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LEUKOCYTE FUNCTION IN VITRO AFTER ADDING VITAMINS A, E, AND β-CAROTENE

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Summary

Blood neutrophils and pulmonary alveolar macrophages isolated from calves at 3 and 6 wk of age were cultured in medium without added vitamins or supplemented with vitamin A, vitamin E, vitamin A and vitamin E, or ß-carotene and vitamin E. Macrophage bactericidal activity improved with A-E supplementation compared to ß-carotene-E supplementation at wk 3. Neutrophil bactericidal activity decreased with all vitamin E treatments at wk 3 and with vitamins E and A-E at wk 6. Neutrophil phagocytosis improved at wk 3 with A, E, and A-E supplementations. The chemotactic index improved at wk 3 with ß-carotene-E compared to vitamin E alone and at wk 6 with vitamin E compared to vitamin A and control treatments. The retinol content of neutrophils at wk 3 was variable, but by wk 6, cells supplemented with A, E, or A-E had higher retinol concentrations than control cells. Neutrophil α-tocopherol concentrations at 3 wk increased over controls with vitamin E or ß-carotene-E supplementation, but at wk 6, vitamin E-supplemented cells were different only from vitamin A-supplemented cells. These data suggest that there are optimum plasma concentrations of vitamins A and E for leukocyte functions.

(Key Words: Retinol, α-Tocopherol, ß-Carotene, Neutrophils, Macrophages, Calves.)

Introduction

Neonatal calf loss from enteric and respiratory illness is a major problem in the dairy industry. The young animal's first immune defenses are antibodies absorbed from colostrum and phagocytic cell activity. Vitamins A and E have been implicated in enhancement of phagocytic functions of leukocytes. In previous research, increased supplemental vitamin E enhanced the chemotactic index, but had no effect on antibody-dependent cellular cytotoxicity. Increased supplementation of vitamins A and E together improved bactericidal responses more than with either vitamin alone. The vitamin E concentrations used in that research were below recommendations for improved immune functions, thereby limiting possible benefits. Mastitis research with dairy cows has suggested a role in the immune response for ß-carotene, independent of vitamin A.

The objectives of this study were to determine the effects of supplemental vitamins A, E, or ß-carotene on blood neutrophil and pulmonary alveolar macrophage functions in vitro and to determine concentrations of vitamins A and E in neutrophils supplemented in vitro with vitamins A, E, or ß-carotene.

Procedures

Twelve Holstein bull calves were fed a milk replacer containing 5 IU/lb vitamin E and 3636 IU/lb vitamin A. At 3 and 6 wk of age, blood samples and pulmonary alveolar macrophages were collected. Neutrophils and

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macrophages were separated and resuspended in medium with no additional vitamin supplementation (0) or containing the following vitamin supplementation: 100 ug/dl retinyl palmitate (A), 1000 ug/dl þ-tocopherol acetate (E), 100 ug/dl retinyl palmitate and 1000 ug/dl þ-tocopherol (AE), or .25 IU/dl ß-carotene and 1000 ug/dl α-tocopherol (BC). Neutrophils were used in a migration assay to measure movement toward a chemoattractant, in a phagocytosis assay to measure the cell's ability to ingest *S. aureus*, and in a bactericidal assay to measure the cell's ability to kill *S. aureus*. The macrophages were also used in the bactericidal assay. Neutrophils were analyzed for α-tocopherol, retinol, and retinyl palmitate.

Results and Discussion

Neutrophil chemotactic index (directed:random migration) at wk 3 was less with vitamin E added to the medium than with ß-carotene and vitamin E added, but neither treatment was different from the control (Table 1). However, by wk 6, the chemotactic index of neutrophils supplemented with vitamin E was higher than that of control cells or those supplemented with vitamin A, but was not different from that of cells treated with AE and CE. Thus, the chemotactic function of neutrophils from 3-wk-old calves responded differently to vitamin supplementation than did that of neutrophils from the same calves at 6 wk.

Neutrophils of 3-wk-old calves had a greater phagocytic capacity than the control cells when supplemented with vitamins A, E, or A and E together (Table 1). However, supplementation with ß-carotene and vitamin E had no beneficial effect. No differences were seen in the phagocytic functions at wk 6, suggesting that the animals had sufficient vitamin stores in those cells for this function prior to use in this experiment.

Neutrophil bactericidal activity responded negatively to vitamin supplementation in the medium at both 3 and 6 wk (Table 1), with the exception of the wk 3 and 6 A treatment and wk 6 CE treatment. At wk 3, activities of all vitamin E-supplemented treatments were significantly decreased compared to the control. ß-carotene and vitamin E supplemented together decreased bactericidal activity compared to the vitamin A supplementation and the control. After observing similar trends in other species, other researchers have suggested that increased phagocytosis and decreased bactericidal activity may have been due to the antioxidant reducing the free radicals and increasing membrane stability. The more stable membrane improved the cell's phagocytic capacity, but the antioxidant effect that was preserving the cell's membrane probably reduced the superoxide in the cell and, therefore, the cell's capacity to destroy bacteria after ingestion. However, at wk 6, only the vitamin E and AE treatments reduced bactericidal activity compared to controls. Neutrophils responded similarly to vitamin E supplementation in the medium at 3 and 6 wk, with the exception of the CE treatment.

Macrophage bactericidal activity (Table 1) was affected by vitamin supplementation at wk 3, but not at wk 6. At 3 wk, differences occurred between AE treatment and the E or CE treatments. None of those treatments differed from the A treatment and the control. Therefore, neutrophils and macrophages responded differently to vitamins supplemented in the medium at both 3 and 6 wk. For example, at wk 3, vitamins A and E together tended to improve bactericidal activity of macrophages, but decreased bactericidal activity of neutrophils. At wk 6, vitamin E tended to increase neutrophil bactericidal activity, but all supplemental vitamins had no effect on macrophage bactericidal activity.

The vitamin A and E contents of neutrophils are shown in Figure 1. Retinol content of cells at wk 3 was variable between calves. One calf had large values of retinyl palmitate, but others had little or no retinyl palmitate and smaller increases of retinol acetate (data not shown). The vitamin A-supplemented cells were not different from the control cells.

In contrast, the α-tocopherol content of the cells reflected supplementation of that vitamin alone or in conjunction with ß-carotene. When neutrophils were supplemented with retinyl palmitate or retinyl palmitate and α-tocopherol, their α-tocopherol content was not different from that of the controls. Retinyl palmitate tended to inhibit the incorporation of α-tocopherol into the cells, but ß-carotene increased cellular α-tocopherol at wk 3.

At wk 6, α-tocopherol content differed only between the vitamin A-supplemented cells and the vitamin E-supplemented cells. In contrast, the retinol content of neutrophils at wk 6 was increased compared to the control by supplementation with vitamins A, E, or A and E, but not with ß-carotene.

In conclusion, these data suggest that there are optimal plasma concentrations of vitamins A and E for leukocyte function. More research is warranted to determine the exact range that is most beneficial for these leukocytes.

Table 1. Function of Neutrophils and Macrophages from Calves at 3 and 6 Weeks of Age after Incubation in Medium with Vitamin Supplementation

	Vitamin supplementation[1]					
Function	0	A	E	AE	CE	SE
			Week 3			
Neutrophil						
Chemotaxis (directed:random migration)	2.6^{ab}	3.5^{ab}	1.5^{b}	2.9^{ab}	3.9^{a}	1.0
Phagocytosis (% ingestion)	29.7^{b}	50.3^{a}	46.8^{a}	47.3^{a}	35.3^{b}	4.6
Bactericidal kill (%)	43.4^{a}	38.2^{ab}	32.6^{bc}	32.9^{bc}	29.2^{c}	3.5
Macrophage						
Bactericidal kill (%)	36.9^{ab}	39.5^{ab}	34.5^{b}	47.5^{a}	30.3^{b}	5.1
			Week 6			
Neutrophil						
Chemotaxis (directed:random migration)	2.6^{b}	3.0^{b*}	4.6^{a}	3.0^{ab*}	3.6^{ab}	.7
Phagocytosis (% ingestion)	36.6	32.9	39.1	32.9	34.7	6.6
Bactericidal kill (%)	57.7^{a}	53.8^{ab}	46.9^{bc}	40.6^{c}	51.3^{ab}	4.1
Macrophage						
Bactericidal kill (%)	39.3	44.8	37.5	33.3	36.8	5.3

[1]0 = no vitamin supplementation, A = 100 ug/dl vitamin A, E = 1000 ug/dl vitamin E, AE = 100 ug/dl vitamin A and 1000 ug/dl vitamin E, and CE = .25 ug/dl ß-carotene adn 1000 ug/dl vitamin E.

[abc]Means within the same row with different letters differ (P<.10).

[*]Equal values with differing superscripts are a result of decimal rounding.

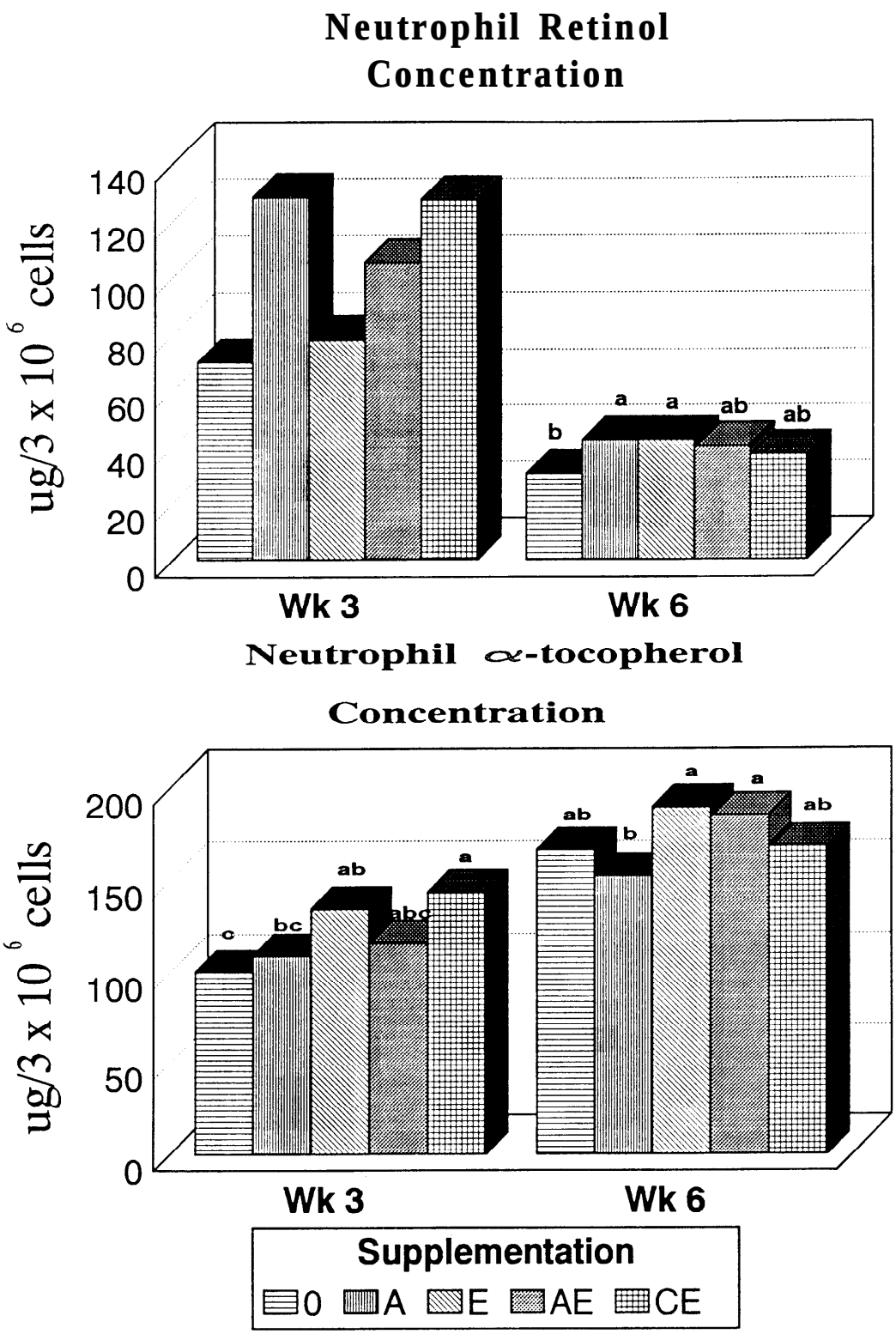

Figure 1. Retinol and α-tocopherol contents of blood neutrophils following incubation in medium without supplemented vitamins (0) or supplemented with 100 ug/dl vitamin A (A), 1000 ug/dl vitamin E (E), 100 ug/dl vitamin A and 1000 ug/dl vitamin E (AE), or .25 ug/dl ß-carotene and 1000 ug/dl vitamin E (CE). Means with different letters differ ($P < .10$).