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Effect of 6-Aminonicotinamide on the Levels of Some Metabolites and Related Enzymes in Rabbit Serum

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The effects of an antimetabolite, 6-aminonicotinamide (6-AN) on the levels of enzymes and metabolites in rabbit serum were investigated. The intraperitoneal administration of 6-AN (multiple doses of 15mg/kg body weight) gave rise to a remarkable increase in glucose and cholesterol levels but did not exert any appreciable influence on the concentration of albumin and total protein. Alkaline phosphatase activity was significantly reduced by administration of 6-AN, whereas creatine phophokinase, serum glutamic oxaloacetate transaminase and serum glutamic pyruvate transaminase activities were markedly enhanced. Nevertheless, the levels of Ca, P, Na, K, Cl and Co were not affected to any extent by 6-AN.

KEY WORDS: 6-Aminonicotinamide, Enzymes, Metabolites.

It has been well documented that certain structural analogues of nicotinic acid or nicotinamide interfere with the synthesis of or action of nicotinamide coenzymes (Hothersall *et al.*, 1981; Balaban, 1985). In general, these synthetic coenzyme analogues have been proved useful in elucidating the chemical and physical nature of enzymes involved in complex formation which reflect specific changes in fluorescence, spectral absorption, and catalytic activity (Woenckhaus, 1974).

As an analogue of nicotinamide, 6-aminonicotinamide (6-AN) is substantially incorporated into NAD and NADP by glycohydrolase to form the antimetabolites 6-amino-NAD and 6-amino-NADP which are incapable of transferring hydrogen in oxido- reduction (Herken and Neuhoff, 1964). In particular, 6-amino-NADP is a competitive inhibitor for NADP-dependent dehydrogenases such as glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and glutathione reductase. The resulting high concentration of 6-phosphogluconate caused by strong inhibition of 6-phosphogluconate dehydrogenase led to an impairment of the glycolysis on the level of glucose 6-phosphate isomerase. The reduced metabolism of glucose via the glycolysis and pentose phosphate shunt pathways could contribute to the elevated levels of glucose, consequently resulting in the hyperglycemia (Kolbe *et al.*, 1977).

In addition, 6-AN was shown to cause a dramatic reduction in enzyme activities of mitochondrial systems such as β-hydroxybutyrate and α-ketoglutarate dehydrogenases and glyceraldehyde 3-phosphate dehydrogenase (Dietrich *et al.*, 1958) and NAD reductase and succinate dehydrogenase (Iglesias-Rozas *et al.*, 1973). This low enzyme activity is believed to be coupled with marked

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lowering of ATP concentration. Furthermore, 6-AN, as a central nervous system depressant, lowered the activity of gamma-aminobutyric acid transaminase intimately related to gamma-aminobutyric acid shunt (Prakash and Baquer, 1981) and of monoamine oxidase mainly responsible for catecholamine metabolism (Mayanil *et al.*, 1984). On the other hand, some enzyme namely either lactate dehydrogenase remains virtually unchanged or acetylcholinesterase activity increaseed significantly (Zeitz *et al.*, 1978).

Although no convincing evidence is available for the present, all these biochemical alterations by 6-AN may be closely associated with some neurological disturbances in both vertebrate and invertebrate nervous systems (Knoll-Kohler *et al.*, 1980). The present investigation attempts to examine effects of 6-AN on the levels of some metabolites and enzymes as well as inorganic minerals in the rabbit serum.

Materials and Methods

Materials

All chemicals used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Treatment of Animals

White adult rabbits weighing approximately 2.5 kg were randomly divided into two groups, 20 test and 20 control. All animals were allowed free access to food and water during the course of the experiment. Rabbits in the test group received intraperitoneally 1 ml saline solution containing 6-AN (15 mg/kg of body weight) every other day, whereas those in the control group received 1 ml saline solution (0.9% NaCl) only.

After the animals were sacrificed by decapitation the blood was drawn into clean centrifuge tubes. The collected blood was left in ice for 1hr and the serum was then obtained by centrifuging the blood at 800 $\times$ g for 10 min. The clear serum sample was kept at $-20^{\circ}C$ until further analysis was required.

Determination of Enzymes and Metabolites

The concentration of glucose was determined by Falis (1963), cholesterol by Abell *et al.* (1959), albumin by Kohn (1958) and protein by Lowry *et al.* (1951).

Alkaline phosphatase activity was assayed by Salomen *et al.* (1964), creatine phosphokinase by Rosalki (1967), lactate dehydrogenase by Fritz (1967), glutamic pyruvic transaminase and glutamic oxaloacetic transaminase by Amador and Wacker (1962).

The levels of mineral elements were determined as follows: Ca by Trudeau anf Freier (1967), P by Goldenberg and Fernandez (1966), Na and K by Valle and Thiers (1965), Cl and Co by Cotlove (1961).

Statistical Analysis

The Student t-test was employed for the determination of statistical significance (Smith, 1962). Differences between means which give probability value (p) smaller than 0.05 are considered to be significant.

Results and Discussion

Normal white rabbits administered the antimetabolite 6-aminonicotinamide showed a gradual reduction in body weight starting from day one experiment. On the 7th day the average body weight of the test group corresponded to approximately 60% of the control group whose body weight remained virtually unchanged despite a slight tendency of the increase in body weight during the latter portion of the experiment (Figure 1). A similar observation was also made with mice (Park *et al.*, 1990).

The administration of 6-AN did not exert any influence on organ weight such as brain, heart and kidney except liver when expressed in the ratio of organ weight divided by body weight (Table 1).

The effect of 6-AN on levels of some metabolites are presented in Table 2. 6-AN was shown to cause a marked increase in glucose level as compared with the control. This kind of hyperglycemic effect was very well demonstrated in previous stu-

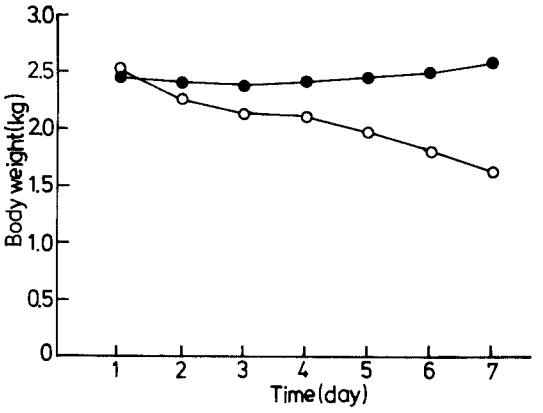

Fig. 1. Effects of intraperitoneal administration of 6-aminonicotinamide on body weight change in rabbit. Saline or 6-AN solution was injected intraperitoneally. Control (● – ●), 6-AN (○ – ○).

Table 1. Effect of 6-aminonicotinamide on the organ weight (g) in rabbit

Organ	Treatment	
	Control	6-Aminonicotinamide
Brain	7.50(3.95)	6.32(3.61)
Heart	5.60(2.96)	4.43(2.48)
Kidney	13.43(7.10)	12.54(7.17)
Liver	69.57(36.62)	49.08(28.05)

Values are given as mean ±S.D. of 4 experiments. Values in parenthesis are derived from $\frac{\text{Organ weight(g)}}{\text{Body weight(g)}} \times 1000$ for better understanding.

Table 2. Effect of 6-aminonicotinamide on the levels of metabolites (mg/ml) in rabbit serum

Metabolites	Treatment	
	Control	6-Aminonicotinamide
Glucose	1.14 ± 0.12	2.02 ± 0.16***
Cholesterol	0.40 ± 0.05	0.93 ± 0.09***
Albumin	25 ± 5	22 ± 4
Total protein	69 ± 11	54 ± 6*
Creatine	0.01 ± 0	0.01 ± 0

Values are given as mean ±S.D. of 4 experiments. *$p < 0.05$ ***$p < 0.001$. The student's t-test was employed to estimate the significance of differences between two means of control versus test.

dies by Baba *et al.* (1978) and Park *et al.*, (1990). It has been strongly suggested that the hyperglycemic action of 6-AN was essentially triggered by either the lowering of insulin (Tanese *et al.*, 1983) or the enhancement of epinephrine release from the adrenal medulla which may be responsible for the breakdown of glycogen. Another possibility is that 6-AN may produce an inhibitory action on 6-phosphogluconate dehydrogenase in the pentose phosphate shunt pathway eventually leading to the massive accumulation of 6-phosphogluconate. 6-phosphogluconate at high concentration inhibits phosphoglucose isomerase in the glycolysis which results in a subsequent reduction of glycolytic flux and ATP content (Griffiths *et al.*, 1981). In addition, 6-AN was also similarly effective in inducing the hyperglycemia in other tissue such as brain (Chung and Park, unpublished data).

It is of interest that cholesterol levels in 6-AN-treated animals increased significantly as compared to control animals. Similar results were also obtained with mice administered 6-AN (Park *et al.*, 1990). Although the precise mechanism underlying the effects of 6-AN on changes of cholesterol level is still unclear it can be speculated that either 3-hydroxy-3-methylglutaryl-CoA reductase, one of the key enzymes in cholesterol synthesis, may be activated (Hothersall *et al.*, 1981) or the cholesterol catabolism may be retarded.

The concentration of total protein following 6-AN is slightly lower than that in the control whereas the concentration of albumin is not affected. In the mouse C-1300 neuroblastoma cell the 6-AN was demonstrated to lower the protein content (Zeitz *et al.*, 1978) and to promote the protein degradation in the rat cerebral cortex (Benzi *et al.*, 1984). The mode of how 6-AN is involved in the reduction of the protein level still needs to be clarified but it may be related to the adaptive metabolic response to the shortage of energy reserves as a result of the rapid decline of glycolytic flux and ATP concentration.

As shown in Table 3, activities of most enzymes are remarkably increased by 6-AN administration with the exception of alkaline phosphatase. In particular, creatine phosphokinase, a unique enzyme involved in ATP synthesis in the muscle tissue,

Table 3. Effect of 6-aminonicotinamide on the levels of enzymes (nmol/min/mg protein) in rabbit

Enzymes	Treatment	
	Control	6-Aminonicotinamide
Alkaline phosphatase	1.13 ± 0.15	1.16 ± 0.20
GOT	0.57 ± 0.06	1.07 ± 0.13**
GPT	0.52 ± 0.06	0.95 + 0.10**
Creatine phosphokinase	8.50 ± 0.70	37.20 ± 8.00***
Lactic dehydrogenase	3.50 ± 0.50	8.40 ± 1.80**

Values are given as mean ±S.D. of 4 experiments. GOT: glutamic oxaloacetate transaminase, GPT: glutamic pyruvate transaminase **$p<0.01$, ***$p<0.001$.

Table 4. Effect of 6-aminonicotinamide on the levels of mineral components (mg/100ml) in rabbit serum

Mineral	Treatment	
	Control	6-Aminonicotinamide
Calcium(mg/100ml)	15.0 ± 1.3	14.2 ± 1.1
Phosphorus(mg/100ml)	6.5 ± 0.8	7.4 ± 0.9
Sodium(mmol/l)	146 ± 13	153 ± 16
Chloride(mmol/l)	105 ± 10	116 ± 11
Potassium(mmol/l)	6.5 ± 0.5	7.0 ± 0.6
Cobalt(mmol/l)	15.5 ± 1.4	17.0 ± 1.7

Values are given as mean ± S.D. of 4 experiment

was the most severely affected. Elevated creatine phosphokinase activity may be indicative of the occurrence of damage of the cardiac or skeletal muscle (McClintic, 1978). Though the creatine phosphokinase activity was not measured in muscle tissues, there is strong possibility that the elevation of this enzyme may be partially involved in the synthesis of ATP in muscle tissues in response to the dramatic fall in ATP content (Griffiths *et al.*, 1981).

Similarly, glutamic oxaloacetate transaminase and glutamic pyruvate transaminase activities after 6-AN treatment are significantly increased relative to the control. The enzyme, normally absent from serum, is liberated into the serum. An elevation of these enzymes is presumably associated with the extensive tissue destruction produced by the antimetabolic action of 6-AN (McClintic, 1978). Some of the resulting by-products are probably mobilized to channel into the initiation of gluconeogenesis ultimately generating the energy source such as ATP. The administration of 6-AN to the normal rabbit causes a significant lowering in lactate dehydrogenase activity but does not affect alkaline phosphatase activity (Table 3). These results are quite similar to those of Iglesias-Rozas et al (1973). They reported that lactate dehydrogenase activity in both grey and white matter of spinal cord and glia is markedly reduced whereas no change in alkaline phosphatase activity was observed upon administration of 6-AN. We believe that the reduction in the activity of some enzymes discussed above may not be a specific effect of 6-AN but it is common consequences of various pathological processes resulting from metabolic failures.

The mineral elements such as Ca, P, Na, K, Cl, and Co were not influenced to any extent by 6-AN administration (Table 4). The results of mineral elements obtained with the current study are in good agreement with the reports of Moreland (1974) and of Harkness (1977).

Overall it can be concluded that 6-AN exerts a pronounced influence on levels of some important metabolites such as glucose and cholesterol as well as some enzymes related to energy generation in rabbit serum.

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6-Aminonicotinamide가 토끼혈청내 효소 및 대사물질에 미치는 영향

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Antimetabolite인 6-aminonicotinamide(6-AN)가 토끼 혈청내 효소 및 대사 물질에 미치는 영향에 관하여 연구하였다. 복강투여시 (15mg / kg의 체중) 포도당과 콜레스테롤농도는 현저이 증가하였으나 알부민과 총단백질량은 크게 변화하지 않았다. Creatine phosphokinase, glutamic oxaloacetate transaminase, glutamic pyruvate transaminase 및 lactate dehydrogenase활성은 매우 증가하였으며 alkaline phosphatase와 Ca, P, Na, K, Cl 및 Co 등의 수준은 영향을 받지 않았다.