Effect of Bordetella Pertussis Vaccine on Glucagon Secretion in Normal and Alloxan Dogs

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Abstract

The effect of Bordetella pertussis vaccine on plasma glucose, insulin and glucagon secretion was investigated in normal and alloxan dogs. On the 8th day after the vaccine injection, in normal and alloxan dogs during the infusion of arginine and glucose, the plasma glucose level was lower and the IRI level was higher than in the saline controls. On the other hand, the plasma IRG level showed no significant difference between the vaccine and saline control groups of normal dogs, but in alloxan dogs this vaccine made the plasma IRG level lower during arginine infusion than in the saline controls and suppressed it significantly during glucose infusion. These effects of the vaccine disappeared on the 30th day after its injection into normal and alloxan dogs.

It is suggested that in normal dogs Bordetella pertussis vaccine decreased plasma glucose through the promotion of insulin secretion without any effect on glucagon secretion, while in alloxan dogs this vaccine might alleviate hyperglycemia through the enhancement of insulin and the inhibition of glucagon secretion.

In 1963 Szentivanyi et al. reported that adrenalin-induced hyperglycemia was prevented in mice pretreated with Bordetella pertussis vaccine. Afterward, regarding this mechanism, Sumi and Ui (1975) found that Bordetella pertussis vaccine promoted insulin secretion by the enhancement of the action of beta-adrenergic receptor and in consequence led to the improvement of hyperglycemia.

Toyota et al. (1978) reported that islet-activating protein (IAP), which was purified from the supernatant of culture medium of Bordetella pertussis cells (Yajima et al., 1978), blocked the stimulatory effect of noradrenalin on glucagon in both diabetic and normal isolated perfused rats pancreas. However, the effect of this vaccine on glucagon secretion has not been clearly explained.

The present study was designed to find out whether glucagon secretion was influenced by Bordetella pertussis vaccine in normal and alloxan dogs.

Materials and Methods

Twenty healthy mongrel dogs weighing 6.0-9.0 kg were used. Under ketamine hydrochloride (Ketalar®) anesthesia, polyethylene catheters were inserted into the femoral vein for blood sampling and into the axillary vein for drug infusion.

a) Ten normal dogs were divided into the two groups; five dogs were given an injection of 1 ml/kg of Bordetella pertussis vaccine (Takeda Pharmaceutical Co. Osaka) containing $2 \times 10^{10}$ organisms per ml subcutaneously, and the other five dogs were given the same amount of saline subcutaneously as controls.
b) For the alloxan experiment, ten dogs were divided into two groups one of which received Bordetella pertussis vaccine and the other was treated in the manner mentioned above.

On the third day after the injection of the vaccine or saline, five pertussis sensitized and five saline control dogs received a single injection of alloxan (Sigma Co. U.S.A.) in a dose of 50 mg/kg through polyethylene catheters into the axillary vein.

All these dogs were fed on dog food and were fasted for 24 h before the experiment.

On the 8th and 30th days after the injection of Bordetella pertussis vaccine or saline, the dogs were given an infusion of 1-arginine (Morishita Pharmaceutical Co. Japan) in a dose of 120 mg/kg/min for 30 min (arginine tolerance test) at 9:00 a.m. and d-glucose (Otsuka Pharmaceutical Co. Japan) in a dose of 30 mg/kg/min for 30 min (glucose tolerance test) at 2:00 pm. The antibody titer against Bordetella pertussis vaccine was measured every week after the vaccine injection.

Blood was drawn from the femoral vein at appropriate intervals, indicated in the figures, and the samples were immediately transferred into a chilled tube containing 1.2 mg of EDTA and 1000 KIU of aprotinin (Trasylol(R), Bayer Co.) per ml of blood. They were centrifuged immediately and the plasma was stored at -20°C until used.

Glucagon was measured by radioimmunoassay with the talc method (Sakurai et al., 1973) using the C-terminal specific glucagon antiserum 30K (obtained from Dr. R. H. Unger, Dallas, Texas). Insulin was measured by radioimmunoassay using the double-antibody method (Hales and Randle, 1963) and glucose by the glucose-oxidase method. The antibody titer against Bordetella pertussis vaccine was measured as follows; 1 ml of serum was serially diluted with saline and the same amount of culture medium of Bordetella pertussis containing 13 × 10^8 organisms per ml was added. They were incubated at 37°C for 2 hrs and then at 4°C overnight, and the antibody titer was measured by the maximum dilution which clearly showed agglutination particle.

All data were shown as mean±SEM. Student’s t-test was used for the statistical analysis and measured values were compared with the basal ones or between the two groups.

Results

Effect of pertussis vaccine on normal dogs
1) Arginine tolerance test on the 8th day after the injection of pertussis vaccine or saline

In the pertussis group, basal plasma glucose, IRI and IRG levels were 82±7 mg/dl, 8±3 μU/ml, and 70±10 pg/ml respectively which did not differ from the corresponding values of 94±7 mg/dl, 7±4 μU/ml and 80±10 pg/ml in the control group. However, after arginine infusion plasma glucose level was significantly lower in the former than the latter throughout the study (p<0.05).

At 5 min after the administration of arginine, the plasma IRI level rose to 75±6 μU/ml in the pertussis group, a value significantly higher than the 40±4 μU/ml observed in the control group (p<0.01).

The plasma IRG level rose significantly during arginine infusion with a peak value of 240±20 pg/ml in the pertussis group and 290±30 pg/ml in the control group. However, there was no substantial difference between the pertussis and control group plasma IRG levels.

2) Glucose tolerance test on the 8th day after the injection of pertussis vaccine or saline

During glucose infusion, the plasma glucose level in the control group from the baseline value of 90±15 mg/dl, which was not distinct from the 85±15 mg/dl in the pertussis group, to a peak value of 418±25 mg/dl at 30 min, but in the latter it was much lower than that of the former at 20 and 30 min (p<0.05).

Plasma IRI level showed a much higher response in the pertussis group than in the control group at 5, 10, 20 and 30 min (p<0.05).

In the pertussis group, the basal plasma IRG level was 70±10 pg/ml which had little distinction from 83±9 pg/ml in the control group and during glucose infusion, the plasma IRG level rose significantly at 30 min in the both groups. But, no significant difference was observed between the pertussis and control group plasma IRG levels (Fig. 1).

3) Arginine and glucose tolerance tests on the 30th day after the injection of pertussis vaccine or saline
Before and after the injection of arginine and glucose, no notable difference in plasma glucose, IRI and IRG levels could be observed between the pertussis and control groups (Fig. 2).

Effect of pertussis vaccine on alloxan dogs

1) Arginine tolerance test on the 8th day after the injection of pertussis vaccine or saline

In the pertussis group, the basal glucose level was $152 \pm 20 \text{ mg/dl}$ which was different from the $202 \pm 15 \text{ mg/dl}$ in the control group, and, during arginine infusion, the plasma glucose level in the former was much lower than the latter ($p<0.05$).

Although there was no difference between the pertussis and control group basal plasma IRI levels ($6 \pm 2$ vs $4 \pm 2 \mu U/ml$), after the administration of arginine the plasma IRI level in the former showed a
Fig. 3. Comparisons of plasma glucose, IRI and IRG levels during arginine and glucose infusion in alloxan dogs on the 8th day after the injection of pertussis vaccine or saline.

much higher response at every corresponding time, except 1 min, than the control group in which there was little response to arginine infusion ($p<0.05$).

In the pertussis group, the plasma IRG level was elevated significantly from a baseline value of $210\pm20$ pg/ml, which was not different from the $270\pm30$ pg/ml in the control group, to a peak value of $770\pm60$ pg/ml and had a much lower response than that of the latter throughout the observation ($p<0.01$) (Fig. 3).

2) Glucose tolerance test on the 8th day after the injection of pertussis vaccine or saline

In the pertussis group, the basal plasma glucose level was $160\pm15$ mg/dl, which was considerably lower than the $190\pm15$ mg/dl in the control group, and, during glucose infusion, the plasma glucose level showed a lower response than the latter ($p<0.05$).

Although there was no difference between the basal plasma IRI levels in the pertussis and control groups ($7\pm2$ vs $4\pm2$ $\mu$U/ml), after glucose infusion the plasma IRI level in the former rose notable to a peak value of $48\pm8$ $\mu$U/ml, but in the latter there was little response to glucose infusion (Fig. 3).

No significant difference between the basal plasma IRG levels in the pertussis and control groups ($210\pm25$ vs $270\pm32$ pg/ml) could be seen, and although in the control group plasma IRG level showed almost no change during glucose infusion, in the pertussis group it significantly decreased to a nadir of $145\pm25$ pg/ml which was much lower than $255\pm30$ pg/ml in the control group at 30 min ($p<0.05$) (Fig. 3).

3) Arginine and glucose tolerance tests on the 30th day after the injection of pertussis vaccine or saline

After as well as before the infusion of arginine and glucose no substantial difference was observed between the plasma glucose, IRI and IRG levels in the pertussis and control groups (Fig. 4).

Antibody titer against Bordetella pertussis vaccine

They were $2\times$ before the injection, $64\times$ on the 4th week after the injection, and $128\times$ on the 8th week after the injection (Fig. 5).
**Discussion**

Sumi and Ui (1975) found that in pertussis sensitized rats the enhancement of insulin secretion was observed during the administration of not only beta-adrenergic agents but also glucose, arginine, glucagon and sulfonylureas. Our present study confirmed that pertussis sensitization causes the enhancement of insulin secretion during arginine and glucose infusion also.

On the other hand, concerning the effect Bordetella petrussis vaccine on glucagon...
secretion, our present results showed that in alloxan dogs the vaccine injection inhibited glucagon secretion. These findings agreed with the report of Toyota et al. (1978) as mentioned above and also the recent report of Aoki et al. (1979) which showed the inhibitory effect of IAP on glucagon response to arginine. However, as shown in Figs. 1 and 3, this inhibitory effect that was observed in alloxan dogs could not be found in normal dogs. This difference may be attributed to the "mode of action" of this vaccine.

Several factors should be taken into consideration regarding the suppressive effect of the pertussis vaccine on glucagon secretion.

First, it is well known that in diabetic patients, excessive glucagon response to arginine, which is thought to occur in succession to the metabolic disorder based on the diabetic state, can be normalized after the therapy (Ohneda et al., 1975; Unger et al., 1972). Furthermore, Shichiri et al. (1979) reported that the abnormal glucagon response to oral glucose load in diabetics was normalized by the adequate insulin infusion using the artificial beta cell. Therefore, it is suggested that in the pertussis sensitized alloxan dogs in our experiment the improvement of an insulin deficiency might amend the excessive glucagon response to arginine and make the glucose load significantly suppress glucagon response.

Second, this vaccine may directly affect A cells. Although the "mode of action" of Bordetella pertussis vaccine on A cell is still unclear, it has been reported that Ca++ channel of the cell membrane of B cell is activated by this vaccine and as a result the concentration of Ca++ in B cell increases and insulin secretion is enhanced (Katada and Ui, 1979). Iversen (1976) reported that an increase in the Ca++ concentration enhanced the secretion of glucagon as well as insulin in the isolated perfused dog pancreas. Leclerq-Meyer et al. (1973) reported that a reduction of the Ca++ concentration in the medium increased the glucagon secretion using the isolated pancreas. And Ohneda et al. (1974) reported that Ca++ infusion into the pancreatic artery decreased the glucagon concentration in the pancreatic vein. There is, however, a negative report concerning this point (Kuzuya et al., 1974). Therefore, since there is no known correlation between Ca++ and glucagon secretion of A cell at present, the possibility that the increased Ca++ concentration in A cell which was induced by Bordetella pertussis vaccine through the activation of Ca++ channel might inhibit glucagon secretion cannot be denied. However, as an inhibitory effect of this vaccine on glucagon secretion could not be found in normal dogs, this possibility may be slight.

Third, it was demonstrated that despite the hyperglycemia in depancreatized dogs, plasma glucagon (gut GI), which was measured using a C-terminal specific glucagon antibody markedly increased, and this glucagon was inhibited by a small insulin injection (Matsuyama and Foa, 1974; Vranic et al., 1974; Mashiter et al., 1975). Yoshida (1977) reported the possibility of the existence of gut GI in blood in normal dogs, judging from the difference between IRG response to arginine in gastroenterectomized and normal dogs. Watanabe (1977) demonstrated that gut GI was present in normal dogs immediately after pancreatectomy and increased significantly during arginine administration. Furthermore, Ohneda et al. (1977) reported that after pancreatectomy in alloxan dogs IRG (gut GI) increased more significantly on arginine administration than in normal dogs, in which there was no significant increase. Therefore, it is suggested that gut GI is secreted in alloxan dogs with a lack of insulin. In our study, the insulin response was seen more significant during the infusion of arginine and glucose into pertussis
sensitized alloxan dogs than in saline control alloxan dogs. Therefore, this vaccine activated the remaining B cells and the increased insulin might suppress gut GI which increased due to the lack of insulin in alloxan dogs. This may explain why the inhibitory effect on glucagon secretion could not be observed in normal dogs without a lack of insulin.

Incidentally, as shown in Figs. 2 and 4, why the effect of the vaccine on the secretion of insulin and glucagon disappeared on the 30th day after the vaccine injection is not still known. It may be possible that the increase in the antibody titer against Bordetella pertussis vaccine is one of the reasons why the effect of the vaccine was lost. The antibody titer rapidly increased after the second week of the vaccine injection. Therefore, after that stage, this antibody might work gradually on B cells and almost entirely eliminate the effect of the vaccine after the fourth week.

Acknowledgements

We are indebted to the Takeda Pharmaceutical Co. for generously supplying the Bordetella pertussis vaccine.

References


Unger, R. H., L. L. Madison and W. A. Muller (1972). Diabetes 21, 301.


