Pharmacological Contributions to Vitamin B Preparations commonly used in the Oral Area

Part II. Comparative Effects of Pyridoxine and Pyridoxal-5-Phosphate on Toxopyrimidine, together with Antidotic Action of Pyridoxal on the Latter

by

Toyoyuki TAMURA and Yoshiko WAKAMATSU**

The present paper is concerned with a follow-up on a similar study previously published [1].

Experiments

1. Comparative Effects of Pyridoxine and Pyridoxal-5-Phosphate on Toxopyrimidine.

In the decomposition process of vitamin B₁ in a vital body, there will be produced out of pyrimidine phase 2-methyl-4-amino-5-hydroxymethyl-pyrimidine through the hydrolysis. Since its toxicity was pointed out by ALBERHALDEN, there have been published many research results including those of MAKINO and ARAMAKI, the latter throwing light on the relationship of this toxic substance and vitamin B₆. In view of its high toxicity, MAKINO designated this substance "*Toxopyrimidine*" (usually abbreviated as TXP) and similarly vitamin B₆ which acts antidotically on toxopyrimidine came to be named "*Atoxopyrimidine*" by the same investigator.

In a series of tests in which rats which were fed on a B₁-deficient feed were given an injection of toxopyrimidine (0.12-0.2 mg/g) and thiazol in an effort to inquire after possible synthesis of vitamin B₁ in a vital body, it was revealed that there took place a convulsion in the rats after a few hours of an injection. The cause of convulsion is ascribed to toxopyrimidine, which gives rise to convulsion when it is administered not only as a shot but also orally if a dosage is large enough.

On the other hand, vitamin B₆ which is an antidotic agent of this toxopyrimidine or atoxopyrimidine is found in three forms; pyridoxamine (PAM), pyridoxine (PIN) and pyridoxal (PAL). Hydrochloric salt of pyridoxine is in the ordinary use. This is the drug also used in our present study.

On the other hand, recent advances in biochemistry have confirmed the fact that B₆, B₁ and B₂ are alike produced ultimately from the phosphorus ester. The present paper is concerned with an effect to find out whether pyridoxal would have the same

* 田村 豊幸, 若松 佳子: *Dept. of Pharmacology, Nihon Univ. School of Dentistry (Director: Prof. Mikio Sato).*

TABLE 1
Chemical Structures of Test Drugs

effect as that of B₆, focusing attention on atoxopyrimidine action which is representative of B₆.

1-1. Materials and Method.

A group of mature mice was injected with toxopyrimidine subcutaneously through the dorsal region, while the other group was subjected to a combined dosage of toxopyrimidine with other drugs such as pyridoxine. Count was taken of the number of mice who gave rise to convulsion at a certain level (minimum convulsant amount) and measurement was made at the level where they came to die in an effort to look into effect of a combined usage of these drugs.

1-2. Result.

a. The frequency of intermittent convulsion on the part of mice and their subsequent death are given in Table 2. The dosage of toxopyrimidine was within the range of 100–500 γ /g.

The time to be taken before a convulsion occurs is surprisingly long. For instance, No. 3 subject (0.1 mg/g) took as long as 2 hours 31 minutes before a single convulsion took place and it gradually reverted to the normal condition without further symptom. Of the test mice, the shortest length of time before convulsion was 1 hour 39 minutes (No. 2, 0.5 mg/g) and the longest was 3 hours 51 minutes (No. 8, 0.5 mg/g). In terms of the total number of convulsions, the frequency of convulsion seems to increase within the range of 0.1 mg to 0.5 mg but beyond 0.75 mg the tendency reverses and starts to decrease somewhat. Part of the reason for this fact is that the subjects which suffered from a severe convulsion in the early stage died immediately.

Fig. 1 gives a percentile classification of deaths owing to a convulsion. At a dosage of 0.1 mg/g the death rate was 10 per cent, while at the level of 0.5 mg/g there took place the death rate of 100 per cent.

b. The result of experiment to see how much death rate could be inhibited through a combined dosage of toxopyrimidine and pyridoxine, the latter being used as a premedication anywhere from 0.5 to 5.0 γ /g, is given in Fig. 2. Although no difference was observed at the level of 0.5 γ /g, survival rates at the levels of 1.0 γ /g and 5.0 γ /g were respectively 20 per cent and 100 per cent, thus attesting an efficacy of pyridoxine as an antidotic agent of toxopyrimidine.

Similarly, an effort was made to look into an efficacy of pyridoxal used as premedi-

TABLE 2
Frequency of Intermittent Convulsions

No.	mg/g			
	0.1	0.3	0.5	0.75
1	0 —	0 —	0 +	0 +
2	0 —	0 —	1 +	0 +
3	1 —	0 —	0 +	4 +
4	0 —	4 —	0 +	0 +
5	0 —	0 +	0 +	1 +
6	7 —	0 —	5 +	0 —
7	0 +	0 +	5 +	0 +
8	0 —	0 —	5 +	4 +
9	0 —	6 +	0 +	7 +
10	1 —	3 —	4 +	1 +
Total convulsions	8	13	19	16

(+) Deaths immediately after convulsion.

(—) Survivals.

Fig. 1. Death rate by toxopyrimidine

cation within the range of 0.3–0.7 γ /g. Here, although no effect was seen at a dosage of 0.3 γ /g, survival rates at the levels of 0.4 γ /g and 0.7 γ /g were respectively 20 per cent and 100 per cent (Fig. 3).

1-3. Considerations.

As is seen from the results in Figs. 2 and 3, both pyridoxine and pyridoxal possess a potent antidotic action against the toxicity of toxopyrimidine, the latter far stronger than the former. That is to say, while the minimum lethal dosage of toxopyrimidine

Fig. 2. Anti-toxopyrimidine action of pyrimidine

Fig. 3. Anti-toxopyrimidine action of pyridoxal

is 0.5 mg/g, pyridoxal corresponding to 1/700 of toxopyrimidine, i. e., 0.7 γ and pyridoxine corresponding to 1/100 of the same, i. e., 5.0 γ , can completely restore the animals to life with a rate of 100 per cent. Though the fact that pyridoxal has a higher potency than pyrimidine can be fairly surmised from its being an activator of B_6 in a vital body, this part of our study fully confirms it.

2. An Antidotic Action of Pyridoxal against Toxopyrimidine through the Skin Absorption.

As toxopyrimidine brings about a convulsion by acting upon the central nervous system, sometimes to the extent of death, we thought that we might diminish its toxicity through the skin administration of pyridoxal, the subcutaneous administration of which was tried previously.

2-1. Materials and Method.

The same mature mice were used for experimental purposes and a retentive device was constructed so that the animals were kept in the stable position without holding them too tightly (Fig. 4).

A group of mice was immersed in a solution of pyridoxal in the beakers which was prepared in the same temperature as their bodily temperature, as if in a bath. Another group of control mice was immersed in mere hot water. The beakers to be used must have a sufficient depth (65 cc in the present case) so that the blood circulation in the neck region might not be interfered with.

Fig. 4. A retentive device to hold animals

a.

b.

c.

d.

2-2. *Result.*

After 30 minutes of hot water immersion, control mice were given an injection of toxopyrimidine in the dosage of 0.5 mg/g and subjected to serial observations for 4 hours. At the end of which period, all the ten cases came to die. An experimental group which was immersed in a 3 per cent pyridoxal solution for the same length of time was also subjected to consecutive observations of 4 hours, after the animals were injected with 0.5 mg/g of toxopyrimidine. As a result, death claimed 8 of 10 cases while the degree of convulsion generally was much weaker than that in the controls. The same was true of the frequency of convulsions that took place.

2-3. *Considerations.*

The experiment described above established the fact that pyridoxal which is administered through the skin has an antidotic action against the toxicity of toxopyrimidine. Although it is not clear within the scope of our study how much can be absorbed through the skin, it may be well imagined that with an adequate adjustment of temperature and concentration of a solution involved far more quantity of pyridoxal will be absorbed.

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