Resumen

Objetivo: El estudio de la lipasa y amilasa total y sus isoenzymas en suero como indicadores bioquímicos de daño pancreático en pacientes tratados con ácido valproico y con otros fármacos antiepilépticos inductores enzimáticos.

Método: Se determinaron las actividades séricas de lipasa y amilasa total y sus isoenzymas en 41 pacientes tratados con ácido valproico en monoterapia, 50 pacientes tratados en mono/politerapia con fenitoína, fenobarbital y carbamazepina y 30 controles clínicamente sanos.

Resultados: En el primer grupo de pacientes no se encontró una diferencia clínicamente significativa en relación al grupo control para ninguna de las actividades enzimáticas; sin embargo, en el grupo tratado con fármacos antiepilépticos inductores se encontró una diferencia clínicamente significativa para la lipasa y amilasa de tipo pancreático. En este grupo de pacientes, en 2 casos (4%) la actividad de amilasa pancreática estaba claramente aumentada con niveles que sugerían la existencia de un daño pancreático. La amilasa total presentó una deficiente especificidad como marcador bioquímico de daño pancreático en los pacientes estudiados, correspondiendo la mayor actividad encontrada a un caso con aumento de la isoenzima de tipo salivar. Por su parte la lipasa parece presentar una menor sensibilidad.

Conclusiones: En pacientes tratados con fármacos antiepilépticos la determinación de la isoenzima de tipo pancreático de la amilasa podría ser de interés aun en ausencia de signos clínicos de pancreatitis aguda.


Summary

Objective: The study of the serum lipase and total amylase and its isoenzymes as biochemical markers of pancreatic injury in patients treated with valproic acid and other enzyme-inducing antiepileptic drugs.

Method: The serum activities of lipase and total amylase and its isoenzymes were determined in 41 patients treated in monotherapy with valproic acid, 50 patients in mono/polytherapy with phenytoin, phenobarbital and carbamazepine, and 30 healthy controls.

Results: In the first group of patients a clinically significant difference in relation to the control group was not obtained for any of the enzyme activities studied; however, in the group of patients treated with enzyme-inducing antiepileptic drugs clinically significant differences were obtained for lipase and pancreatic amylase. In this group of patients, the activity of pancreatic amylase was clearly increased in two cases (4%), suggesting the existence of a pancreatic damage. In the patients studied, the total amylase showed a poor specificity as a biochemical marker for pancreatic injury, and the greater serum activity observed in one case corresponds to an increase of the salivar isoenzyme. The sensitivity of the lipase is smaller than amylase pancreatic isoenzyme.

Conclusions: In patients treated with antiepileptic drugs, the determination of the pancreatic isoenzyme of amylase would be of interest even in absence of clinical signs for acute pancreatitis.

Key words: Antiepileptic drugs. Pancreatitis. Lipase. Total amylase. Pancreatic amylase.

INTRODUCTION

The link between administering valproic acid and pancreatitis has been widely documented1-4. However, recent studies have revealed that there is a similar risk of acute pancreatitis in patients treated with other antiepileptic drugs as the estimated risk associated with valproic acid5. The hypothesis that the aforementioned drug con-
stitutes an independent risk factor for pancreatitis must therefore be reconsidered.

Most patients treated with valproic acid present normal or slightly elevated serum amylase values\(^{23,6,9}\). Even though enzymatic activity is raised slightly during treatment\(^{23}\) its clinical significance is uncertain\(^{13}\). The possible effect of other anticonvulsant drugs, such as phenytoin, phenobarbital or carbamazepine, on the serum activity of pancreatic enzymes has received little attention\(^{10,11}\).

Serum lipase is mainly secreted by the pancreas. Amylases however are not specifically originated in the pancreas. Other organs, such as the salivary glands, may be involved in causing higher serum activity. As a consequence, the determination of salivary and pancreatic amylase isoenzymes increases the sensitivity and diagnostic specificity of the test. This article presents the results obtained from total amylase and its isoenzymes and serum lipase in two groups of epileptic patients; one group administered valproic acid only in monotherapy and another group in monotherapy or in polytherapy receiving phenobarbital-type enzyme-inducing anticonvulsant drugs (phenytoin, phenobarbital and carbamazepine).

**METHOD**

Ninety-one (91) epileptic patients were studied (52 men and 39 women) with an average age of (± standard error of the mean) of 41.8 ± 2.9 years (range of 18-85 years), distributed across two groups depending on whether they were receiving valproic acid in monotherapy (n = 41) or phenytoin, phenobarbital and carbamazepine (n = 50). As an inclusion criterion, all patients had to be receiving treatment with doses that remained unchanged for a period of at least three months. Given that those patients in the second group were being administered drugs in polytherapy, doses were expressed in units/day in accordance with a previously determined points system\(^{12,13}\). None of the patients showed any sign of acute pancreatitis. The control group included 30 clinically healthy individuals (19 men and 11 women) with an age of 36.5 ± 2.9 years (range of 18-58 years) who were not receiving any form of drug treatment.

Total amylase (EC 3.2.1.1) was determined using 4,6-ethylidene-p-nitrophenyl-α-D-maltoheptaoside as a substrate in an Advia 1650 (Bayer Diagnostics) Analyzer. The pancreatic isoenzyme was determined using the same substrate with previous immunoinhibition of the salivary isoenzyme in Cobas Integra 400 (Roche Diagnostics) analyzer and, consequently, the difference between the total and pancreatic amylase activities corresponds to the salivary isoenzyme. Lipase (EC 3.1.1.3) is determined using 2-chlorine-4-nitrophenyl-α-D maltotrioside and 1,2-O-dilauryl-rac-glycerol-3-glutaryl-(6’-methylresorufin) ester in the presence of colipase and bile salts on the Dimension Clinical Chemistry System (Dade Behring). The activity of the γ-glutamyltransferase (EC 2.3.2.2) is determined in an Advia 1650 (Bayer Diagnostics) analyzer. The valley concentrations corresponding to the state of equilibrium of valproic, phenytoin, phenobarbital and carbamazepine acid were determined using enzyme immunoassays on a V-Twin (Dade Behring) analyzer.

Microsoft-Excel (v 5.0) is used to statistically analyse the results. The distribution of data was analysed using the Smirnov-Kolmogorov method and the Pearson’s correlation coefficient for Gaussian distributions and Spearman’s rank correlation coefficient for non-Gaussian distributions. The clinical significance of the differences was established using a criterion requiring an eighth of the corresponding reference range to be exceeded\(^{14,15}\). In accordance with this criterion, and considering our laboratory’s reference ranges for these biochemical variables, the clinically significant differences were calculated for lipase (> 21.5 U/l), amylase (> 10.0 U/l) and pancreatic amylase (> 5.4 U/l). The results are expressed as the mean average ± the standard error of the mean (median).

**RESULTS**

The epileptic patients that were studied had an adequate drug compliance with low concentrations of valproic acid of 64.7 ± 2.6 µg/ml (n=41), of phenytoin of 15.1 ± 0.9 µg/ml (n = 34), of phenobarbital of 31 ± 4.2 µg/ml (n = 5) and of carbamazepine of 7.6 ± 0.4 µg/ml (n = 26).

Table I presents the results obtained for the serum activity of lipase, amylase and pancreatic amylase for the control groups and patients. The results for the group of patients treated with phenytoin, phenobarbital and carbamazepine were significantly clinically different compared to those for the control group for lipase and pancreatic amylase. However, no clinically different results were obtained for any of the variables studied for patients treated with valproic acid in monotherapy. No significant correlation was found between the dose or level of the different drugs administered with the serum lipase activities, total amylase and pancreatic amylase (p > 0.05).

In the control group, the serum activities of the enzymes studied were not included in all of the cases in the corresponding reference intervals used in our laboratory. Figure 1 shows the distribution of activity of lipase,
amylase and pancreatic amylase (expressed as multiples of the highest reference limit) for both groups of patients. In the group of patients treated with phenytoin, phenobarbital and carbamazepine, two cases (4%) presented sufficient levels of pancreatic amylase for developing pancreatic injury. By contrast, there were no cases of biochemical pancreatic affection in the group of patients treated with valproic acid in monotherapy.

In the group of patients treated with valproic acid, there was a significant correlation between total amylase and pancreatic amylase ($r=0.595$, $p < 0.001$) and lipase ($r=0.589$, $p < 0.001$) as well as between pancreatic amylase and lipase ($r=0.461$, $p < 0.001$). For the group treated with phenytoin, phenobarbital and carbamazepine, significant correlations were also found between total amylase and pancreatic amylase ($r=0.796$, $p < 0.001$) and lipase ($r=0.630$, $p < 0.001$) and between pancreatic amylase and lipase ($r=0.777$, $p < 0.001$), as well as between lipase and a relative proportion of pancreatic amylase ($r=0.395$, $p < 0.05$). However, there was no significant correlation (positive or negative) in either of the groups of patients for total amylase with a relative proportion of pancreatic amylase. This indicates that, for these patients at a population level, greater serum amylase activity would not necessarily mean a preferable increase in one or the other of the types of pancreatic or salivary isoenzymes.

In patients treated with phenytoin, phenobarbital and carbamazepine, a γ-glutamyltransferase activity was obtained (average $74.7 \pm 13.0$ U/L, mean $33.5$ U/L) that was significantly greater (Mann-Whitney test, $p < 0.001$) than that obtained for the group treated with valproic acid (average $28.0 \pm 6.1$ U/L, mean $10$ U/L). The correlation between pancreatic amylase and γ-glutamyltransferase was statistically significant ($r=0.292$, $p < 0.05$).

**DISCUSSION**

Acute pancreatitis caused by valproic acid may be due to some form of an idiosyncratic complication and arguably related to the patient’s age and length of duration of treatment and increasing the risk of combining treatment with olanzapine. It has been suggested that one possible cause is that the drug has a direct toxic effect due to the free radicals. However, it has also been suggested that the depletion of carnitine due to valproic acid may also have played an important part in pancreatic injury. Nevertheless, other antiepileptic drugs may also contribute to the creation of free radicals and carnitine depletion. On the other hand, oxidative stress may also be increased by greater tobacco intake in epileptic patients. These data may help to explain the recent results obtained by Norgaard et al. demonstrating a similar risk of pancreatitis linked to administering valproic acid or other antiepileptic drugs.

In the group of patients treated with valproic acid in monotherapy, no clinically significant difference was obtained for any of the variables studied with regard to the control group (Table I). There were no cases in which the activity of serum lipase or pancreatic amylase was found that may have suggested pancreatic injury. However, the group of patients that were treated with phenytoin, phenobarbital or carbamazepine did present higher levels of lipase and pancreatic amylase than the control group and the difference was statistically significant. Two patients showed signs of pancreatic injury, at least biochemically, with clearly increased pancreatic amylase activities (Fig. 1). In these patients, the total amylase and lipase values were also higher than the corresponding highest reference, but with less noticeable increases.

Voudris et al. discovered an increase in salivary amylase in a group of children treated with carbamazepine for 6 months. One year into the treatment, enzymatic activity became stabilised. As indicated above, no significant correlation was found between the activity of total amylase and the relative pancreatic isoenzyme proportion in either of the two groups of adult patients studied. This suggests that the increases in enzyme activity would not necessarily depend on one isoform or another. The highest level of total amylase activity of all of the patients studied was in one 34-year-old male patient, treated with carbamazepine in monotherapy (serum concentration $9.9 \mu g/mL$). This patient showed an increase in the salivary type isoenzyme with pancreatic amylase and lipase within a normal range. These results demonstrate the limited specificity of total amylase as a marker for pancreatic injury in these patients. These results also corroborate the previous observations which show an uncertain clinical significance to the increase of this enzyme in patients treated with valproic acid. Lipase appears to present less sensitivity.

The greatest γ-glutamyltransferase activity was in the group of patients treated with phenytoin, phenobarbital and

Fig. 1. The distribution of serum total amylase (O), pancreatic amylase (●) and lipase (△) activities in the groups of patients treated with valproic acid and antiepileptic enzyme inducers.
carbamazepine due to the enzymatic hepatocyte inducer nature of these drugs. This enzyme, however, has a limited use as a marker because its serum activity is modulated by additional factors other than induction itself. The alteration of the lipid composition and the permeability of the plasmatic membranes by phenobarbital inducer drugs encourages membrane and cytosolic enzymes to be released into the extracellular medium and enter the circulatory flow. Given that the pancreas contains a large amount of γ-glutamyltransferase, this type of action may help to explain the strong correlation between γ-glutamyltransferase serum activities and pancreatic amylase in patients treated with phenytoin, phenobarbital and carbamazepine.

By studying the results obtained, periodically determining pancreatic amylase in epileptic patients may be of interest in clinical practice. Those cases with high serum activity of this isoenzyme, even if there are no clinical signs of acute pancreatitis (such as vomiting or abdominal pains), must be closely biochemically monitored. This will enable the prognostic value for the pancreatic amylase for these patients to be established.

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References