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Rifampicin, Halothane and Glucose as Mediators of Lysosomal Enzyme Release and Tissue Damage

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Abstract — It is suggested that the important drugs rifampicin and halothane and the raised glucose levels in diabetes mellitus exert injurous effects on cells through a lysosomal mechanism. Further evidence is given of by time rifampicin induction of β-glucuronidase and β-N acetylglucosaminidase and its possible relation to hepatitis and pancreatitis. On the basis of preliminary data halothane may cause hepatitis connected to lysosomal enzyme release in the presence of other aggravating factors common to the perioperative period. The onset of diabetic vascular complications may be related to the similar raised levels of lysosomal enzymes found in insulin, drug and diet controlled disease.

Release of these enzymes into plasma may be a marker of important changes in the lysosome, whether due to enzyme induction or damage, and could be a primary mechanism of many disease processes including some thought to be mainly autoimmune in character. Routine estimation in the clinical laboratory along with existing cytoplasmic and microsomally derived enzymes in the chemical screen would be a useful way of surveying lysosomal changes in the wide spectrum of disease in a general hospital.

Introduction

One of the simplest and most attractive mechanisms of cell injury or death is disruption of the lysosomal organelle and release of its potent hydrolytic enzymes into the cell. Although this idea was frequently raised and in some cases documented in the experimental lysosomal literature the evidence for such a mechanism in man is still uncertain. It is probably most suggestive in rheumatoid arthritis (1) and in certain forms of photosensitisation (2). Evidence for various organisms occupying this vacuolar system is strong (3) but the precise role this plays in the pathogenesis of the infection is not clear. Only in the genetic storage diseases (4) can lysosomal pathology be considered the main cause of cell injury and death.

Drug accumulation in lysosomes is well documented (3) but the in vivo effects in man are not clear. In this paper I wish to raise the hypothesis that there is an important lysosomal dysfunction in the pathogenesis of two clinical areas. First cell damage due to specific drugs and second the vascular disease of diabetes mellitus. The lyso-

somal enzymes β-glucuronidase and β-N acetylglucosaminidase (5, 6) can be measured simply in plasma (7, 8) and are probably largely hepatic in origin. However further isoenzyme analysis will probably show other tissue involvement in the case of diabetes and where drugs have their effect on the pancreas.

Rifampicin induction of β-glucuronidase and β-N acetylgluc osaminidase.

When we showed that these enzymes were raised in the plasma of patients receiving rifampicin for tuberculosis (9) the drug combination included isoniazid and it was not clear whether that might be contributing to the effect. Figure 1 shows the rise when streptomycin is the only combining drug and although it can accumulate in lysosomes under experimental in vitro conditions (10) there is no evidence of an effect at dose levels used in man. The rise in β-N acetylglucosaminidase appears more consistent than that of β-glucuronidase and as this is a purely lysosomal enzyme (6) it may be the enzyme of choice for screening purposes.

Although levels of these enzymes do not rise after the first month of treatment (9) the early rate of rise to maximum values is shown in a patient in Figure 2. Ten to 14 days appear necessary when the effect is observed in plasma thus the maximum cellular effect is probably a little earlier. If this is the rate of intracellular enzyme protein induction it will explain the similar by time excretion of D-glucaric acid and plasma antipyrine half-life decline (11, 12) standard indices of hepatic microsomal enzyme induction.

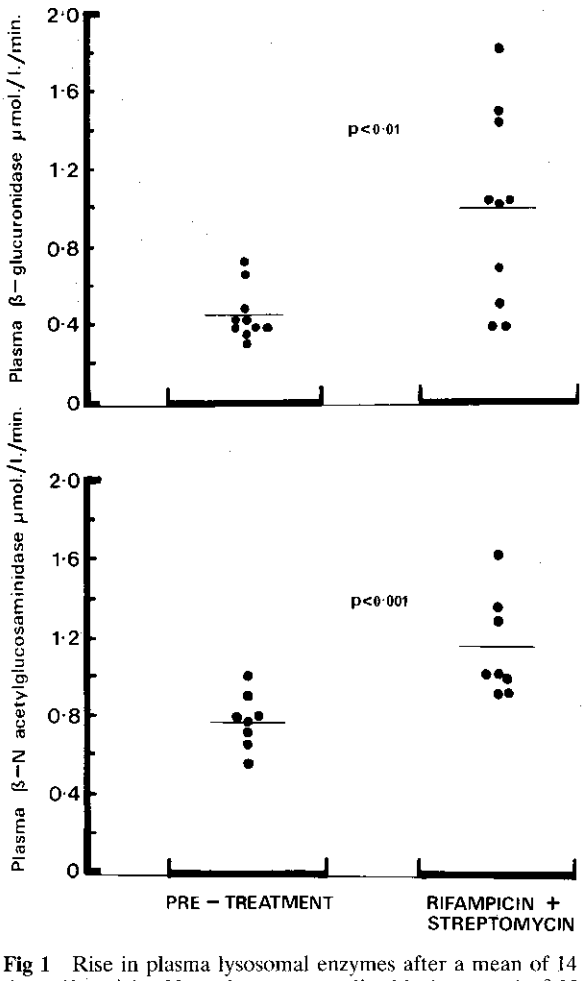

Fig 1 Rise in plasma lysosomal enzymes after a mean of 14 days rifampicin. Note the more predictable increase in β-N acetylglucosaminidase.

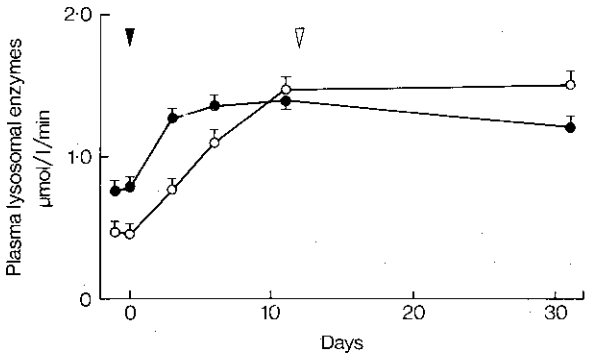

Fig 2 By time plasma rise in β-glucuronidase (open circles) and β-N acetylglucosaminidase (closed circles) with rifampicin 600 mg and streptomycin 1g daily (▼). Note the absence of change when isoniazid 300 mg daily is substituted for streptomycin (▽). Values are shown as the mean ± SEM.

One patient who was followed serially with rifampicin developed severe pancreatitis after 14 days therapy and this falls neatly into the period of maximum induction. Other observations have also suggested this time sequence in rifampicin induced fulminant hepatitis (13, 14) but usually associated with the time response seen in autoimmune reactions. The mechanism could be accumulation of rifampicin or its metabolites in the lysosome with discharge of enzymes. For severe tissue damage to occur other aggravating factors may need to be present such as previous anaesthesia, other hepatotoxic drugs, previous sub-clinical liver damage or high alcohol intake.

Autoimmune and lysosomal theory may be combined in that antibody reactions to rifampicin (15) might exert their most harmful effect at the level of lysosome.

Anticonvulsants also raise β-glucuronidase (11) and β-N acetyl glucosaminidase (unpublished observation) but the mechanism here is probably entirely microsomal thus explaining their relative freedom from dangerous reactions. Levels are similar to rifampicin induction i.e. 2–5 fold above control values. It is of interest that even in severe hepatitis levels do not rise above 5 fold (16).

Halothane may cause lysosomal enzyme release.

In looking for a further drug in which the mechanism of lysosomal-mediated damage may explain toxic effects halothane appeared an interesting candidate. Much work has revolved around a possible humoral or cell-mediated cause for the hepatitis but the answer is still unclear (17). It is known to have enzyme inducing properties (18) and its metabolites can also cause liver injury (19). Figure 3 shows some preliminary data in which we followed both lysosomal enzymes after an orthopaedic procedure in which halothane was the main anaesthetic. There was a marked rise in β-N acetyl glucosaminidase above our normal range (9) on the 5th postoperative. Two further patients out of 6 showed similar changes including the microsomally derived gamma glutamyl-transferase (unpublished data). None of these patients received blood transfusions. The problem of controlling these observations has proved severe. The large number of drugs administered other than halothane in the perioperative period, the infusion procedures, length of anaesthetic time and the difficulty of finding comparable controls without halothane has prevented us from taking this work further. The possibility of lysosomal enzyme induction leading to hepatitis should be considered in the competing theories of halothane injury.

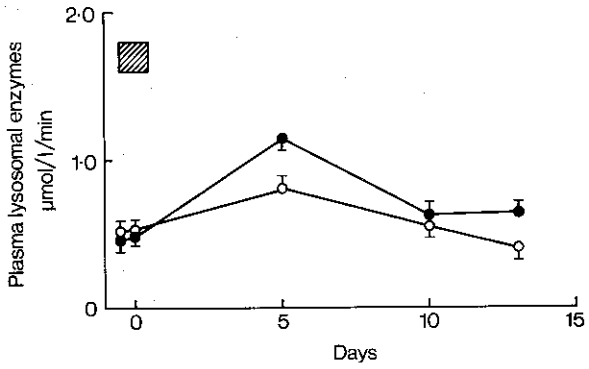

Fig 3 Rise in β-N acetylglucosaminidase (closed circles) following halothane anaesthesia. The shaded area indicates the perioperative period. Values are the mean ± SEM.

The Main Diabetic Sub-groups have Similar Raised Lysosomal Enzymes.

It has been known for some time that plasma lysosomal enzymes are raised in diabetes mellitus and it was proposed that the degree of elevation might be related to the severity of diabetic retinopathy, a microvascular disease (20). Tissue levels of these enzymes appear to be depleted and one could reasonably assume that raised lysosomal glucose caused an increase in enzyme discharge as shown experimentally (21). There is also a correlation between the level of enzymes and the height of the blood glucose (22). In the Table three main sub-groups of diabetes were examined. There was little difference in their high levels of β-N acetylglucosaminidase regardless of the type of diabetes. This is interesting for it may favour the view that the vascular disease is related to other metabolic disturbances than purely glucose though this may still be an underlying cause. Two mechanisms might lead to endothelial damage and atherosclerosis or microvascular disease. First simple toxicity of released enzymes into the cell due to lysosomal glucose accumulation. Second through a lipid effect e.g. cholesterol esterase is a lysosomal enzyme essential for normal cholesterol metabolism and if this failed to work normally either through increased discharge or some other mechanism an increase in cholesterol deposition in the vessel wall might result. There is an extension to such a theory for the general rise in lysosomal plasma enzymes with age may reflect a normal breakdown in function leading to atherosclerosis. Levels of the enzyme at any age are higher in males than females (16) and the former suffer from atherogenic vascular disease earlier.

Conclusions

The role of hepatic lysosomes in drug metabolism could be an important factor in understanding drug-induced tissue damage and among the simplest screening tests would be plasma β-N acetylglucosaminidase. The vascular disease of

Table

	Insulin	*Chlorpropamide*	*Diet*
Number	58	24	13
Mean age (yrs)	39.7	56.6	58.2
SD	13.1	13.1	11.2
β-N acetylglucosaminidase (μmol/l/min)			
Mean	1.153	1.285	1.262
SD	0.321	0.385	0.383
β-glucuronidase μmol/l/min)			
Mean	0.449	0.546	0.675
SD	0.227	0.247	0.460

Plasma lysosomal enzymes in 3 outpatient diabetic groups. Using t-test only significant difference is that for glucos aminidase in diet v insulin treated patients ($p<.005$). When allowance is made for the age difference of the two groups using the regression equations for normal subjects (8) the difference disappeared. (The data is taken from the work of Dr J. W. Woollen and colleagues and is gratefully acknowledged).

diabetes could be linked to inadequate control of blood glucose through a lysosomal malfunction in the arterial wall. The raised levels in these conditions require further explanation and here I have attempted to implicate them as a main cause of tissue injury.

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