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SHORT COMMUNICATION

Effect of Diisopropyl Phosphorofluoridate in Some Aspects of Carbohydrate Metabolism

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ABSTRACT

An acute dose of DFP equivalent to 50 per cent of the LD_{50} causes glycogenolysis and hyperglycemia in male albino rats. The hyperglycemic effect can atleast be partially suppressed by the administration of insulin. Under sub-acute dose equivalent to 5 per cent of the LD_{50}, there is glycogenolysis but no change in blood glucose. The action of DFP on carbohydrate metabolism seems to be mediated through adrenal gland. DFP also increases the glycolytic rate, suppresses the LDH activity and is hepatotoxic.

Diisopropyl phosphorofluoridate (DFP) is a highly toxic organophosphorus compound (OPC) categorised under acetylcholinesterase inhibitors. Amongst earlier literature on the effect of OPCs on carbohydrate metabolism, Matin and Siddiqui[1] found increase in blood glucose and reduction in glycogen in various brain structures in rats after treatment with malathion. Samson, *et al*[2] observed that *Soman* has greater impact on brain regional glucose use than DFP.

Present investigation is aimed at obtaining more insight into the effect of DFP on certain aspects of carbohydrate metabolism. The first aspect comprised of study of the effect of acute and sub-acute doses of DFP on liver glycogen and blood glucose levels in male albino rats and the effect of simultaneous administration of insulin. The second aspect consisted of study of the effect of an acute dose of DFP on liver glycogen, total free (unconjugated) catecholamine levels in blood and lactate

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dehydrogenase levels in serum. While the third aspect consisted of study of glycolytic rate by determination of blood and liver lactate and pyruvate levels and lactate pyruvate ratios. The statistical analyses were done applying student *'t'* test.

For the first phase of study (Table 1), male albino rats (body weights 150±10 g) were divided into four groups of eight animals each. The group 1 served as control. The animals of this group were injected sc with equivalent diluents of DFP. The group 2 animals were similarly injected with an acute dose (50 per cent of the LD_{50}) of DFP. The animals of group 3 received the same dose of DFP as group 2, together with 1.0 unit/kg of insulin. The group 4 animals received, daily sub-acute doses (5 per cent of the LD_{50}) of DFP for a 22 day period. The dilutions of DFP were made in distilled water from a 10 per cent stock solution of the pure compound in propylene glycol. The LD_{50} of DFP was predetermined[3] in this laboratory and found to be 3.3 mg/kg by sc route. A portion of liver tissue (approx 2 g) was collected from each animal in tubes containing 30 per cent *KOH* (6 ml) for glycogen estimations and specimens of blood (approx 4 ml) for blood glucose estimations in tubes containing a small quantity (20 mg) of *NaF* as anticoagulent, either after 75 min or 24 hr from the last dose DFP depending whether the studies were made under acute or sub-acute toxicity conditions. Glycogen was isolated from liver as described by Hawk[4], hydrolysed and estimated as glucose by Dubois method[5]. Blood glucose was estimated by glucose oxidase method[6].

Table 1. Effect of an acute dose of DFP on liver glycogen and blood glucose followed by administration of insulin + effect of daily, sub-acute doses of DFP for a 22-day period on liver glycogen and blood glucose*

Group	Treatment	Liver glycogen (g glucose/100 g liver)	Blood glucose (mg %)
	Control (no treatment)	6.34 ± 0.64	114.0 ± 5.32
	Single acute, sc dose of DFP (50 per cent of the LD_{50})	1.13 ± 0.42[a]	187.0 ± 4.60[a]
	Same dose of DFP as in group 2 + insulin (1.0 ml/kg dose, sc)	1.94 ± 0.32[a]	154.4 ± 7.10[b] (as compared to group 1 and group 2)
	Subacute, sc., daily doses (5 per cent of the LD_{50}) of DFP for a 22 day period)	2.09 ± 0.20[a]	104.0 ± 5.71

* The values are mean ± SE; a : $P < 0.001$; b : $P < 0.05$

For the second phase (Table 2), the procedure up to collection of blood and liver samples were the same as described above under acute toxicity conditions (studies confined to group 1 and group 2) except that in group 2 the dose of DFP was 75 per cent of the LD_{50} value and a separate sample of blood (approx 1 ml) was collected

from each animal and made into serum for lactate dehydrogenase (LDH) activity determinations by colorimetric method[7]. The total free catecholamine levels in blood were estimated by differential spectrophotofluorimetry[8].

Table 2. Variation of liver glycogen, total blood free catecholamine levels and serum LDH levels in male albino rats under the action of DFP*

Group	Treatment	Liver glycogen (g glucose/100 g liver)	Total free catecholamine in blood (adrenaline μ g/l)	Serum LDH activity at 25 °C (IU/l)
1	Control (no treatment)	5.86 ± 0.79	25.96 ± 4.40	423.50 ± 7.20
2	Single, acute, sc dose of DFP (75 per cent of the LD_{50})	1.54 ± 0.34[a]	83.90 ± 4.25[a]	336.86 ± 17.0[a]

* The values are mean ± SE; a : $P < 0.00$

For the third phase, the initial procedures up to the collection of blood and liver samples were the same as described above for Table 2 except that 50 per cent LD_{50} dose of DFP was used. For actual determination of liver lactate and pyruvate levels, approx 2 g of liver from each animal was made into a 20 per cent homogenate by taking in tubes containing 8 ml of 0.85 per cent ice-cold *NaCl* solution and homogenising using a tissue homogeniser. It was deproteinised, using appropriate protein precipitants (for pyruvate and lactate) and the filtrate used (as serum) for blood, and liver lactate and pyruvate determinations by methods of Barker and Summerson[9] and Friedmann[10] respectively.

Table 1 shows that due to an acute dose of DFP there was depletion ($P < 0.001$) of liver glycogen associated with rise ($P < 0.001$) in blood glucose levels (cf group 2 versus group 1). For treatment with dichlorovos also (another OPC) decreased glycogen levels were observed in rat liver[11] under similar conditions. Hyperglycemia due to OPCs like parathion and dichlorovos have been reported[12,13]. However, present finding is in variance with that of Kleinrok and Rajtar[14] who reported hypoglycemic effect of DFP. In group 3 animals where 1.0 unit/kg dose of insulin was administered alongwith an acute dose of DFP, depletion ($P < 0.001$) of liver glycogen associated with rise ($P < 0.01$) in blood glucose levels were observed. However, this rise in blood glucose levels was less ($P < 0.05$) than that of in group 2 where DFP was used alone. This partial reversal of hyperglycemic effect of DFP by insulin (1.0 unit/kg) was similar to that reported by Krystyna, *et al*[13] for dichlorovos. In group 4 where DFP was administered in sub-acute doses for a period of 22 days, again there was depletion of liver glycogen ($P < 0.001$) but did not show up as rise in blood glucose levels. This could probably be due to the fact that in group 4, the specimens were collected 24 hr after the last dose of DFP, by which time the blood glucose levels in

animals were probably raised but came down to normal level at the time of collection of blood. Lowering of glycogen level in this group indicates that the dose of DFP was not too low which could have been the other possibility. This is in agreement with the findings of Krystyna, *et al*[13] for dichlorovos.

Table 2 shows that under the action of an acute dose (75 per cent of the LD_{50}) of DFP there was depletion ($P < 0.001$) of liver glycogen associated with elevation ($P < 0.001$) of total free catecholamine levels of blood and inhibition ($P < 0.001$) of serum LDH activity (cf group 2 versus group 1). DFP probably has an effect on adrenal glands producing increased secretion of catecholamines in blood which activated[15] the inactive phosphorylase *'b'* to active phosphorylase *'a'* which in turn was responsible for glycogenolysis. Reduction of catecholamine contents in adrenal glands associated with depletion of liver glycogen by parathion after acute intoxication in chick embryo have been reported[16,17]. Inhibition of serum LDH activity was indicative of accumulation of lactic acid. Domagk[18] reported inhibition of glycolytic enzymes including LDH by DFP in frog muscle extract.

Results also showed that there is an increase ($P < 0.05$) in blood and liver lactate levels and lactate/pyruvate ratios together with a fall ($P < 0.05$) in blood pyruvate level by DFP (cf group 2 versus group 1). This increase in lactate levels and lactate/pyruvate ratios point towards an increased rate of anaerobic glycolysis[19]. Since liver is the site where lactic and pyruvic acids are converted into liver glycogen, there is excess accumulation of these acids in liver disease. Rise in lactate/pyruvate ratio was indicative of hepatotoxicity.

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