EFFECTS OF EXCESSIVE DOSING OF RETINOL ON SERUM LIPID COMPOSITION IN RABBITS

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SUMMARY

It has been tried to make use of retinol for improvement of quality of the depot fat of the beef cattle in the area of animal science. Therefore, research is needed to see how excessive dosing of retinol influence on lipid metabolism.

The author investigated changes with the passage of time of serum lipid components and correlation of phospholipid, cholesterol, lipid peroxide in serum with fatty acid composition when rabbits are administrated excessive doses of retinol.

MATERIALS and METHODS

The laboratory animal used were male rabbits of New Zealand White Kind. The agents administered were retinyl palmitate and α-tocopherol acetate. The agents were administered by intramuscular injection at 4 day intervals. The blood samples were collected once every 4 days (at 10:00 a.m.).

The items measured were total lipid, triglyceride, phospholipid, total-cholesterol, HDL-cholesterol and lipid peroxide. Furthermore, for a group showing hypervitaminosis, glucose, total-protein, hemoglobin, hematocrit, enzyme activity in serum and fatty acid composition were analyzed.
RESULTS

As for mice and rats, there is a report by Misra et al. on hypervitaminosis caused by the administration of 15,000 IU/kg B.W. of retinol. However, for the rabbits, my results did not show anemia, weight loss and depilation etc., by the administration of the dose of retinol which is toxic to mice and rats. Also, any remarkable change in serum lipid composition was not observed at this dose. Therefore, in this experiment, the rabbits were divided into two groups, one administered with 150,000 IU/kg B.W. retinol alone which caused hypervitaminosis A and the other given a combined treatment with 150,000 IU/kg B.W. retinol and 100 mg/kg $\alpha$-tocopherol (as antioxidizing agent for retinol). The rabbits showed anemia, weight loss and depilation etc., by the administration of 150,000 IU/kg B.W. of retinol. The weight of rabbits decreased just after the administration. At the end of was 70 % as compared with the weight before the administration. The severity of hypervitaminosis by retinol was reduced when the rabbits were given combined treatment with $\alpha$-tocopherol.

The concentration of retinol in the serum remarkably increased just after the administration, becoming 85,000±5,000 IU/dl after the 4th dosage in spite of continuing an administration. The group of the concurrent administration of $\alpha$-tocopherol showed a higher concentration than the group of retinol alone. It was suggested that $\alpha$-tocopherol inhibited oxidation of retinol. The lipids level in the serum was shown to increase by administration of excessive doses of retinol.

Triglyceride in serum drastically increased until the 5th dosage in the group given retinol alone. But, after the 6th dosage, the level gradually decreased. The group of the concurrent
administration with α-tocopherol showed greater increase than the group given retinol alone.

Phospholipid, total-cholesterol and free-cholesterol, abruptly increased by the administration of retinol alone. The higher levels of the serum concentration of the lipids continued for a long time. The increase of phospholipid, total-cholesterol and free-cholesterol were suppressed by α-tocopherol.

For both groups, HDL-cholesterol was shown to abruptly increase until the 4th dosage of retinol. But the group given retinol alone showed a decrease after the 5th dosage. The group of the concurrent administration with α-tocopherol continued to increase for a long time.

Lipid peroxide significantly increased just after the administration of retinol alone, and remained at a high concentration until the experiment was finished. On the other hand, lipid peroxide was controlled by α-tocopherol. The author analyzed with gas-chromatography the fatty acid composition of serum lipids, in order to clarify the influence of administration of excessive dosing of retinol.

Fatty acids (less than 14:0, 18:0) were shown a significant disease by retinol. In particular, fatty acid (less than 14:0) abruptly decreased by the administration of α-tocopherol.

Fatty acids (18:2,18:3,20:4) were shown to significant increase for the both groups of excessive dosing of retinol and the concurrent administration with α-tocopherol. In particular, fatty acid (20:4) abruptly increased by α-tocopherol.

Therefore, as for the influence on fatty acids of excessive
dosing of retinol, unsaturated fatty acids were more sensitive than saturated fatty acids.

CONCLUSION

1) The rabbits had power of resistance against excessive doses of retinol because even though the rabbits were administered 15,000 IU/kg B.W. of retinol, they did not show hypervitaminosis A. Also, the lipid composition in serum did not change. The reason is probably as concluded below. Under the physiological conditions, retinol specifically binds to RBP (retinol binding protein) and is supplied to proper tissues. Therefore, when retinol is supplied excessively the production of RBP is enhanced and thus inactivate retinol specifically. If retinol binding-capacity of RBP is low, retinolester occurs in serum by a slight increase of retinol. This is a cause of hypervitaminosis A. Therefore, it is guessed that the difference of power of resistance to excessive dosing of retinol between mice, rats and rabbits depends on the difference of retinol binding-capacity of RBP.

2) With the administration of excessive doses of retinol, lipid concentration in the serum (total lipid, phospholipid, cholesterol, HDL-cholesterol, lipid peroxide) were significantly increased. It was guessed that retinylester administered beyond the binding-capacity of RBP bound lipoprotein (LDL fraction), and supplied non-specifically to the tissues other than proper tissues and used their machinery for lipid metabolism. Retinylester of non-binding RBP has a strong surface activity. In the case of direct action on the membrane, it causes cell lesion by hypervitaminosis A. The cause of increase of lipid peroxide is oxidation shortening of carbon chain of unsaturated fatty acid
and it is because of decrease of \( \alpha \)-tocopherol in serum by excessive dosing of retinol. The \( \alpha \)-tocopherol inhibits the incorporation of long-chain unsaturated fatty acids into the membrane, which causes lesion of the membrane by the surface active action of retinolester.

3) The levels of total lipid, phospholipid, cholesterol and lipid peroxide were decreased by the concurrent administration of retinol and \( \alpha \)-tocopherol. This is because the level of serum concentration of \( \alpha \)-tocopherol was maintained by the concurrent administration of \( \alpha \)-tocopherol. As a natural consequence, long-chain unsaturated fatty acids in serum were not oxidized.

4) Retinol concentration in serum was \( 85,000 \pm 5,000 \) IU/dl by administration of excessive doses of retinol, because the retinol-ester concentration in serum was increased by decrease of \( \alpha \)-tocopherol. It was suggested that lipid peroxide was increased and oxidation of retinol was promoted.

5) Unsaturated fatty acids (18:2, 18:3, 20:4) in serum were increased, short chain fatty acids (less than 14:0) were decreased, and polyunsaturated fatty acids (especially 20:4 fatty acid was drastic) were shown to increase by excessive dosing of retinol. It was suggested that long chain unsaturated fatty acids in serum were increased because of inhibition of their incorporation into the membrane by excessive dosing of retinol. The reason of low level of short-chain fatty acid can be explained by existence of a mechanism controlling shortening of the carbon chain of unsaturated fatty acid.

Therefore, the excessive dosing of retinol alone caused increase
of lipid peroxide, and eventually it brought about cell lesion. That is the cause of hypervitaminosis A. However, these lesion can be controlled by the concurrent administration of α-tocopherol and retinol. It is obvious that polyunsaturated fatty acids (especially arachidonic acid) in serum significantly increase by administration of α-tocopherol.

As my results indicate, by using retinol, it is possible that fatty acid composition of intramuscular fat is changed without hypervitaminosis A. If this knowledge is applied for improvement of meat, it will be possible to produce meat rich in long-chain unsaturated fatty acids on purpose.