THE *IN VITRO* GENOTOXIC ACTIVITY OF ALBENDAZOLE AND PRAZIQUANTEL, SINGLY AND IN COMBINATION ON HUMAN BLOOD LYMPHOCYTES

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السميّة الوراثية للألبندازول والبرازيكوانتل مجتمعان
أو على انفراد في مستنبتات خلايا دم الإنسان اللمفيّة

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تم بحث السميّة الوراثية لجرعات مختلفة (٥ ، ١٠ ، ٢٠، ٤٠ ، ١٠٠ ميكروغرام لكل
ميلليلتر) من الألبندازول والبرازيكوانتل ، وهما يستخدمان في علاج بعض الأمراض التي
تسببها مجموعة من الديدان المفلطحة ، على انفراد أو مجتمعين معاً ، في مستنبتات
الخلايا اللمفيّة المأخوذة من دم الإنسان ، وذلك باستقصاء التبادلات بين شقائق الأجسام
الصبغية (الكروموسومات) ، ومتابعة معاملي انقسام الخلايا وتضاعفها . وبمقارنة خلايا
المستنبتات التجريبيّة بخلايا المستنبتات الشاهدة ، لوحظت زيادة ليست ذات دلالة
إحصائية في التبادلات الشق – صبغية عند إضافة المادتين معاً أو على انفراد . وبالمثل، لم
يكن هناك فرق إحصائي بين تأثير المادتين سواء كانتا معاً أو على انفراد . وعلاوة على
ذلك ، انخفض معاملا الانقسام والتضاعف في الخلايا التجريبيّة ، غير أن هذا الانخفاض
لم يكن ذا دلالة إحصائية . وتشير هذه النتائج إلى أن استخدام الألبندازول والبرازيكوانتل
قد يحدث تغييرات في المادة الوراثية . وعليه ينصح بإجراء مزيد من التجارب لتحديد
المخاطر المحتملة لهما قبل التوسع بتداولهما لأغراض الوقاية من الديدان المفلطحة .

Keywords: Genotoxicity activity, Albendazole, Praziquantel

ABSTRACT

Two anthelmintic drugs; Albendazole (ABZ) and Praziquantel (PZQ), applied individually or in combination, were tested at varying concentrations (5, 10, 20, 40 and 100 ug/ml) for their ability to induce sister-chromatid exchanges (SCE) and to inhibit the cell proliferation and mitotic indices (CPI and MI, respectively) in cultured human peripheral blood lymphocytes. The data demonstrated that treatment of cells with ABZ and PZQ simultaneously induced slightly higher, but not significant (P>0.05), SCE incidence than did constituent agent alone. Similarly, the combined treatment did not cause significant reductions in CPI and MI values. Furthermore, the two drugs can be considered as weak mutagens regardless of their mode of administration. However, it is suggested to undertake further experimentations, both *in vitro* and *in vivo* using various cellular systems and genetic parameters, to determine the potential risks of these drugs before their widespread release in the human market.

INTRODUCTION

In the last two decades, there has been an increasing number of people treated with anthelmintic drugs. The use of two drugs; albendazole (ABZ) and praziquantel (PZQ) has revolutionized the management of several veterinary and human helmintic infections.

Albendazole is used for the treatment of neurocysticercosis [1,2] and hydatidosis [3,4]. Praziquantel has been used for the therapy of schistosomiasis [5,6]. Furthermore, this antishistosomal drug has been proved to be a fast-acting drug on *Echinococcus granulosus* protoscolices [7]. Recently, a combination therapy of ABZ and PZQ has been reported to be better and much quicker compared to either drug alone in the treatment of hydatid disease [8,9] and neurocysticercosis [10,11].

Although ABZ and PZQ are almost free of side-effects when given for a short course, the safety of longer courses of chemotherapy has been questioned. Albendazole at a dose of 10 or 15 mg/kg did not cause severe effects in developing bovine embryos [12], but *in vitro* it was found to be potential developmental toxicant in rat embryo midbrain and limb bud cells [13]. More recently, dose-related embryolethality and growth reductions as well as morphologic alterations (mainly shape abnormalities and development of forelimb buds) have been observed in pregnant Sprague-Dawley rats treated with ABZ between gestational days 10 and 12 [14]. Moreover, ABZ elicited an accumulation of cultured differentiating rodent embryonic cells in the mitotic phase of the cell cycle [13]. In human, several side reactions such as hepatotoxicity, neutropenia and alopecia were reported in some patients given ABZ [15]. In cultured human hepatocytes and hepatoma cell lines; Hep G_2 , and Hep 3B, ABZ was shown to be cytotoxic and resulted in a rise in mitotic index due to blocking of cells at the prophase or at the metaphase stages [16]. Based on these studies, conservative use of courses of ABZ in pregnant women and in females of reproductive age has been suggested [17].

In case of PZQ, the situation is even more controversial [For review see 5 and 18]. No mutagenic effects were observed both in prokaryotes and eukaryotes [19]. Later investigations confirmed these findings and PZQ neither did significantly change the DNA content and the level of ploidy in hepatocytes of *Schistosoma mansoni* - infected mice [20] nor could induce transformations in Chinese hamster cells [21]. In man, PZQ did not significantly increase the frequency of micronuclei (MN) and chromosomal aberrations (CA) in peripheral blood lymphocytes from schistosomiasis patients [18]. In this context, PZQ did not induce mutations at the hprt locus in patients with neurocysticercosis [22].

In contrast, PZQ has been found to have a high affinity for DNA and to inhibit the *in vitro* RNA synthesis in isolated hamster liver nuclei [23]. In addition, PZQ was reported to be mutagenic using Ames assay [14]. It caused significant induction of chromosomal breaks in cysticercotic swine [25] and MN in the Syrian hamster embryo (SHE) cells [26]. In the latter study, there was no effect on cell survival and the unscheduled DNA synthesis (UDS) was not potentiated. However, in the same study, aneugenic activity of PZQ was demonstrated and suggested to be responsible for morphological transformation and cancer induction. In this regard, PZQ has been implicated as an etiological factor in the process of carcinogenesis associated with mice schistosomiasis infection [27]. Other studies have indicated that PZQ enhances the effects of mutagens and carcinogens in bacterial and mammalian cells [18].

Added to the above contradictions are the reports that the frequency of MN in urothelial cells was reduced significantly after treatment with PZQ [18]. Other investigations have found no potential for PZQ on nitrosamine - induced lesions in the Syrian hamster liver cells [28] and it was even effective at reducing hepatocellular nodule development [29].

The present work was carried out to have insight into one aspect of potential drawbacks of utilizing ABZ and PZQ in human populations for the control of helmintic diseases. For this purpose, the SCE assay, which is considered the most sensitive indicator of genetic toxicity, has been used in cultured human lymphocytes. In addition, two end-points; MI and CPI were evaluated under the regime of exposure of the lymphocytes.

MATERIALS AND METHODS

Chemicals

Albendazole (Andzol) was from Biofarma Ilac, Samandira, Istanbul, Turkey. Praziquantel was acquired from Shin Poong Pharm. Co. Seoul, Korea. Dimethylsulphoxide (DMSO; Purity > 99%) was obtained from Fisher Chemical Co. U.S.A. The test chemicals were dissolved in DMSO and diluted with saline (0.1 M phosphate pH 7.2; 0.1 M sodium chloride). Further dilutions were made in the culture medium according to the requirement of the experimental protocol. The volume of the medium was adjusted so that each test culture had the specified concentration (5, 10, 20, 40 or 100 ug/ml), either as separate compound or mixed (1:1), in 10 ml final volume per culture flask. The final concentration of DMSO did not exceed 0.1%. Untreated (negative control) cultures received DMSO plus saline on the same volumetric basis.

Lymphocyte cultures

Blood was obtained by venipuncture from three healthy male smokers (15-20 cigaretts a day) aging from 20 to 25 years. Whole blood cultures were set up by addition of 0.5 ml of heparinized blood to 9.5 ml of complete RPMI 1640 medium (Flow) supplemented with fetal bovine serum, antibiotics and antimycotics as well as hepes buffer as reported before [30]. Prior to lymphocyte stimulation by phytohemagglutinin (PHA-M, 3% v/v; Flow), bromodeoxyuridine (10 ug/ml; Fluka) was added. During the last 3h of the total culture period (72 h), colchicine was present at a final concentration of 0.5 ug/ml. In addition to the experimental cultures, one negative control, prepared as described above, and another positive control (1 h treatment with 0.5 ug/ml mitomycin C; MMC, Jenssen, Belgium) were established in parallel. All cultures were made in three replicates.

Chromosome preparation and cytogenetic analysis

Chromosome spreads were made by the air-drying method and stained using the fluorescence plus Giemsa (FPG) technique as indicated previously [30]. Blindly coded slides were analyzed as follows: 200 metaphases per culture were identified by their differentially stained chromatids (Figure 1) and classified into first , second , third or subsequent metaphase. Then, the CPI was calculated using the following formula: CPI = $(\%M_1 + 2 \times \%M_2 + 3 \times \%M_3)/100$. Twenty five well differentiated cells from each

104

culture were screened for SCE. Finally, the MI was found by the proportion of mitotic nuclei among 4000 cells per culture.

Statistical analysis

The SCE data were evaluated by ANOVA followed by the Dunnet's multiple comparison test [31]. The Chi-square analysis was used to compare the MI and CPI results. Differences with P < 0.05 were considered statistically significant.

RESULTS

Since the data within each test treatment were not widely dispersed, they were analyzed on the basis of their mean and standard error. Table (1) shows the distribution of SCE frequencies in lymphocytes from treated and control cultures. Although the results suggest a general trend towards an increase in SCE level in treated cultures, none of the ABZ- or PZQ-treated cultures (Figure 1B and C) showed significant SCE induction over the level obtained in the negative control (range from 6.50 ± 0.41 to 8.20 ± 0.37 and from 6.32 ± 0.35 to 7.69 ± 0.69 for ABZ and PZQ, respectively, versus a control average of 6.18 ± 0.44). Similarly, no significant elevations in the SCE frequency occurred when combined treatments were compared either with individual corresponding treatments or with the DMSO control ($P > 0.05$). On the other hand, 1 h exposure of lymphocytes to 0.5 ug/ml MMC (the positive control; figure 1D) resulted in a significantly higher SCE level (17.92 ± 0.71; $P < 0.01$) than the negative control.
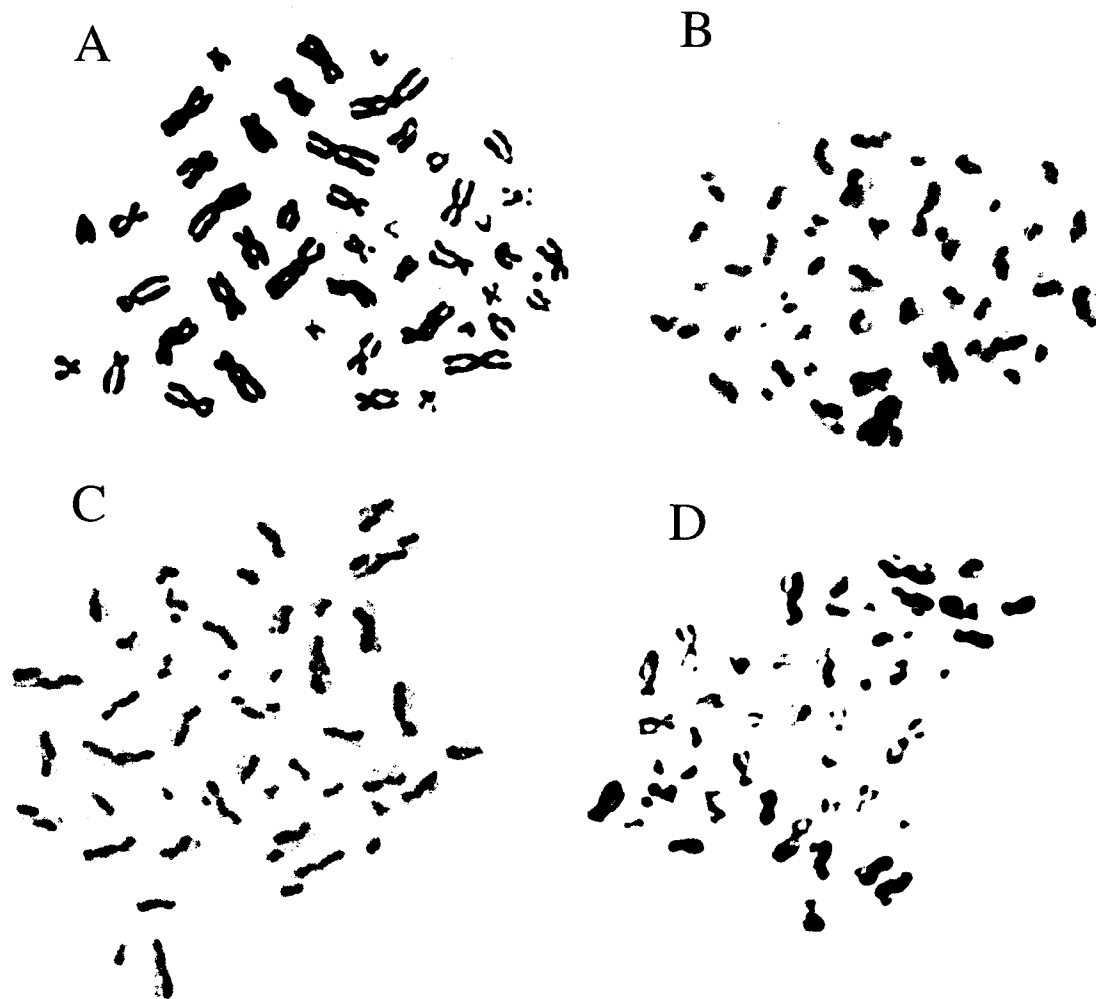

Figure 1. Photographs of FPG-stained human blood lymphocytes. A- First division metaphase (M_1); all chromosomes stained uniformly dark. B and C- second division metaphases (M_2); in each chromosome, one chromatid stained darkly and its sister-chromatid stained lightly. B- The cell was treated with 100 µg/ml ABZ, it shows about 10 SCE. C- This cell was treated with 0.5 µg/ml MMC, at least 17 SCE are scorable. D- Third division metaphase (M_3); roughly 50% of the chromosomes have differentially stained chromatids, the remaining chromosomes are stained uniformly light.

Table 1
TheFrequency of Sister-chromatid exchanges, Cell proliferation Index
and Mitotic Index in Cultures of Human Peripheral Blood Lymphocytes
treated with Albendazol, Praziquantel or their Combination
(For details see materials and methods)

Treatment	Concentration ug/ml per cell ± S.E.	Sister-chromatid Exchanges (SCE)	Cell Proliferation Index (CPI) ± S.E.	Mitotic Index (MI) ± S.E.
Albendazol (ABZ)	5	6.50±0.41	2.00±0.09	2.97±0.21
	10	6.90±0.82	1.84±0.05	2.76±0.09
	20	6.86±0.33	1.66±0.07	2.64±0.04
	40	7.33±0.55	1.73±0.09	2.52±0.09
	100	8.20±0.37	1.56±0.07	2.40±0.15
Praziqantel (PZQ)	5	6.32±0.35	2.02±0.05	3.00±0.10
	10	6.66±0.26	1.93±0.09	2.86±0.06
	20	6.40±0.16	1.75±0.03	2.68±0.10
	40	7.40±0.22	1.68±0.07	2.57±0.15
	100	7.69±0.69	1.64±0.04	2.56±0.20
ABZ±PZQ (1:1)	5	6.69±0.11	1.87±0.05	2.80±0.03
	10	6.88±0.05	1.80±0.05	2.60±0.10
	20	7.20±0.20	1.73±0.04	2.42±0.18
	40	7.57±0.10	1.64±0.07	2.34±0.11
	100	9.38±0.83	1.47±0.10	2.28±0.08
Mitomycin C (MMC)	0.5	17.92±0.71*	1.19±0.06*	1.03±0.13*
DMSO	-	6.18±0.44	2.22±0.10	3.25±0.10

* Significant at P < 0.5 using ANOVA followed by Dunnet's multiple comparison for SCE and Chi-square for CPI and MI data.

Furthermore, with the exception of the MMC cultures, the reductions in the CPI and MI values were not significant relative to the solvent (DMSO) treatment alone (Table 1). However, the depressions in these indices were greater, but not significant, when both ABZ and PZQ were applied together.

DISCUSSION

Though the exact mechanism(s) involved in the SCE genesis is still largely unknown, it is generally agreed that DNA lesions and their ongoing repair processes are associated with SCE initiation [32] and are related to the slowing of the cell cycle, most particularly in G_2-phase [33]. It is important to note that to determine whether an agent is able to induce SCE, two-fold increase in the SCE level over the control have to be detected in the experimental cultures [34]. Therefore, the data presented here suggest that both ABZ and PZQ are not clastogenic to cultured human lymphocytes taken from healthy individuals. The results from the current study confirm and extend the previous reports both *in vitro* [19,21] and *in vivo* [18,20] that PZQ is not genotoxic.

The low level of SCE which was observed in this investigation is consistent with the absence of UDS reported in SHE cells after treatment with PZQ [26] but not with significant induction of MN in the same study [26].

In addition, we have demonstrated that human lymphocytes cultured in the presence of PZQ alone had slightly lower SCE frequency than in case of ABZ. Together, ABZ and PZQ exhibited slightly higher incidence of SCE than did either drug alone. This finding could be related to the enhancement effect that has been reported *in vivo* when mice were exposed to benzene followed by PZQ treatment [18]. This effect may be relevant to the increase in cell membrane permeability by PZQ [35] to ABZ or its active metabolite; ABZ sulphoxide. Alternatively, PZQ may have modified metabolism of ABZ. Whether one or both of these mechanisms is (are) working has to be found out.

The cells incubated with PZQ and ABZ either separately or in combination had a slower rate of growth as evidenced by the reductions in the CPI and MI. The reduction in MI seems

contradictory to the ABZ-induced accumulation of cultured rodent embryonic cells [13] and human hepatoma cell lines [16] in the mitotic phase of the cell. However, the present findings are in keeping with the notion that slower lymphocyte proliferation kinetics have been detected in some patients with neurocysticercosis following PZQ therapy [36]. It is well known that cestodes are capable of depressing cellular response to mitogenes [37,38]. The in vitro response of lymphocytes from infected individuals to PHA may not be identical to that seen in the present study.

In conclusion, the data reported here may be somewhat preliminary, but provacative since they directly address at chromosomal level, for the first time, how a combined therapy of ABZ and PZQ may act. The magnitude of genotoxicity following chemotherapy is an important issue, thus it is imperative that further studies, with more appropriate human subjects, should be undertaken to unequivocally define the risk-benefits of the promising use of intermittent combined therapeutic treatment of ABZ and PZQ before the wide release of the two drugs in the human market to control systemic helmintic infections.

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