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How Does Plasma Therapy Approach Affect VDR Expression in the Elderly?

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ABSTRACT

Objective: Young plasma is rich in rejuvenating factors that are diminished in aged mice, making it a promising treatment for organ regeneration and hormonal secretion support. Enhancing hormonal renewal suggests that this therapy may influence the expression of vitamin D receptor (VDR), which declines with aging. In this study, plasma therapy was applied to aged rats to evaluate its effects on histomorphological parameters and VDR levels in the jejunum.

Materials and Methods: Aged female Wistar rats (12–15 months, n=7) were treated with pooled plasma collected from younger rats (6 months, n=28). Post-treatment, villus height, total mucosal thickness, crypt depth, and surface absorption areas were measured in the jejunum of rats in the experimental group (n=7), control group (n=7), and a positive control young group (YPC) (5 weeks, n=7). VDR expression was assessed using immunohistochemistry, and the number of VDR-positive cells was quantified within 0.01 mm^2 areas for all groups.

Results: Histomorphological evaluation revealed no statistically significant differences between the experimental group and the YPC group in villus height, total mucosal thickness, and surface absorption area parameters. However, the experimental group exhibited the highest crypt depth compared to the YPC group. Both the YPC and experimental groups demonstrated increases in all parameters, including histological and VDR evaluations, compared to the control group.

Conclusion: The plasma treatment supported parameters that facilitate digestion and increased VDR expression, which decreases with aging, thereby exerting a beneficial influence.

Keywords: Histomorphology, immunohistochemistry, jejunum, plasma therapy, vitamin D receptor.

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INTRODUCTION

A vitamin is an organic molecule considered an essential micronutrient required by an organism.[1] Vitamin D is part of the fat-soluble vitamin group, which includes vitamins A, E, and K. However, it is also regarded as a hormone because it is synthesized within the body, affects target tissues, and its levels in the circulatory system are regulated by feedback mechanisms.[2] Vitamin D (VD) plays an active role in regulating levels of phosphorus (P) and calcium (Ca).[3] It is also associated with various diseases, including autoimmunity, allergy, cancer, Type 1 diabetes mellitus, depression, cardiovascular diseases, and pregnancy complications. One of the most important functions of VD is maintaining serum CA and phosphorus levels within a healthy physiological range to support metabolic and bone health. Vitamin D receptor (VDR) in the digestive system facilitates increased intestinal absorption of phosphorus and CA.[4] The 1-alpha hydroxylase enzyme gene and the VDR gene, which is directly related to it, are expressed in numerous cells and tissues, including the prostate, kidney, skin, colon, lung, breast tissue, macrophages, and monocytes. It has been reported that active VD generally functions as an intacrine or paracrine factor in tissues.[5,6] Vitamin D deficiency is more commonly observed in older individuals.[7,8] In a study on VD, Kweder and Eidi (2018) reported that the issue cannot be resolved through dietary changes alone. Consequently, various pharmacological supplements have been introduced to address VD deficiency.[9]

CA plays an active role in many biological processes. The primary function of VD is closely linked to the intestine, particularly in maintaining CA homeostasis, which is essential for skeletal health. VD increases calcium absorption in the intestine. Therefore, its role is critical in this regard. Additionally, numerous expressions of $1,25(OH)_2D_3$ (VDR) have been identified in the intestine, independent of CA absorption. Multiple functions of VDR in the intestine have been highlighted, including its active role in regulating barrier function, supporting intestinal stem cells, maintaining homeostasis, regulating drug-metabolizing enzymes, and mediating anti-inflammatory effects.[10]

Recent studies suggest that VD may influence the aging process. It enhances neurotrophin gene expression by stimulating VDR and supports anti-inflammatory signaling pathways and survival in the brain. Evidence also indicates that VD has a protective effect against premature aging and safeguards cell metabolism from toxic agents. Research on the nervous system has shown that combined lipoic acid and calcitriol treatment can protect the brain from damage caused by iron accumulation, mitigating aging and neurodegeneration.[11] Additionally, VDR has been shown to reduce ischemia-induced oxidative stress and brain damage through reciprocal activation of SMAD3 and VDR transcription factors.[12] Another study suggested that VD's regulatory effects on aging stem from its pleiotropic mechanisms, and that VD deficiency may contribute to the development of some age-related diseases, including Parkinson's and Alzheimer's diseases.[13] Some preliminary studies suggest that VD, D3 may play a role in the biological regulation of aging from a cellular perspective.[14] On the other hand, studies have also highlighted that approximately 3% of the mouse and human genomes are directly or indirectly regulated by VD. This suggests that VD, and consequently the VDR, plays an active role in various disease mechanisms.[15–17]

KEY MESSAGES

- The interest in the newly discovered plasma therapy is increasing day by day. Plasma therapy applied to old tissues from a young subject made the jejunum in the digestive system more functional.
- The evaluation results of most of the digestive system parameters we used in the evaluation show this.
- We also presented evidence that plasma therapy supports VDR synthesis in the intestines, which is the main target organ of vitamin D receptor synthesis that decreases due to aging.

Blood plasma treatments have garnered attention for their potential therapeutic benefits, which are just beginning to be explored. These treatments primarily aim to mitigate age-related damage. It has been suggested that the diminished regenerative capacity in tissues due to aging can be reversed through younger plasma blood therapy. Conboy et al.[18] (2005) demonstrated that young serum modulates progenitor cell activity. Plasma therapy, which has recently been applied to neurogenesis, cardiovascular diseases, and metabolic syndrome, is now being investigated for its potential across various organ systems.[10,19,21] In addition to studies on organ systems, it has been shown that young plasma injection improves antioxidant enzyme activity and reduces protein carboxylation in aged tissues.[22] This indicates that this method has potential anti-aging benefits. The digestive system, particularly the intestines, plays a critical role in overall health by facilitating nutrient and water absorption, energy production, and waste excretion.[23] In a recent study, young plasma treatment was applied to aged rats to enhance bacterial diversity and support the intestinal microbiota, yielding successful results.[24] Enhanced crypt depth and villus height in the intestines are indicators of angiogenesis, improved tissue oxygenation, mucosal growth, and increased nutrient absorption.[25]

Aging leads to significant decline in vitamin D levels and VD expression. In recent years, the effects of plasma treatment on aged tissues have garnered considerable attention. Young plasma is rich in rejuvenating factors that decline in aged mice, making this treatment particularly intriguing for its potential to support organ regeneration and hormone secretion. Supporting hormonal renewal suggests that young plasma therapy may also positively affect VDR expression, which decreases with age. This study aims to investigate the effects of plasma treatment on the jejunum of aged rats. Specifically, VDR levels in the jejunum, which decline due to aging, will be compared between a control group and an experimental group of aged rats treated with plasma obtained from younger rats. Additionally, histomorphometric analyses will be conducted to evaluate parameters related to digestion, including villus height, crypt depth, total mucosal thickness, and surface absorption areas.

MATERIALS AND METHODS

Animal Studies: Female Wistar rats were used in this study. Aged rats (n=7, 12–15 months old), randomized from different groups, were treated with pooled plasma (0.3 mL daily, intravenously (iv) for 30 days from September 1 to September 30) collected from randomized younger rats (n=28, 6 months old). Following plasma administration, rats in the experimental group (aged rats treated with young plasma) (n=7), the control group (aged rats) (n=7), and the young positive control group (YPC) (5 weeks old, n=7) were sacrificed under ether anesthesia.[24,26] Jejuna from the sacrificed animals were collected for analysis. All animals included in the experiment were housed in an environment with free access to adequate food and water, maintained under standard animal care conditions. The study was conducted at the Saki Yenilli Experimental Animal Production and Practice Laboratory and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (approval number: 2022/05/06/21).

Plasma Collection

Prior to plasma extraction from the experimental cohort, vaginal smears were conducted to confirm that the 6-month-old rats, from which plasma was to be obtained, were in the diestrus, proestrus, estrus, and metestrus phases. Subsequently, seven subjects from each phase were euthanized, and their blood plasma was collected.[27] Plasma aliquots were stored at -80 °C until use.

Histological Analyses

Jejunal from the sacrificed animals were collected, placed in 10% buffered neutral formalin solution in numbered cassettes, and fixed. Standard histological procedures were performed.[28] Tissue sections obtained from paraffin blocks were stained using Crossmonn triple stain, and morphological changes in the samples were examined under a light microscope (Nikon 80i microscope).[29] Morphometric parameters, including villus height (µm), total mucosal thickness (µm), surface absorption area (mm^2), and crypt depth (µm), were measured using Carl Zeiss-GmbH ZEN 3.5 software. The measurement of villus height was taken from the uppermost tip of the villus to the villus-crypt junction. Crypt depth was measured from the villus-crypt junction to the lower border of the crypt, reaching the submucosa. Measurements were performed for 15 randomly selected villi and 15 crypt glands at their base from serial sections. The total mucosal thickness was also calculated for each segment. Surface absorption area was determined using the formula: Villus absorptive surface area = 2π (6.28) × (half of the average villus width) × villus height.[30] Some sections were reserved for immunohistochemical analysis to determine VDR expression.

Immunohistochemical Analysis

Tissue sections were deparaffinized and processed through an alcohol series and sodium citrate buffer. To inhibit endogenous peroxidase activity, a 3% hydrogen peroxide solution was applied, and nonspecific protein binding was prevented using secondary blocking serum. Subsequently, the primary antibody (anti-VDR; Rabbit Vitamin D Receptor Monoclonal Antibody MBS8534510, 1:200 dilution) was applied to the sections and maintained at +4 °C overnight. After overnight incubation, sections were treated with the secondary antibody (MP7401; ImmPRESS reagent, Vector Laboratories, Inc.) and the chromogen 3,3'-diaminobenzidine (DAB; Zymed Laboratories, USA) was applied. Preparations were counterstained with hematoxylin and covered with entellan.

Evaluation was performed using a scoring system by two independent observers:

- 0: No immune reaction;
- 1: Weak immune reaction;
- 2: Moderate immune reaction;
- 3: Strong immune reaction.

Scoring was based on staining intensity.[31]

To calculate the mean values of VDR-positive cells, these cells were counted in five randomly selected areas from five sections per group, totaling 25 fields. The mean number of VDR-positive cells per view (over areas of 0.01 mm^2) was then calculated for each group.

Table 1. Morphometric analysis of villus height, crypt depth, total mucosal thickness, and villus surface absorption area in the jejunum of control and experimental groups[1–3]

Groups	N	Villus height	Crypt depth	Total mucosal thickness	Surface area
Control	15	965.2 (711.2–1021.3)[a]	220.2 (190.3–251.5)[a]	1172.6 (1092.3–1212.4)[a]	1.10 (0.95–1.20)[a]
Experimental	15	1344.4 (1271.2–1434.6)[b]	390.4 (353.3–411.5)[b]	1490.2 (1456.4–1530.6)[b]	1.60 (1.45–1.72)[b]
YPC	15	1322.12 (1298.3–1421.8)[b]	314.3 (287.2–34.8)[c]	1440.8 (1435.4–1505.2)[b]	1.50 (1.30–1.65)[b]
*p value		**0.001**	**0.001**	**0.001**	**0.046**

*Kruskal Wallis test; data presented as median (min–max). YPC: Young positive control. Different superscript letters (a, b, c) in the same column indicate statistical significance.

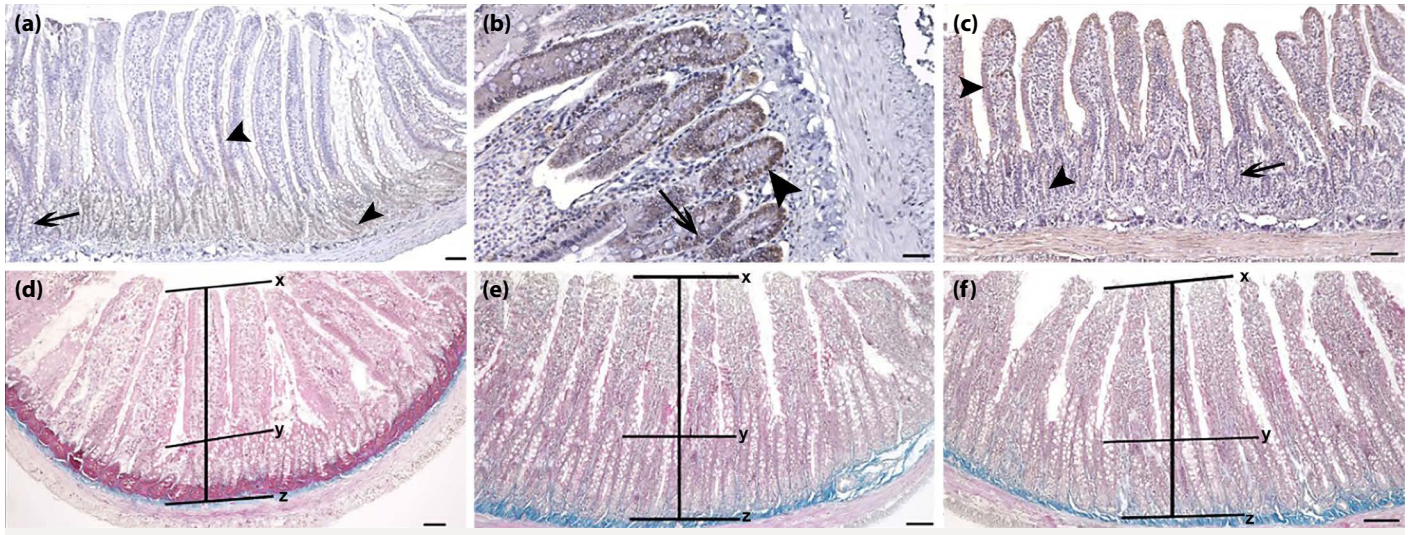

Figure 1. Jejunum sections from experimental and control groups. **(a–c)** Vitamin D receptor (VDR) expression; **(d–f)** Histomorphological measurements. **(a, d)** Control group; **(b, e)** Young plasma control (YPC) group; **(c, f)** Experimental group. Measurements include villus height (x-y), crypt depth (y-z), and total mucosal thickness (x-z). Arrowhead: VDR positive cells; arrow: VDR-negative cells (scale bar: 50 µm).

Statistical Analysis

At the start of the study, the minimum sample size was calculated as 21 animals in total, providing 80% test power at a 95% confidence interval with an effect size of f=0.78, based on the Analysis of Variance (ANOVA) test. Since parametric test assumptions were not met, descriptive statistics for numerical variables were presented as medians (minimum–maximum). Since the assumptions for parametric tests were not met to assess differences between groups in terms of numerical variables, the Kruskal-Wallis test was used. The Dunn-Bonferroni test was performed as a post hoc analysis to identify which groups contributed to the observed differences. A p value of <0.05 was considered statistically significant. The analyses were conducted using IBM SPSS v29 software.

RESULTS

Histological Analyses

Histomorphometric parameters were evaluated for the control, experimental, and YPC groups. The lowest villus height was observed in the control group of aged rats, consistent with expectations based on age-related degeneration. Villus height in the experimental and YPC groups was significantly higher compared to the control group (p<0.001) (Table 1, Fig. 1a–c). Mitotic activity associated with cell renewal, observed in the crypt glands and essential for supporting and protecting digestion, is known to be more active in younger organs. In our study, we investigated the effect of plasma treatments derived from younger rats and administered to aged mice on the crypt glands, where this mitotic activity

Table 2. Vitamin D receptor expression intensity and cell count in the jejunum of control and experimental groups[4–6]

Groups	N	VDR expression intensity	VDR cell count
Control	25	1.10 (0.90–1.30)[a]	45.20 (39.10–54.12)[a]
Experimental	25	2.40 (2.20–2.50)[b]	124.60 (110.20–129.2)[b]
YPC	25	2.24 (2.16–2.33)[b]	130.30 (112.10–145.20)[b]
*p value		**0.001**	**0.001**

*Kruskal Wallis test; data presented as median (min–max). VDR: Vitamin D receptor; YPC: Young positive control. Different superscript letters (a, b, c) in the same column indicate statistical significance.

occurs. Crypt depth, which is associated with cell proliferation and mitotic activity, was measured. The lowest crypt depth was observed in the control group ($p<0.001$, $p<0.05$) (Table 1, Fig. 1–c). A highly significant difference was observed between the control and experimental groups ($p<0.001$). Additionally, a statistical difference was found between the YPC and control groups ($p<0.001$) as well as between the YPC and the experimental group ($p<0.05$) (Table 1). The highest crypt depth was observed in the experimental group. Total mucosal thickness was measured from the lamina epithelialis to the submucosa. No statistically significant difference was found between the experimental and YPC groups, whereas the control group exhibited a lower total mucosal thickness compared to both groups ($p<0.001$) (Table 1). An increase in surface area was observed in both the experimental and YPC groups. Statistically, both groups demonstrated a larger surface absorption area compared to the control group ($p<0.05$) (Fig. 1a–c, Table 1).

Immunohistochemical Analysis

Considering that vitamin D and the expression of the vitamin D receptor, to which vitamin D binds, decrease with aging, we examined changes in VDR expressions following young plasma treatment applied to aged rats. This treatment is expected to have a reversal effect. VDR expression was observed in both the jejunal epithelium and crypt cells (Fig. 1d–f). The lowest VDR expression intensity was found in the control group (Table 2, Fig. 1d–f). While the control group exhibited a low immunoreaction, the experimental and YPC groups demonstrated moderate VDR expression ($p<0.001$) (Fig. 1d–f, Table 2). The number of VDR-positive cells was found to increase in the experimental group following plasma treatment (Table 2). However, no statistically significant difference was observed between the experimental group and the YPC group (Fig. 1d–f, Table 2).

DISCUSSION

The prevalence of VD deficiency is not well-documented. Studies on VD levels in elderly populations have shown that only 15% of elderly individuals have normal serum 25(OH) D levels.[32] A study on VD deficiency in Europe has reported that over 50% of adults aged 18–74 have serum 25(OH) D levels below 50 nmol/L, whereas the normal range is 75–175 nmol/L.[33] These findings indicate that VD deficiency is a widespread health concern, particularly among the elderly. This condition is associated with reduced physical activity and limited sun exposure in older adults.[34] Additionally, it has been reported that natural VD synthesis, particularly in the skin, decreases significantly in the elderly.[35] In our study, we aimed to evaluate VDR expression in the jejunum tissue of aged rats. When the plasma treatment data were compared with the YPC values used as a positive control, the results demonstrated that the treatment could reverse the age-related decline in VD and VDR expression in aged tissue.

It is well-known that the function of VDR in the digestive system is to regulate CA absorption. Interestingly, earlier studies reported that VDR expression in the intestine was limited to the surface or tip of the villus, with no detectable expression in the crypt glands. However, recent studies have shown that VDR is expressed in both the villi and crypts in mice.[36] In the present study, we observed VDR in both the villi and crypt glands. Crypt cells, which exhibit the highest mitotic activity, are frequently studied as key determinants in cell regeneration.[29] In our study, plasma application from younger rats to aged rats was observed to increase crypt depth. This indicates that our treatment supports cell mitotic activity. Studies also report that VD plays an important regulatory role in the function of intestinal stem cells, which may contribute to maintaining intestinal homeostasis.[37,38] In our study, the intense detection of VDR in crypt cells suggests that plasma treatment also supports intestinal homeostasis. It has been reported that VD or VDR deficiency in mice with intestinal inflammation exacerbates enterocolitis symptoms, leading to increased disease severity and mortality rates due to heightened immune activity mediated by T cells, which are associated with intestinal immunity. The findings of this study suggest that VD may help prevent inflammatory diseases.[39] In our study, the increase in VDR expression observed in the experimental group suggests that the applied treatment method may also support gastrointestinal (GIT) health in terms of immunity. However, we have not yet examined immune-related markers such as CD3, CD4, CD8, or Immunoglobulin A (IgA). Therefore, further investigation is needed to assess changes in the expression of various immune-related markers following our treatment methodology.

In a study examining intestinal bacterial diversity following young plasma treatment, it was suggested that this treatment could improve bacterial diversity in the intestinal microbiota of aged rats.[17] This provides evidence that plasma treatments applied to aged tissues contribute positively to the aging process.

Aging impairs intestinal epithelial regeneration and barrier integrity.[40] Our study demonstrates that young plasma treatment applied to aged mice enhances gastrointestinal parameters, including increased villus height and crypt depth.

These findings provide strong evidence supporting the therapeutic potential of young plasma in counteracting age-related degeneration in the gastrointestinal system, promoting improved digestive function and nutrient utilization in aged organisms.

CONCLUSION

Histological analyses revealed improvements in parameters that positively impact digestion. We observed that increased villus height, crypt depth, total mucosal thickness, and surface absorption area after the applied treatment support the digestive system, enhance nutrient utilization, and facilitate digestion. Furthermore, the fact that in our study there was no difference in both histological and immunohistochemical parameters between the experimental group treated with young plasma and the YPC group indicates that the applied treatment may be effective in tissue regeneration. This research provides innovative insights into treatment strategies for age-related diseases in human clinical sciences by presenting a novel regenerative therapeutic approach to alleviate age-related declines in gastrointestinal VD and VDR expression and to support GIT health. We believe that the treatment method applied in this study will contribute to the literature on anti-aging treatments.

Ethics Committee Approval: The Saki Yenilli Experimental Animal Production and Practice Laboratory Ethics Committee granted approval for this study (date: 22.12.2022, number: 21).

Author Contributions: Concept – HTT, TC, EDA; Design – EDA; Supervision – EDA, HTT, TC; Resource – HTT, TC, EDA; Materials – HTT, TC, EDA; Data Collection and/or Processing – EDA; Analysis and/or Interpretation – EDA; Literature Search – EDA; Writing – EDA; Critical Reviews – TC.

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