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In Vitro Effect of Dipyrone on Several Peroxidase Labelled Immunoassays

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Summary: We studied the *in vitro* effect of dipyrone on the determination of free triiodothyronine (free T_3), cortisol, progesterone, estradiol, carcinoembryonic antigen, human chorionic gonadotropin and α-fetoprotein measured with an immunoenzyme assay based on enhanced luminescence that uses peroxidase as label. We found significant interference from dipyrone ($p < 0.01$) in the determination of all the analytes mentioned: for progesterone and estradiol the interference was present at high doses of dipyrone; for free T_3 and cortisol the minimum dipyrone concentration producing interference was 712 µmol/l and for carcinoembryonic antigen, human chorionic gonadotropin and α-fetoprotein 44 µmol/l. Dipyrone has an analytically and statistically significant interference effect on the determination of the mentioned analytes.

Introduction

Dipyrone (noramidopyrine methanesulfonate), one of the oldest synthetic pyrazolone derivatives, is an effective analgesic, antipyretic and anti-inflammatory drug (1). Due to its high solubility, it can be administered intravenously. Some of the secondary effects dipyrone can produce are: allergic reactions, skin eruptions and central nervous system alterations. In spite of its infrequent undesirable effect (agranulocytosis) (2), dipyrone is one of the most widely used analgesic drugs in Spain. It is mainly administered for the treatment of acute postsurgical or postraumatic pain and renal colic pain (3).

We have demonstrated the negative interference by dipyrone, *in vitro* and *in vivo*, in the determination of several biochemical analytes (4, 5). The common feature of the majority of these methods was a final reaction involving the enzyme peroxidase[1]).

Peroxidases consist of a wide group of enzymes with different functions. The longest and the best studied is horseradish peroxidase (6) which is used in many immunoassay techniques.

The objective of this work is to examine the *in vitro* effect of dipyrone on several analytes measured by immunoassay techniques using peroxidase as a label.

Materials and Methods

Instrumentation

We measured the following analytes in serum: free T_3, cortisol, progesterone, estradiol, carcinoembryonic antigen, human chorionic gonadotropin and α-fetoprotein. For all the measurements we used Amerlite reagents on the Amerlite Analyzer (Kodak Clinical Diagnostics Ltd, Amersham, UK).

Procedures

The determination of free T_3, cortisol, progesterone and estradiol is based on a competitive technique using enhanced luminescence. The method uses coated wells as solid phase and horseradish peroxidase as label. After removing the unbound fraction, the horseradish peroxidase activity of the bound conjugate is measured by an enhanced luminescence reaction obtained with the addition of the signal reagent that contains hydrogen peroxide as substrate, luminol as luminogen and a compound that enhances the intensity of light produced and prolongs its emission (the substituted phenol *p*-iodophenol). The amount of conjugate bound is inversely proportional to the concentration of analyte present in the sample.

[1]) Peroxidase: EC 1.11.1.7

The Amerlite carcinoembryonic antigen, human chorionic gonadotropin and α-fetoprotein assays are based on a non-competitive design involving a polyclonal antibody coated on to the wells and a horseradish peroxidase-conjugated monoclonal antibody. The horseradish peroxidase activity of the bound conjugate is measured by the enhanced luminescence reaction and the amount of conjugate bound is directly proportional to the concentration of analyte present in the sample.

Study *in vitro*

The possible interference of dipyrone on the different analytical methods was studied following the recommendations of the Sociedad Española de Química Clínica (7) and the guidelines of the National Committee for Clinical Laboratory Standards (8) for the detection and quantification of *in vitro* drug interference. We prepared a homogenous pool of fresh serum samples and we added dipyrone (Sigma, St. Louis, MO; cat. no. D-8890) to one half of this pool to a final concentration of 10 g/l (28 460 μmol/l), ten-fold higher than the expected maximum concentration obtained after a standard dose of dipyrone, as suggested by *Melvin R. Glick* (9); the rest of the pool of serum containing different concentrations of the analytes studied was free from the interferent. Both pools were analyzed 15 times for each analyte. The outliers were eliminated by *Dixon*'s criteria (10, 11). We tested the normality and homoscedasticity of the data by using the *Agostino* normality and *Snedecor* F tests, respectively, so that we could compare the means of both groups with results from the *Mann-Whitney* U test. If the differences between means were significant ($p < 0.01$), the drug was considered to be an interferent.

To quantify the interference, we diluted the pooled sera containing 10 g/l (28 460 μmol/l) of dipyrone with serum from the drug-free pool to obtain a series of samples containing dipyrone at 14 230, 7115, 2846, 1523, 712, 356, 178, 89 and 44 μmol/l. Five replicates of each sample were analyzed. Differences of mean concentrations of the analytes between the drug-free pool and the samples containing the interferent were evaluated for each method by using the *Mann-Whitney* U test.

Results

Table 1 shows the original concentrations determined for the serum samples we used in these studies and the minimum concentration of dipyrone that produced any interference with measurements of the different analytes.

Tab. 1 Original concentrations of the quantities and Minimum Dipyrone Concentration (MDC) producing interference *in vitro*

Constituent	Original concentration	MDC (μmol/l)	p
Free T_3	10.36 pmol/l	712	0.0079
Cortisol	529.8 nmol/l	712	0.0079
Progesterone	50.94 nmol/l	14230	0.0079
Estradiol	0.71 nmol/l	2846	0.0079
Carcinoembryonic antigen	256.5 μg/l	44	0.0079
Human chorionic gonadotropin	859.0 IU/l	44	0.0079
α-Fetoprotein	312.7 kIU/l	44	0.0079

The results obtained from the quantification of the interference are represented by the interferographs, an easy format to present interference data obtained in the laboratory (9): the average assay value for each specimen was calculated as a fraction of the original concentration (without interferent); by plotting these fractions vs the concentration of potential interferant added, we drew these graphs (figs. 1–7).

Discussion

The main goal of an interference study is to determine if interference is present, and if so, to distinguish between a statistically significant effect and clinically significant interference. Lastly, the establishment of the type of interference offers a clearer understanding of its chemical nature.

The results from the *Mann-Whitney* U test obtained in the studies *in vitro* were consistent with the presence of significant interference ($p < 0.01$) by dipyrone for all the analytes listed in tab. 1. It is important to note that the interference produced by dipyrone on the determination of progesterone and estradiol (at concentrations shown in table 1) is present only at dipyrone concentrations higher than the maximum concentration obtained after a standard dipyrone dose. For the rest of the quantities studied, the minimum dipyrone concentrations producing interference ranged from 44 to 712 μmol/l. This range includes dipyrone concentrations that can be observed in a patient after a standard dipyrone dose (1).

The interference is positive or negative depending on the type of technology studied: positive interference in competitive methods and negative interference in non-competitive ones. Dipyrone therefore interferes positively with the determination of free T_3, cortisol, estradiol and progesterone and negatively on the measurement of carcinoembryonic antigen, human chorionic gonadotropin and α-fetoprotein.

From this data we demonstrate that dipyrone has an analytically and statistically significant *in vitro* interference on the concentration of several analytes determined by peroxidase labelled immunoassays. This interference is produced through a direct and specific inhibitory effect on the enzyme label since dipyrone can cause a varying loss of activity in peroxidase (12). We conclude that clinical laboratory data obtained by these methods in patients taking dipyrone should be carefully evaluated.

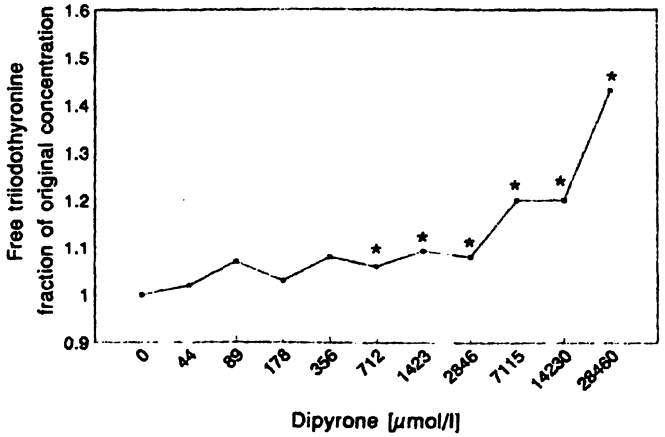

Fig. 1 Interferograph for free T_3

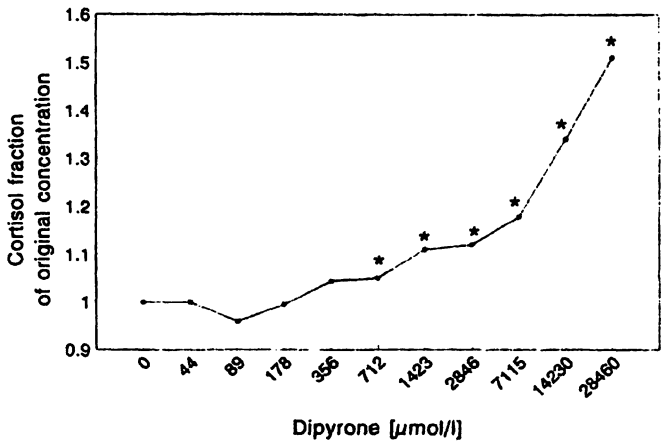

Fig. 2 Interferograph for cortisol

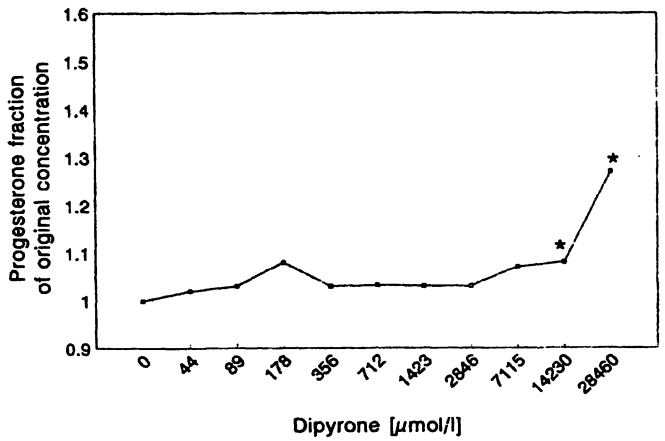

Fig. 3 Interferograph for progesterone

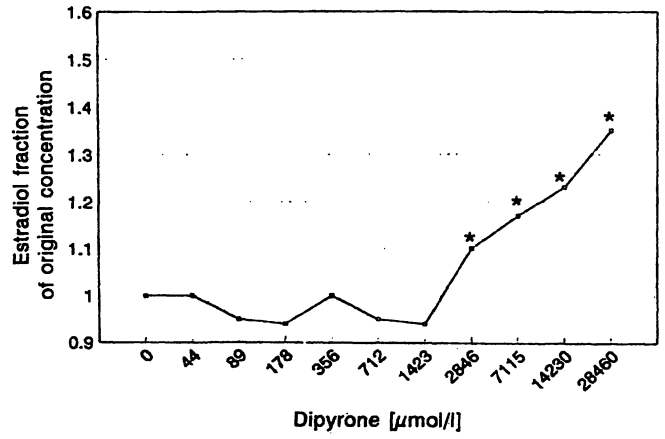

Fig. 4 Interferograph for estradiol

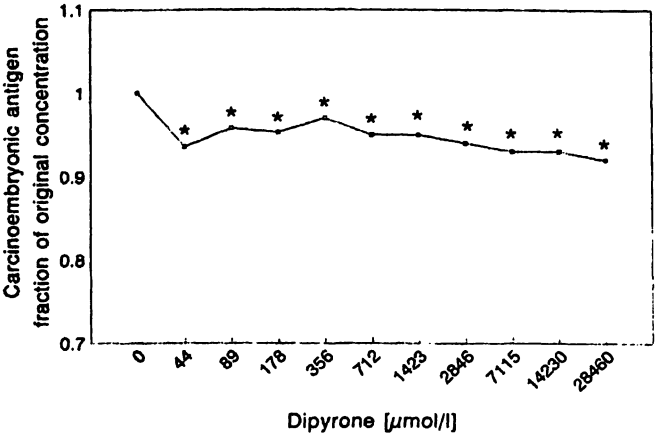

Fig. 5 Interferograph for carcinoembryonic antigen

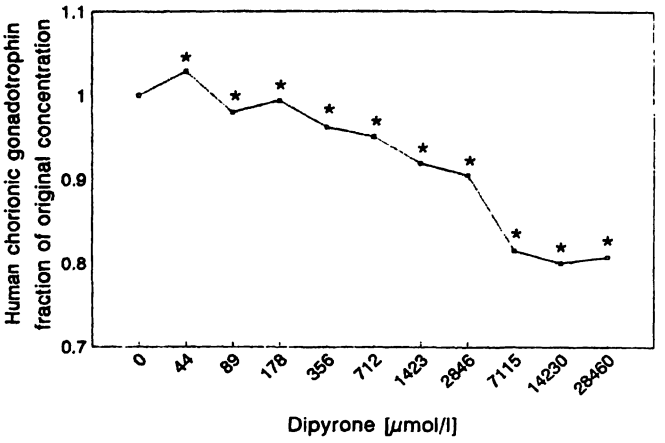

Fig. 6 Interferograph for human chorionic gonadotropin

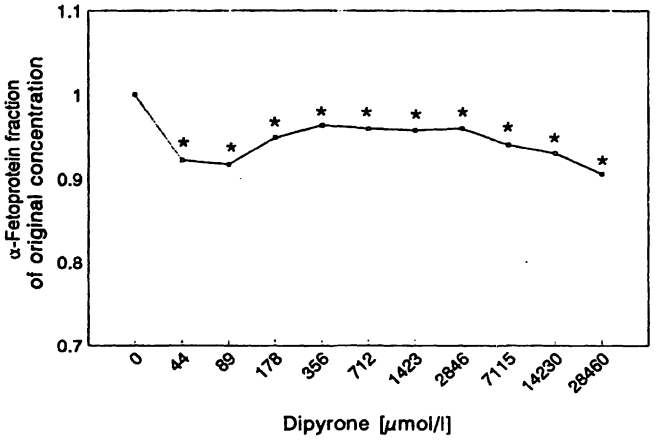

Fig. 7 Interferograph for α-fetoprotein

Figs. 1–7
The starlets indicate significant ($p < 0.01$) interference

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