EFFECT OF SINGLE ORAL DOSE OF PHENOBARBITONE ON LYMPHOCYTE BLASTOGENIC RESPONSE IN MAN

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SUMMARY

A single oral dose of phenobarbitone 1.5 mg kg\(^{-1}\) was given to 18 normal subjects to evaluate the effects on lymphocyte function. Serum barbiturate concentrations and lymphocyte blastogenic response to three mitogens (PHA, Con A, PWM) were tested before and at 2, 12 and 36 h after drug administration. A serum barbiturate plateau of about 2 ng ml\(^{-1}\) was maintained from 2 to 36 h. Lymphocyte blastogenesis was reduced with barbiturate compared with controls. However, the reduction was significant only at 36 h (\(P < 0.01\)).

Lymphocyte response to non-specific mitogens, T and B lymphocyte counts and cutaneous delayed hypersensitivity to antigens, have been found to decrease to a variable extent in the first few days following surgery (Cullen and Van Belle, 1975; Slade et al., 1975; Miller et al., 1976; Roth et al., 1976; Tarpley et al., 1977; Sochat, Miller and Snyder, 1979). Surgical stress and anaesthetic drugs have been suggested as likely factors in postoperative immunosuppression (Bruce and Wingard, 1971; Howard and Simmons, 1974; Roth et al., 1976; Tarpley et al., 1977; Sochat, Miller and Snyder, 1979; Walton, 1979).

In a previous study, the in vitro lymphocyte blastogenic response was found to decrease slightly during the 3 days preceding surgery (Adami et al., 1980). An impending operation is a stressing condition and may be a possible cause. However, such an effect might be a result of phenobarbitone, usually given to our patients as hypnotic. This study was undertaken since the few published reports do not agree on the immunosuppressive action of barbiturates (Park and Brody, 1971; Gabourel, Davies and Rittenberg, 1977).

METHODS

Eighteen normal volunteers (nine male), aged 21–58 yr and who were not taking drugs, were studied. Each subject received a single oral dose of phenobarbitone 1.5 mg kg\(^{-1}\) at 8 p.m. At 8.00 a.m., before administration of barbiturate (−12 h), venous blood samples were taken for measurement of serum barbiturate concentration and lymphocyte blastogenic response. Measurements were repeated 2, 12 and 36 h after drug administration, but no lymphocyte cultures were made at 2 h.

Venous blood was drawn into 10-ml disposable syringes coated with preservative-free heparin 10 u ml\(^{-1}\) (Calbiochem, La Jolla, USA). Lymphocytes were collected after centrifugation at 400 \(g\) for 20 min through a Ficoll-Urovison gradient (sp.gr.t. 1.077) and washed three times in Hanks balanced salt solution (Eurobio, Labtek, Paris). Cells were resuspended at a concentration of 2 x 10\(^6\) ml\(^{-1}\) in TC 199 medium buffered with Heps and 7% bicarbonate (Eurobio, Labtek, Paris), supplemented with 1% L-glutamine, penicillin 100 i.u. ml\(^{-1}\), streptomycin 100 \(\mu\)g ml\(^{-1}\) and 20% heat-inactivated autologous plasma.

Cultures were performed in microtitre plates with U-bottomed wells (Linbro, Hamden, USA). One hundred microlitre of the cell suspension was added to each well with 100 ulitre of solution containing TC 199 (control cultures), phytohaemagglutinin 100 \(\mu\)g ml\(^{-1}\) (PHA, Wellcome), concanavalin A 100 \(\mu\)g ml\(^{-1}\) (Con A, Pharmacia AB, Upsala) or pokeweed mitogen (PWM, Grand Island Biological Co., New York) diluted 1 : 20. The same batches of mitogens were used for the entire study. Cultures were performed in triplicate and plates incubated at 37 °C in a humidified atmosphere for 55 h. Methyl-\(^3\)H-thymidine 0.5 \(\mu\)Ci (specific activity 2 Ci mmol\(^{-1}\), Amersham,
England) were added to each well. Seventeen hours later, cultures were harvested on to glass fibre filters with a semiautomatic device (Sacrificator, Labtek). Filters were added to 2.5 ml of scintillation fluid and counted in an LKB scintillation spectrometer. Blastogenic response was expressed as counts per min (c.p.m.). Our normal laboratory ranges are: 29 000–56 000; 14 000–33 000 and 7000–31 000 c.p.m. respectively for PHA, Con A and PWM stimulation.

Gas–liquid chromatography was used for measurement of serum phenobarbitone concentration (Kupferberg, 1970).

Analysis of variance was performed between c.p.m. values and between serum barbiturate concentrations at the times sampled.

RESULTS

Serum barbiturate concentrations. Values at −12, 2, 12 and 36 h were, respectively: 0, 1.95 ± 0.53 μg ml⁻¹, 1.96 ± 0.39 μg ml⁻¹ and 2.01 ± 0.66 μg ml⁻¹. There was no significant difference between 2, 12 and 36 h.

Lymphocyte blastogenesis (table I). Basal c.p.m. values of all subjects were in the normal range for the three mitogens. From −12 to 12 h, c.p.m. decreased, but not significantly. Significant decreases in c.p.m. were found between −12 and 36 h and between 12 h and 36 h (P<0.01 for all mitogens tested). Mean c.p.m. percent decreases at 36 h showed no differences between mitogens.

DISCUSSION

Oral administration of a single dose of phenobarbitone 1.5 mg kg⁻¹ resulted in serum concentrations of about 2 μg ml⁻¹. This value was achieved 2 h after drug intake and maintained 36 h, so that the serum concentration of phenobarbitone was assumed to be constant for the period of study (Buchtal and Svensmark, 1971). Mean lymphocyte blastogenic response to three different mitogens (PHA, Con A and PWM) was reduced in the presence of barbiturate compared with control values, the effect increasing with time. However, the mean decrease was significant only at 36 h. The changes were the same with the different mitogens used.

Our data suggest that phenobarbitone has an immunosuppressive action and this can follow a single oral hypnotic dose which results in low serum drug concentrations compared with values required for control of epilepsy (Svensmark and Buchtal, 1963). However, the serum concentrations achieved in this study are similar to that used in lymphocyte cultures by Park and Brody (1971) which was associated with a significant decrease in DNA synthesis under PHA stimulation.

There is a latency of 12 h or more in the in vivo appearance of phenobarbitone immunosuppression. This delay may represent the time required to inhibit the biosynthetic pathway leading to DNA synthesis (Park and Brody, 1971). Immunosuppression observed before operation in surgical patients (Adami et al., 1980) can be attributed to phenobarbitone only in the few patients receiving this hypnotic two or three nights before surgery.

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REFERENCES


EFFET D'UNE DOSE ORALE UNIQUE DE PHENOBARBITONE SUR LA REPONSE BLASTOGENIQUE DES LYMPHOCYTES CHEZ L'HOMME

**RESUME**

On administra à 18 sujets normaux une dose orale unique de phénobarbital de 1,5 mg kg⁻¹ pour en évaluer les effets sur la fonction lymphocytaire. Des essais portant sur des concentrations de barbituriques dans le sérum et sur la réponse blastogénique des lymphocytes *vit-à-vit* de trois mitogènes (PHA, Con A, PWM) ont été effectués avant l'administration de la substance et 2, 12 et 36 h après celle-ci. Un palier d'environ 2 µg ml⁻¹ de barbiturique de sérum se maintint de 2 à 36 h. La blastogénèse lymphocytaire diminua sous l'effet du barbiturique en comparaison avec les contrôles. Toutefois, cette diminution ne devint significative qu'à partir de 36 h (*P* < 0,01).

WIRKUNG EINER EINZELNEN MÜNDLICHEN DOSIS VON PHENOBARBITON AUF DIE LYMPHOZYTBLASTOGENISCHE REAKTION BEIM MENSCHEN

**ZUSAMMENFASSUNG**

Eine einzelne mündliche Dosis von Phenobarbiton 1,5 mg kg⁻¹ wurde bei 18 normalen Versuchspersonen verabreicht, um die Wirkung auf die Lymphozytfunktion auszuwerten. Serumbarbiturkonzentrationen und die lymphozytoblastogenische Reaktion auf drei Mitogene (PHA, Con A, PWM) wurden vor und 2, 12 und 36 Stunden nach Verabreichung getestet. Eine Serumbarbiturplateau von ca. 2 µg ml⁻¹ wurde von 2 bis 36 Stunden aufrechterhalten. Die Lymphozytoblastogénesis wurde mit Barbiturpräparaten im Vergleich zu Kontrollen reduziert. Die Reduktion war jedoch nur bei 36 Stunden bedeutend (*P* < 0,01).

EFECTO DE UNA DOSIS ORAL UNICA DE FENOBARBITONA EN LA RESPUESTA BLASTOGENICA DE LOS LINFOCITOS EN EL HOMBRE

**SUMARIO**

Se administró una dosis oral única de fenobarbitona de 1,5 mg kg⁻¹ a 18 sujetos normales a fin de evaluar los efectos sobre la función de los linfocitos. Se llevaron a cabo ensayos con concentraciones de barbituratos en el suero y la respuesta blastogénica de los linfocitos a tres mitogénes (PHA, Con A, PWM) antes y 2, 12 y 36 h después de la administración de la substancia. Se mantuvo una plataforma de barbiturato en el suero de alrededor de 2 µg ml⁻¹ de 2 a 36 h. La blastogénesis de los linfocitos se redujo con el barbiturato en comparación con los controles. Sin embargo, la reducción llegó a ser significante a las 36 h solamente (*P* < 0,01).