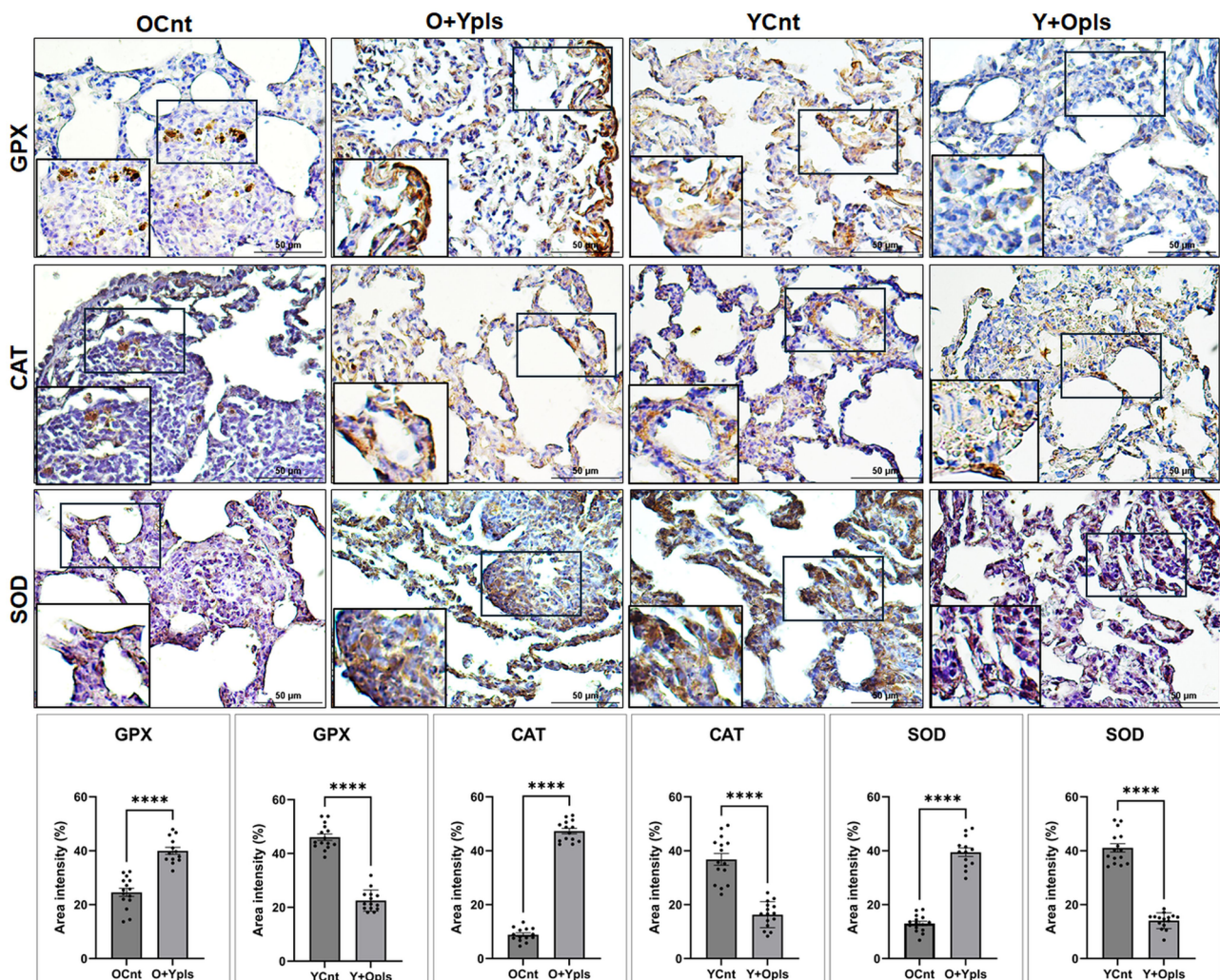


Figure 5. Immunohistochemical validation of antioxidant enzyme expression in lung tissue following heterochronic plasma exchange. Representative immunohistochemical staining for glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD) in lung sections from old control (OCnt), old rats receiving young plasma (O+Ypls), young control (YCnt), and young rats receiving old plasma (Y+Opls) groups. Aged rats transfused with young plasma (O+Ypls) displayed markedly increased immunoreactivity for all three antioxidant enzymes compared with age-matched controls (OCnt), whereas young rats receiving old plasma (Y+Opls) exhibited a distinct reduction in staining intensity relative to young controls (YCnt). Insets show higher magnification of representative alveolar regions. Quantitative analysis of staining area (% intensity) revealed significant group differences (**** $p < 0.0001$ for all comparisons). Scale bars: 50 μ m. These findings confirm that heterochronic plasma exchange modulates antioxidant enzyme expression in the lung at both transcriptional and protein levels.

This reinforcement of redox homeostasis suggests that systemic factors in young plasma can restore antioxidant capacity in senescent lung tissue. Comparable effects have been reported in liver and intestine, where young plasma bolstered antioxidant responses and reduced oxidative burden.^{12,35} Our prior studies, which demonstrate that young plasma similarly enhances antioxidant responses in the liver¹⁵ and small intestine,¹³ corroborate the consistency of these findings across various organs. Conversely, aged plasma suppressed antioxidant defenses in young rats, reducing CAT activity by ~35% and impairing other enzymes a pattern consistent with elevated reactive oxygen species (ROS) and systemic oxidative imbalance driven by pro-aging circulating factors. For instance, increased β 2-microglobulin levels have been identified as pro-aging plasma constituents detrimentally affecting tissue regeneration and cognitive function.³⁶ These bidirectional outcomes emphasize that young plasma conveys resilience, while old plasma transmits molecular signatures of senescence.

Histological analyses have directly confirmed these molecular effects. In the old+young plasma group, lung sections demonstrated preserved alveolar integrity, reduced septal thickening, and diminished inflammatory infiltration. In contrast, the young+old plasma transfer resulted in alveolar disruption and parenchymal inflammation. These findings align with heterochronic parabiosis studies that have shown structural restoration in muscle, liver, and brain.^{5,8} Notably, our previous research has demonstrated similar benefits in other organs: reduced hepatic fibrosis with restored hepatocyte morphology,¹⁵ preserved intestinal mucosal architecture with attenuated collagen deposition,¹³ egression of collagen accumulation in the jejunal mucosa and increase in proliferative capacity³⁷ and improved seminiferous-tubule organization with revitalized spermatogenesis.¹⁴ Collectively, these data suggest that young plasma broadly preserves tissue integrity, with the lung being a particularly vulnerable yet responsive target. Emerging evidence suggests that the mechanisms observed in our

heterochronic plasma model may have direct relevance to chronic lung diseases of aging, such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). Both disorders are characterized by persistent oxidative stress, impaired antioxidant enzyme activity, and maladaptive remodeling of the extracellular matrix, which collectively mirror the senescence-associated alterations seen in aged lung tissue. The restoration of SOD, CAT, GPX, and TXN2 activities observed in the present study implies that youthful systemic factors can counteract the oxidative-inflammatory cascade implicated in COPD and IPF pathogenesis. Prior studies have shown that redox imbalance amplifies TGF- β -driven fibrotic signaling and mitochondrial dysfunction in IPF, and that antioxidant repletion can mitigate these effects.^{16,38} Likewise, the reduction in mast cell density and collagen deposition following young plasma transfer parallels therapeutic goals in anti-fibrotic strategies, suggesting that circulating rejuvenating factors may act upstream of fibroblast activation and matrix remodeling. Taken together, these findings position heterochronic plasma exchange as a potential model for understanding systemic regulation of lung aging and as a conceptual framework for developing plasma-derived interventions in chronic pulmonary diseases.

In addition to the global reduction of fibrotic remodeling demonstrated by Masson's trichrome staining, microscopic examination revealed that heterochronic plasma exchange modulates specific alveolar epithelial subpopulations, particularly type I (AT1) and type II (AT2) cells. Aged lungs typically exhibited thickened alveolar septa, discontinuous AT1 barriers, and hyperplastic AT2 cells hallmarks of senescence-associated epithelial stress and maladaptive repair activation.^{39,40} In contrast, aged rats receiving young plasma displayed reestablished AT1 continuity and normalized AT2 morphology, indicating restoration of epithelial homeostasis and improved alveolar integrity. These findings suggest that rejuvenating plasma factors not only suppress extracellular matrix deposition but also act at the epithelial stromal interface to rebalance redox and regenerative signaling.^{41,42} Given that AT2 cells serve as progenitors for AT1 cells and play a central role in surfactant production and alveolar repair, their normalization provides additional evidence for systemic rejuvenation of the pulmonary microenvironment.³⁹ Collectively, these observations imply that heterochronic plasma exchange mitigates age-related fibrotic remodeling through suppression of type I/III collagen accumulation and modulation of the AT1-AT2 axis, thereby linking molecular antioxidant responses with epithelial regeneration.⁴³⁻⁴⁵

Beyond antioxidant and structural benefits, Toluidine Blue staining revealed a reduction in mast-cell density in aged lungs following young-plasma therapy, indicating an anti-inflammatory mechanism. Given the pivotal role of mast cells in cytokine release, extracellular matrix remodeling, and tissue aging particularly in non-allergic pulmonary pathologies such as IPF and COPD^{38,46} their suppression suggests that youthful circulating factors recalibrate immune stromal cross-talk in the lung microenvironment. This is consistent with our multi-organ findings, where young plasma suppressed hepatic NLRP3 inflammasome activation,¹⁵ attenuated intestinal fibrosis,¹³ improved spermatogenesis,¹⁴ and normalized gut microbiota. Collectively, these results support a model in which youthful plasma factors attenuate sterile inflammation and

maladaptive remodeling across organs, consistent with evidence that systemic modulation of the NLRP3 inflammasome can recalibrate age-associated inflammatory tone.^{47,48} This aligns immunomodulation with the histological and redox improvements observed in the lung.

At the mechanistic level, several candidate mediators may underpin these benefits. Growth differentiation factor 11 (GDF11), TIMP2, exosomal miRNAs, and NAD⁺ precursors have been proposed as rejuvenating molecules,^{35,49} while pro-aging mediators such as β 2-microglobulin and CCL11 accelerate senescence.^{12,36} Our data suggest that similar mediators influence the pulmonary microenvironment, shifting the balance between inflammaging and regeneration. Although the specific plasma factors responsible for these changes remain to be identified, several circulating molecules present in young plasma such as insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and growth differentiation factor 11 (GDF11) have been shown to contribute to systemic rejuvenation and antioxidant defense in other tissues.^{8,49} It is therefore likely that a coordinated action of these growth factors, together with plasma antioxidant proteins, underlies the improved pulmonary redox balance and structural preservation observed in the present study. The immunohistochemical validation of GPX, CAT, and SOD expression further supports the consistency between transcriptional activation and protein-level regulation, indicating that young plasma exerts a coordinated upregulation of antioxidant defenses across molecular and cellular dimensions.

Finally, the translational relevance is highlighted by parallels with human datasets. GEPIA and UALCAN/CPTAC analyses in lung adenocarcinoma (LUAD) cohorts reveal elevated GR, SOD, and TXN transcripts alongside reduced GST, GPX, and CAT transcripts, mirroring trends observed in our plasma-transfer model. The overall decline in antioxidant proteins in LUAD, and age-related reductions in GST, CAT, and SOD, converge with the impaired antioxidant responses in old plasma treated rats. These similarities reinforce the applicability of our preclinical model to human lung pathophysiology and underscore the potential of identifying youthful plasma signatures to inform therapeutic development. When considered together with our previous findings in the liver, intestine, and testis, the present data indicate that the lung exhibits a comparable antioxidant and anti-inflammatory response to heterochronic plasma exchange. However, the pulmonary tissue displays distinct sensitivity to redox imbalance, likely reflecting its high oxygen exposure and unique immune-vascular microenvironment, distinguishing its response profile from that of other organs.

While the present study provides robust evidence that young plasma exerts antioxidant and anti-inflammatory effects in the aged lung, certain limitations should be acknowledged. The treatment duration was relatively short, and thus long-term or cumulative effects remain to be clarified. In addition, although molecular and histological parameters were comprehensively assessed, functional respiratory outcomes such as lung compliance were not included, which may further strengthen translational interpretation in future studies. Moreover, putative mediators such as circulating cytokines, extracellular vesicles, or specific rejuvenating factors (eg, GDF11, TIMP2) were not directly quantified, leaving mechanistic underpinnings open for

deeper exploration. Finally, as this work was performed in a rodent model, careful extrapolation to human physiology is warranted. Despite these considerations, the findings strongly support the lung as a promising and responsive target of systemic rejuvenation strategies and provide a foundation for mechanistic and translational extensions. It should also be acknowledged that while heterochronic plasma exchange represents a promising rejuvenation model, the clinical translation of plasma-based therapies is not without risk. In human applications, complications such as transfusion-related acute lung injury (TRALI), allergic reactions, or immune incompatibility have been reported following plasma transfusion, particularly in sensitized or critically ill patients.^{50,51} Although our study did not involve direct plasma transfusion but rather plasma exchange under controlled experimental conditions, future translational approaches must carefully consider these potential adverse effects. Furthermore, the current design focused on terminal tissue-level analyses, future studies integrating pulmonary function tests such as compliance, elastance, and gas exchange measurements would be valuable to establish direct functional correlations with the observed structural and molecular improvements.

Conclusion

The present study has elucidated that heterochronic plasma exchange between young and aged rats exerts bidirectional and significant effects on oxidative stress, inflammation, and histological integrity within lung tissue. Specifically, young plasma enhanced antioxidant defense mechanisms, mitigated the inflammatory response, and preserved alveolar architecture in aged recipients. Conversely, old plasma disrupted oxidative balance and heightened inflammatory burden in young recipients. Immunohistochemical analyses further validated the molecular findings, showing increased protein expression of GPX, SOD, and CAT in aged rats treated with young plasma, consistent with transcriptional activation of antioxidant enzymes. Additionally, young plasma reduced collagen deposition and restored the structural balance between alveolar epithelial Type I (AT1) and Type II (AT2) cells, indicating improved epithelial homeostasis. These findings align with the “regenerative–anti-inflammatory” responses previously documented in liver, intestinal and testicular tissues under similar experimental conditions. Nevertheless, the study is subject to certain limitations. The exclusive use of a single rat species and sex, the limited selection of molecular markers, and the absence of a dose response analysis in plasma administration may constrain the generalizability of the results. Although parallels with human data enhance translational potential, interspecies biological differences warrant consideration. Future research should incorporate large-scale omics analyses, diverse age–sex combinations, and longitudinal follow-up to elucidate more comprehensively the mechanisms and therapeutic potential of plasma exchange in addressing age-related pulmonary function decline.

Supplementary material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

Data will be made available on request.

Author contributions

Taha Ceylani: Conceptualization, Writing—original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Supervision, Writing—review & editing. Hikmet Taner Teker: Conceptualization, Writing—original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Supervision, Writing—review & editing. Özlem Düvenci Birben: Writing—original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. Burcu Baba: Methodology, Investigation, Formal analysis, Data curation, Supervision, Writing—review & editing. Enver Fehim Koçpinar: Methodology, Investigation, Formal analysis, Data curation, Supervision, Writing—review & editing. Vesila Yıldırım: Visualization, Methodology, Investigation, Formal analysis, Data curation. Sevcin Kabayer: Visualization, Methodology, Investigation, Formal analysis, Data curation. Seda Keskin: Writing—original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Writing—review & editing. Neslihan Külahlıoğlu: Visualization, Methodology, Investigation, Formal analysis, Data curation, Writing—review & editing.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Paperpal for academic language refinement and stylistic improvement. After using this tool, the author(s) thoroughly reviewed, revised, and edited the content, and take full responsibility for the integrity and accuracy of the final manuscript.

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