**Original Article**

**Effects of Prolonged Intravenous Phosphate Administration On Rabbit’s Serum Calcium**

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**ABSTRACT**

Phosphate administration mediates the genesis of the parathyroid gland hyperplasia thus modulating the parathyroid hormone synthesis. Phosphate retention will consequently lead to hyperparathyroidism which can be detected by markedly high serum calcium levels. The objective of this study is to evaluate the effects of prolonged intravenous phosphate administration on serum calcium and to establish the hyperparathyroidism animal model of rabbits. Ten healthy New Zealand White does (female rabbits) aged 4 months with body mass between 2.0 to 2.5 kg were used. Their bloods were withdrawn 1 week before and 5 months after the intravenous phosphate administration for serum calcium evaluation. Result from the study showed that the rabbit’s serum calcium level after 5 months of the intravenous phosphate administration has increased compared to 1 week before the administration. Prolonged intravenous phosphate administration increased the serum calcium level and can be suggested as one of the ways to establish hyperparathyroidism in the animal model.

**Keywords**: Hyperparathyroidism, phosphate, rabbit

1. **Introduction**

Parathyroid Hormone (PTH) plays an important role in maintaining bone and mineral homeostasis. Hyperparathyroidism (HPT) is defined as unregulated overproduction of PTH, resulting in abnormal calcium homeostasis. There are three main types of HPT namely primary, secondary and tertiary hyperparathyroidism. Primary hyperparathyroidism (PHPT) is the third most common endocrine disorder\(^1\) due to unregulated overproduction of PTH by one or more overactive parathyroid glands that will lead to hypercalcaemia\(^2\). On the other hand, secondary hyperparathyroidism (SHPT) is an excessive secretion of PTH caused by chronic biochemical stimulus, such as chronic kidney disease that normally leads to all four glands becoming hyperplasia\(^2\). Finally tertiary hyperparathyroidism (THPT) is a result of prolonged SHPT in patients with successful kidney transplantation due to renal failure but parathyroid glands remains overactive and autonomous\(^3\). Complications of HPT are primarily related to the long-term effects which include osteoporosis, kidney stones and cardio-vascular diseases.
Human PTH is a single chain 84-amino acid peptide (PTH 1–84) which is exclusively secreted by the chief cells of each parathyroid gland [4] and functions actively as early as 12 weeks of gestation [5]. PTH is responsible in regulating the levels of calcium (Ca\(^{2+}\)), phosphate (HPO\(_4\)\(^{2-}\)) and magnesium (Mg\(^{2+}\)) and increasing the number and activity of osteoclast [6]. In normal calcium concentration, PTH is secreted and synthesized continuously in a pulsatile fashion [7] with a peak plasma level in the morning [8, 9]. Chief cells in parathyroid gland are very sensitive to changes in extracellular ionized calcium concentration and even slight reductions of calcium concentration (<1-2%) will prompt a raise in the PTH secretion [10].

Nowadays HPT is no longer considered a rare disease because there has been a great increase in the rate of HPT in recent years [11]. HPT is considered a benign disease of the elderly but when it occurs during pregnancy it is a life – threatening disease [11, 12]. According to Schnatz and Curry [13], 25% of HPT cases are diagnosed in woman during their child bearing age. The relationship between phosphate administration and hyperparathyroidism was first discussed in 1936. It was reported as the increment of inferior parathyroid gland weight and hyperplasia after the parenteral administration of phosphate [14]. In cases of renal failure, the development of secondary hyperparathyroidism can be caused by alterations in phosphate homeostasis [15]. High phosphorus retention induced parathyroid TGF\(_\alpha\) expression, which functions as an autocrine signal to further stimulate uremia thus inducing the parathyroids’ cell growth and modulating the parathyroid hormone synthesis through indirect mechanisms affecting calcium regulation and calcitriol synthesis [16, 17].

In this study, the phosphorus were intravenously administered to healthy New Zealand White does (female rabbits) for 5 months to establish hyperparathyroidism animal models. Serum calcium levels were measured at both pre- and post- treatment period to compare the effect of prolonged intravenous phosphate administration on serum calcium level.

2. Materials and Methods

The research was approved by the University’s Committee on Animal Research & Ethics. All the experimental procedures were carried out in Laboratory Animal Facility and Management, Faculty of Pharmacy (LAFAM), Universiti Teknologi MARA.

Solution Preparation

The stock buffered solution of sodium phosphate was prepared as suggested by Drake [8]. The stock solution was diluted with distilled water producing a pH 7.3 solution that is isotonic with the blood. The solution was prepared in chemically cleaned glasswares and after autoclaving was kept in a refrigerator to prevent growth of possible contaminants.

Study Design

Ten healthy New Zealand White does aged 4 months with body mass between 2.0 to 2.5 kg were used. They were individually housed and fed with commercial food pellets (Cargill, Malaysia) and deionized water ad libitum. They were acclimatized for a week in order to adjust to the new environment. One week before the initiation of the study, blood was drawn from the peripheral auricular veins of each rabbit, and the serum levels of calcium was measured to determine the baseline biomarker value.

The treatment was carried out according to the method by Drake et al., where each rabbit was injected intravenously with 25 milligram of inorganic phosphorus, three times a day for 5 months [18]. Following the 5 months of treatment, blood samples were collected via peripheral auricular veins once again for serum calcium examination. After 1 hour, the blood was centrifuged at 5000 rpm for 10 minutes and the serum was separated prior to storage at temperature of -80 °C. The serum were sent to Faculty of Veterinary Medicine Universiti Putra Malaysia for serum calcium examination.

Statistical Analysis

The results were expressed as mean ± standard error mean (SEM). Analysis was done using Statistical Package for Social Sciences software (SPSS version 21). Normality of the difference between calcium level in pre-treatment and post-treatment was tested using Kolmogorov-Smirnov test. Paired sample t-test was performed to compare the mean since the data were normally distributed data. The level of significance was taken as p<0.05.

3. Result

The baseline value for serum calcium level for both pre-treatment and post-treatment are presented in table 1. The rabbit’s serum calcium level after 5 months of the intravenous phosphate administration was significantly increased (P<0.05) compared to 1 week before the administration.

4. Discussion

In young New Zealand White does, prolonged administration of intravenous phosphate resulted in significant rise in the serum calcium level. This is very similar to what has been previously reported by Bas and colleague which stated that prolong intake of high phosphorus diet for several months resulted in an increase in both the serum calcium and PTH hormone levels above normal range [19]. Short-term phosphate loading increased parathyroid hormone (PTH) secretion in both humans [20] and in rats [21] while long-term phosphate loading could induce parathyroid enlargement in normal animals [18, 22, 23].
<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-treatment Mean</th>
<th>Post-treatment Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/L)</td>
<td>3.13 ± 0.01</td>
<td>4.61 ± 0.02</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 1: Serum calcium concentrations before and after treatment with intravenous phosphate.
Most of the short-term phosphate administration will demonstrate a mild hypocalcemia condition [24-26]. This was supported by Krook’s theory which explained that the excess dietary phosphate filtering into the blood stream will result in a mild depression of plasma Ca caused by stimulation of PTH secretion [27]. Administration of phosphate either orally [24] or intravenously [25, 26] can cause a decrease in serum calcium level in hypercalcemic and normocalcemic persons.

Many studies have reported that hyperparathyroidism rabbit model can be obtained by feeding high phosphorus diet [19, 23, 28, 29, 30, 31, 32, 33]. Animal model of hyperparathyroidism using adult rat has been established by feeding high phosphate diet for 3 months [23]. It is well known that phosphorus plays a pivotal role in modulating the parathyroid cell proliferation and hyperphosphatemia may have contributed to the development of parathyroid hyperplasia in the rabbit [15-18]. Bas and her colleagues [28] emphasized that adjusting the mineral content from normal diet will also lead to hyperparathyroidism.

5. Conclusion
A rabbit HPT animal model can be successfully established by prolonged administration of phosphate intravenously or orally since both routes will promote the same effects. This animal model allowed us to carry out researches on other hyperparathyroidism-related diseases in the future.

6. Acknowledgment
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