



In-vitro lymphocyte toxicity to a phenytoin metabolite in phenytoin induced cutaneous adverse drug eruptions

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ABSTRACT

Background: Phenytoin, one of the most commonly used antiepileptic drug, is associated with a wide spectrum of adverse drug eruptions. It is metabolized by the hepatic microsomal enzymes. The intermediate metabolites are arene oxides which accumulate due to deficiency of the enzyme epoxide hydrolase. These are postulated to be associated with phenytoin induced hepatotoxicity and antiepileptic hypersensitivity syndrome. **Aim:** We tried to correlate the in vitro lymphocyte toxicity of arene oxide metabolites with phenytoin induced drug eruptions and hence develop it as a predictive test for the same. **Methods:** Clinically diagnosed cases of phenytoin induced drug eruptions were selected in this hospital based study. Lymphocytes from the subjects and controls were exposed to the phenytoin metabolites generated by a murine hepatic microsomal system. The toxicity was assayed by trypan blue dye exclusion test. The results were analyzed by a linear orthogonal curve and were compared for the subject and control. **Results:** The results showed increased toxicity to lymphocytes from the patients when compared to those from controls. The toxicity was directly proportional to the severity of the drug eruption. **Conclusion:** In vitro lymphocyte cytotoxicity to phenytoin metabolites tested in this animal system could possibly predict phenytoin induced drug eruptions.

KEY WORDS: Phenytoin, adverse drug eruption, arene oxide metabolites, epoxide hydrolase, in vitro lymphocyte toxicity test

INTRODUCTION

Ever since its introduction for the treatment of seizures in 1938,¹ phenytoin has remained one of the most commonly prescribed anticonvulsant drugs. It is known to be associated with a wide spectrum of drug eruptions ranging from maculopapular rash, urticarial rash to potentially life threatening ones like Stevens-Johnson syndrome, toxic epidermal necrolysis and anticonvulsant hypersensitivity syndrome. In fact phenytoin accounted for as much as 12% of total cases

of adverse drug eruptions in a report from PGI, Chandigarh.²

Although the mechanism of these eruptions is not completely characterized, toxic intermediates of phenytoin have been implicated as a likely contributing factor.³ Phenytoin is metabolized by hepatic cytochrome p450 enzymes to reactive aromatic intermediates (arene oxides). These arene oxides, which may be responsible for cellular toxicity and for initiation of immunological reactions, are normally detoxified by

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a specific cellular enzyme called epoxide hydrolase. A defect in this enzyme could lead to an accumulation of reactive metabolites and adverse drug eruptions.⁴ It has been suggested that the metabolites may bind to cellular macromolecules that could directly lead to cell necrosis or apoptosis, or may initiate secondary immunological responses.⁵ In a study by Spielberg et al, the predisposition to phenytoin hepatotoxicity was assessed in vitro by using the lymphocyte toxicity test.⁶ Patients with phenytoin induced hepatotoxicity had greater lymphocyte toxicity than normal controls.⁶

We tried to correlate the role of these intermediate metabolites in phenytoin induced cutaneous adverse drug eruptions by using the cellular model developed by Spielberg et al using the lymphocyte toxicity test.⁶

METHODS

This was a hospital based, technician blinded, controlled study. The patients selected were clinically diagnosed cases of phenytoin induced drug eruption presenting to the skin out-patient department or referred from other specialities. Eleven patients with the following eruptions were included in the study: Stevens-Johnson syndrome (5), erythroderma (4), lichenoid rash (1), and morbilliform rash (1). Their peripheral blood was sent to the laboratory along with blood from 11 controls selected randomly from normal volunteers who were not on phenytoin or those on phenytoin who had not developed any eruption. Each blood sample from a patient was accompanied by a control blood sample.

The diagnosis of phenytoin induced drug eruptions was made on clinical grounds in all the patients studied using an in vitro model first used by Shear et al in 1981.⁷ This model reproduces in vitro the metabolic pathway of abnormal phenytoin detoxification that might predispose patients to toxicity. Human lymphocytes were isolated from peripheral blood as they are readily accessible and contain epoxide hydrolase, the enzyme required to metabolize the implicated biologically active intermediate metabolite of phenytoin (arene oxides). Next, they were mixed with mouse microsomes (a source of CYP 450 enzyme). After 24 hours, the per

cent cell death was observed by Trypan blue dye exclusion by the cells by conventional microscopy. Patients not treated with phenytoin as well as those on phenytoin who had not developed the eruption served as controls. The technician performing the test was blinded about the details of the patient.

The dose response curve for lymphocyte toxicity was evaluated through analysis of variance with linear orthogonal contrasts. Curves were compared for their linear trends (slopes) and for increased toxicity of the lowest dose over the baseline percentage of dead cells from the control

RESULTS

The toxicity of phenytoin metabolites to lymphocytes from patients and controls is shown in (Table 1) and (Figure 1). Cells from the patients showed significantly more dose related toxicity than those from controls. The range of values in controls was much below that of patients with phenytoin induced rashes. Although the number of patients included in the study was not enough to derive a definite conclusion, the degree of cellular toxicity appeared to be related to the type of

Table 1: Lymphocyte Toxicity Test Result

No.	Diagnosis	No. of Patients	% Cell death Patient Range	% Cell death Control Range
1.	SJS	5	12 - 26	2.6-3
2.	Erythrodermic	4	7 - 13	2.6 - 3.5
3.	Morbilliform	1	7	3
4.	Lichenoid	1	13	2.8

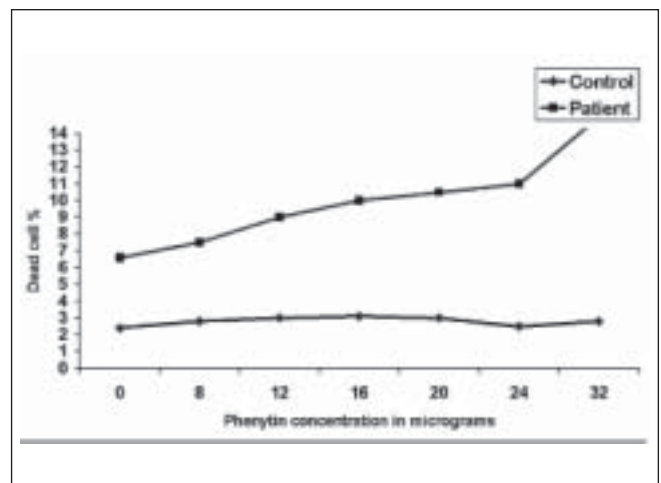


Figure 1: Result of Lymphocytes toxicity Test



drug eruption. The mean value of per cent of dead lymphocytes at 32 microgram concentration of phenytoin in various eruptions was: Stevens-Johnson syndrome - 17%, erythroderma - 12.5%, and morbilliform rash – 6%.

DISCUSSION

Cutaneous adverse eruptions to phenytoin have traditionally been classified as Type B (bizarre) with uncertain pathogenesis.⁶ Recognition of the role of toxic electrophilic intermediates in chemical carcinogenesis⁸ and non-dose related acetaminophen hepatotoxicity⁹ has suggested a link between such intermediates and unsuspected toxicity unrelated to dosage. Spielberg et al have elucidated a role for such intermediates in phenytoin induced hepatotoxicity and anticonvulsant hypersensitivity syndrome.^{6,7} A proposed scheme relating the arene oxide metabolites of phenytoin to adverse drug eruption is shown in (Figure 2).

The arene oxide metabolites may covalently bind to tissue macromolecules, thereby directly causing cell death and when these metabolites act as haptens, they alter endogenous molecules and may lead to a secondary immune response leading to various manifestations of the adverse eruption.

The result of the study shows that there is greater in vitro toxicity to lymphocytes from patients with an

eruption due to phenytoin than those from controls. Moreover, there was no significant toxicity to lymphocyte from two patients having drug eruptions to drugs not sharing a metabolic pathway with phenytoin (data not shown in tables). This means that patients with a phenytoin induced drug rash had a greater accumulation of reactive metabolites probably because of a deficiency of the metabolizing enzyme.

We observed slightly greater cell toxicity in patients with severer pattern of eruption like Stevens-Johnson syndrome as compared to a relatively milder pattern like maculopapular rash. This may be because of greater deficiency of the metabolizing enzyme epoxide hydrolase resulting in more accumulation of toxic metabolites and a different pattern of eruption. However, due to the small number of cases, this difference in cytotoxicity in the two groups is not statistically significant. The relatively high cell death in the patient with lichenoid drug eruption was clinically reflected as the patient developing erythroderma secondary to a lichenoid rash. As the test is based on the principle that there is a relative deficiency in the metabolizing enzyme epoxide hydrolase, leading to accumulation of reactive metabolites, causing adverse drug eruptions, it can be used as a screening test before starting any patient on phenytoin. If a patient is predisposed to develop an eruption with phenytoin, there is a theoretical possibility of him being reactive to other drugs sharing the same metabolic pathway (e.g. carbamazepine, phenobarbitone, lamotrigine and INH).¹⁰

Hence this test may guide us in selecting safer anticonvulsants like sodium valproate in predisposed patients. The present procedure of lymphocyte toxicity test is labor and time intensive and its ability to discriminate among other anticonvulsants and predict cross-reactivity is still to be investigated. A novel method for the lymphocyte toxicity assay has been devised by Neuman which is based on mitochondrial succinate dehydrogenase activity.¹¹ The new assay is objective, faster and has been reproducible in assessing cytotoxicity. The lymphocyte toxicity test has also been used along with the acetylation status of the patient in cases with an adverse reaction to sulfonamides. This test holds a new ray of hope for the prevention of

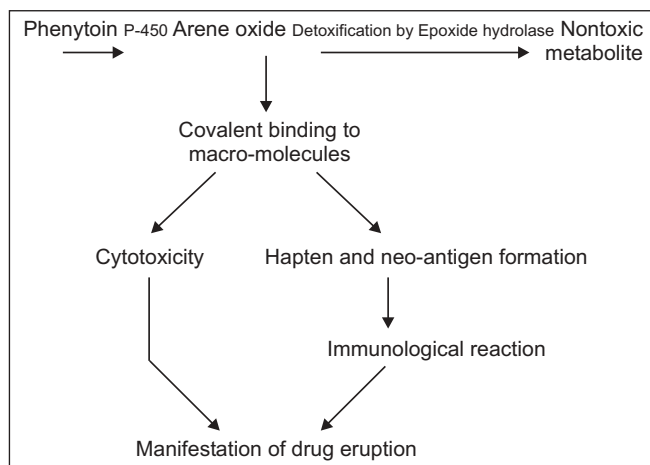


Figure 2: Pathway of phenytoin metabolism and proposed role of the arene oxide metabolites in the pathogenesis of drug eruption



adverse drug reactions to phenytoin and related compounds. A further controlled study with a larger sample population is required to standardize the role of this assay for the diagnosis of phenytoin induced eruptions as well as for predicting them.

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