Cytotechnology (2011) 63:239–245
DOI 10.1007/s10616-011-9334-0

ORIGINAL RESEARCH

In vitro genotoxicity of rocuronium bromide in human peripheral lymphocytes

Umit Zan · Mehmet Topaktas · Erman Salih Istifli

Received: 14 June 2010 / Accepted: 3 January 2011 / Published online: 21 January 2011

Abstract Rocuronium bromide (RB), an aminosteroid type neuromuscular blocking agent, acts by reducing or inhibiting the depolarising effect of acetylcholine on the terminal disc of the muscle cell. To our knowledge, there is no adequate information on the genotoxic effects of RB, up to now. In the present study, possible genotoxic effects of RB have been determined by means of sister chromatid exchange (SCE), chromosome aberration (CA) and micronucleus (MN) analyses in human peripheral blood lymphocytes. The human peripheral blood lymphocytes were exposed to three different concentrations of RB (60, 80 and 100 μg/mL) for 24- and 48-h. In this study, RB increased the frequency of CAs, however, did not increase the frequency of SCEs. RB did not decrease the proliferation index (PI) and mitotic index (MI). Accordingly, RB increased the frequency of micronucleus (MN) but did not decrease the nuclear division index (NDI). Findings from this study suggest that rocuronium bromide is clastogenic but not cytotoxic to cultured human peripheral blood lymphocytes.

U. Zan · E. S. Istifli
Department of Biology, Institute of Basic and Applied Sciences, Çukurova University, Adana, Turkey

M. Topaktas (✉)
Department of Biology, Faculty of Science and Letters, Çukurova University, Adana, Turkey
e-mail: mtopaktas@cu.edu.tr

Keywords Rocuronium bromide · Human peripheral blood lymphocytes · SCE · CA · MN

Introduction

Rocuronium bromide (RB), a derivative of 3-hydroxy metabolite of vecuronium, is a steroidal non-depolarising aminosteroid neuromuscular blocking agent, which couples with the acetylcholine receptors to reduce or inhibit the depolarising effect of acetylcholine on the terminal disc of the muscle cell. A comprehensive literature search showed that RB has never been investigated based on its genotoxic-carcinogenic properties except the data from Physician's Desk Reference which demonstrates negative results for the genotoxicity and mutagenicity of RB (Synder and Green 2001; Synder et al. 2004; Synder 2009). The Norwegian Medicines Agency proposed that RB be withdrawn from routine practice due to frequent reports of anaphylaxis (Laake and Røttingen 2001), and 41 RB-related anaphylactic incidents were reported in France in a survey between 1994 and 1996 (Heier and Guttormsen 2000). Similar anaphylactic and bronchospasm incidents were described in the literature related to the use of other aminosteroid type muscle relaxants (Mishima and Yamamura 1984; O'Callaghan et al. 1986; Farrell et al. 1988; Treuren and Buckley 1990; Kumar et al. 1993; Baird and Futter 1996; Clendenen

et al. 1997; Toh et al. 1999). On the other hand, RB has been demonstrated to have low developmental toxicity in cultured rat embryos (Karabulut et al. 2004). Among the various adverse reactions that a drug may cause, the occurence of genotoxic-carcinogenic effects can not be excluded (Brambilla and Martelli 2009). Chromosome aberrations, both structural and numerical, have been associated with human health (Natarajan 2002). Therefore, standard genotoxicity testing should be performed to identify the potential genotoxic/carcinogenic properties of chemical agents. For this purpose, human peripheral blood lymphocyte is an extremely sensitive indicator of both in vivo (Evans et al. 1979; Evans 1982) and in vitro (Evans 1970; Buckton and Evans 1973) induced chromosome structural and numerical change.

In this study, genotoxic effects of RB have been investigated in order to extend the genotoxicity information on this widely used muscle relaxant by means of sister chromatid exchange (SCE), chromosome aberration (CA) and micronucleus (MN) test systems in human peripheral blood lymphocytes.

Materials and methods

RB (Organon, USA) was used as the test substance for the in vitro assays. The properties of RB are shown as follows:

CAS number: 119302-91-9

Linear Formula: $C_{30}H_{49}BrN_2O_4$

Molecular Weight: 609.69

Rocuronium bromide (RB), in its liquid formulation, was used as the test substance. Test concentrations (60, 80 and 100 μg/mL) were roughly 25–40 times higher than plasma levels achieved in patients under surgery.

The methods of Evans (1984) and Perry and Thomson (1984) were followed for preparation of the CA and the SCE tests with minor modifications. This study was conducted according to the IPCS guidelines (Albertini et al. 2000). Whole blood (0.2 mL) from four healthy donors (two male, age: 18, 20 and two female, age: 19, 20, non-smokers, blood samples not pooled), was added to 2.5 mL chromosome medium B (Biochrom AG, F 5023) supplemented with 10 μg/mL bromodeoxyuridine (Sigma, St. Louis, MO, B5002). Cultures were incubated at 37 °C for 72 h. The cells were treated with 60, 80 and 100 μg/mL concentrations of RB for 24 h (RB was added 48 h after initiating the culture) and 48 h (RB was added 24 h after initiating the culture). A negative control and a positive control (Mitomycin-C (MMC), 0.25 μg/mL, Sigma M-05030) were also used. The cells were exposed to colchicine (0.06 μg/mL, Sigma C9754) 2 h before harvesting. The cells were treated with 0.4% KCl (37 °C) as the hypotonic solution and methanol: glacial acetic acid (3:1) as the fixative (at room temperature 22 ± 1 °C, fixative treatments were repeated three times). The staining of the air-dried slides was performed following the standard methods using 5% Giemsa stain for CA and the modified fluorescence plus Giemsa method for SCE (Speit and Haupter 1985). The slides were irradiated with 30 W, 254 nm UV lamp at 15 cm distance in Sorensen buffer for 30 min, then incubated with 1 × SSC (standard saline citrate) at 60 °C for 50 min and stained with 5% Giemsa prepared with Sorensen buffer. The number of CAs was obtained by calculating the percentage of the metaphases from each concentration and treatment period that showed structural and/or numerical alterations. The CA was classified according to the ISCN (International System for Human Cytogenetic Nomenclature) (Paz-y-Miño et al. 2002). CAs were evaluated in 100 well spread metaphases per donor (totally 400 metaphases per concentration). Gaps were not evaluated as CA according to Mace et al. (1978). CAs were classified as structural aberrations. Structural CAs consisted of the chromatid type (breaks and exchanges) and the chromosome type (breaks, fragments, sister unions, dicentrics) abnormalities. Only the structural CAs were taken into consideration in order to determine the genotoxicity. The scoring of SCE was carried out according to the IPCS guidelines (Albertini et al. 2000). To score SCE, a total of 100 s division metaphases (25 cells per sample) were analyzed. The results were used to determine the mean number of SCE (SCE/cell). In addition, a total of 400 cells (100 cells from each donor) were scored for the proliferation index (PI). The PI was calculated according to the formula as follows: $PI = (M1 \times 1) + (M2 \times 2) + (M3 \times 3)/$ total scored cells. M1, M2, and M3 are the fraction of cells undergoing the first, second, and third mitosis during 72-h cell culture times. The MI was also determined by scoring 3,000 cells per concentration.

For the analysis of MN in binucleated lymphocytes, 0.2 mL of fresh blood was used to establish the cultures and the cultures were incubated for 68 h. The cells were treated with 60, 80 and 100 μg/mL concentrations of RB for 24- and 48-h treatment periods. Cytochalasin B (Sigma, C6762) was added at 44 h of the incubation to a final concentration of 6 μg/mL to block cytokinesis. After an additional 24-h incubation at 37 °C, cells were harvested by centrifugation and processed for the MN test in peripheral blood lymphocytes (Rothfuss et al. 2000; Kirsch-Volders et al. 2003). In all subjects, 2,000 binucleated lymphocytes were scored from each donor (8,000 binucleated cells were scored per concentration). A total of 1,000 cells were scored to determine the frequency of the cells with 1, 2, 3, or 4 nuclei and to calculate the nuclear division index (NDI) for the cytotoxicity of agents using the formula: NDI = (M1 + (2 × M2) + (3 × M3) + (4 × M4))/N, where M1–M4 represent the number of cells with one to four nuclei and N is the total number of the cells scored (Fenech 2000).

T-test was utilized for establishing the statistical significance of all parameters. SCE, CA, PI, MI, micronucleated binuclear (MNBN) cell and NDI data obtained from microscopic analyses were compared to the corresponding negative and positive control groups. Concentration–response relationships were determined from the correlation and regression coefficients for the percentage of cells with CA, as well as for the mean SCE, PI, MI, NDI and micronucleated binuclear (MNBN) cell.

Results

The human peripheral lymphocytes were treated with 60, 80 and 100 μg/mL of RB dissolved in sterile bidistilled water, for 24- and 48-h treatment periods to investigate the effects of RB on SCEs and CAs. Data of structural chromosomal aberrations induced by RB are presented in Table 1. Following treatment with RB, a statistically significant increase in CA frequency was observed in both 24- and 48-h treated lymphocyte cultures (Table 1). Chromatid type breaks were the most common abnormalities.

No statistically significant increases were obtained in the SCE frequency after 24- and 48-h treatment with RB (Table 2).

RB induced statistically significant MNBN cell formation in comparison to the negative control for 24- and 48-h treatment periods. However, the increase was not dose dependent ($p > 0.05$) (Table 3).

Cytotoxic effect of RB was analyzed by investigating the NDI, MI and PI parameters. Treatment of peripheral blood lymphocytes with various concentrations of RB did not lead to a significant alteration in the NDI, for both 24- and 48-h treatment periods (Table 3). Furthermore, there were no significant

Table 1 Effect of rocuronium treatment on CA in human peripheral blood lymphocytes for 24- and 48-h treatment periods

Test substance	Treatment		Structural CA		Percentage of cells with structural aberrations ±SE
	Time (h)	Conc. (μg/mL)	Chromatid type	Chromosome type	
Neg. Control	–	–	19	3	4.50 ± 0.86
MMC	24	0.25	63	42	26.25 ± 1.25
Rocuronium	24	60	19	6	6.25 ± 0.75a_1b_3
Rocuronium	24	80	25	6	7.75 ± 1.43a_1b_3
Rocuronium	24	100	43	4	11.75 ± 2.12a_1b_2
MMC	48	0.25	91	63	38.50 ± 6.73
Rocuronium	48	60	38	6	11.00 ± 2.27a_1b_3
Rocuronium	48	80	30	3	8.25 ± 2.46b_3
Rocuronium	48	100	36	7	10.75 ± 2.56a_1b_2

Data are expressed as the mean values (±S.E) obtained from four donors; $n = 4$

a significant from negative control; *b* significant from positive control

a_1b_1 $p \leq 0.05$, a_2b_2 $p \leq 0.01$, a_3b_3 $p \leq 0.001$

Table 2 Mean SCE values in human peripheral blood lymphocytes treated with rocuronium for 24- and 48-h

Test substance	Treatment (h)	Concentration (μg/mL)	Min–Max SCE	SCE/Cell ± SE
Neg. Control	–	–	1–17	8.58 ± 0.51
MMC	24	0.25	6–64	30.5 ± 3.30
Rocuronium	24	60	4–27	9.25 ± 1.09b_3
Rocuronium	24	80	2–26	8.77 ± 0.09b_3
Rocuronium	24	100	4–16	9.18 ± 0.29b_3
MMC	48	0.25	4–104	48.43 ± 7.34
Rocuronium	48	60	4–18	8.73 ± 0.58b_3
Rocuronium	48	80	3–18	8.42 ± 0.43b_3
Rocuronium	48	100	4–20	8.60 ± 0.08b_3

Data are expressed as the mean values (± S.E) obtained from four donors; $n = 4$

a significant from negative control, *b* significant from positive control

a_1b_1 $p \leq 0.05$, a_2b_2 $p \leq 0.01$, a_3b_3 $p \leq 0.001$

alterations in both the MI and PI after 24- and 48-h treatment periods (Table 4).

Discussion

In this study, the treatment of peripheral blood lymphocytes with various concentrations of RB led to a statistically significant increase in the CA frequency. In contrast, treatment of peripheral blood lymphocytes with diazepam and propofol did not lead to a marked increase in the CA frequency as reported by Karahalil et al. (2005). One reason for the different results can be attributed to the difference of the classes of the anesthetics used in those studies, and the other one can be the test platform which is in vivo in the study of Karahalil et al. (2005). However, an in vitro study demonstrated that sodium thiopental could increase the CA frequency by two-fold when compared to the negative control (Klimova and Paskevich 1984). Similar in vitro increments of the CA frequency in case of diazepam and hexenal were reported by various researchers (Klimova and Paskevich 1984; Huong et al. 1988). SCE frequency after RB treatment was not found to be statistically significant, leading to the conclusion that the SCE-inducing lesions generated by RB in DNA are efficiently repaired before replication. Ishii and Bender (1980) have previously argued that various inhibitors of replication induce SCEs. Thus, it could be proposed that RB is not an inhibitor of replication. Statistically insignificant increase of SCE frequency in human peripheral blood lymphocytes has been also shown for diazepam at a concentration of 0.2 mg/kg (Husum et al. 1985).

In this study, increased MNBN cell frequencies were observed after RB treatment for 24- and 48-h. Furthermore, induced formation of structural CAs was also detected after treatment with RB for 24- and 48-h treatment periods. Thus, it could be inferred that the resulting structural CAs are playing a major role in the formation of micronuclei after RB treatment. Diazepam-induced MN formation was also shown in mice bone-marrow cells in vivo (Leal Garza et al. 1998). In addition, multipolar anaphase-inducing capability (Topaktaş and Rencüzoğulları 1995) of RB should be responsible for the relatively large micronuclei formation that we encountered in this study.

24- and 48-h exposure to RB did not significantly decrease the PI, MI, NDI, as evidenced by the non-toxic behaviour of the employed concentrations. Interestingly, RB increased the CA frequency without decreasing the MI, PI and NDI. We further performed a thorough analysis of the literature in order to find similar reports which may support our finding on the induction of CA without cytotoxicity. Olivier et al. (1998) noted that *p53* wild type tumor cells tolerated unhealed chromosomal breaks and continued cycling after genotoxic insult, suggesting a relaxed/impaired cell-cycle control. In addition to this, etoposide has been shown to induce significant increases in %MN at concentrations >0.01 mg/mL without cytotoxicity (Lynch et al. 2003). Researchers concluded that there was a threshold point between 0.005 and 0.001 mg/

Table 3 The percentage of micronucleated binuclear cell and nuclear division index (NDI) in cultured human peripheral blood lymphocytes treated with rocuronium for 24- and 48-h

Test substance	Treatment		Percentage of micronucleated binuclear cell ± SE	NDI ± SE
	Time (h)	Concentration (μg/mL)		
Neg. Control	–	–	0.35 ± 0.06	1.37 ± 0.12
MMC	24	0.25	1.45 ± 0.19	1.43 ± 0.08
Rocuronium	24	60	0.60 ± 0.04a_2b_3	1.39 ± 0.09
Rocuronium	24	80	0.60 ± 0.04a_2b_3	1.29 ± 0.07
Rocuronium	24	100	0.72 ± 0.09a_1b_2	1.30 ± 0.09
MMC	48	0.25	2.22 ± 0.13	1.30 ± 0.04
Rocuronium	48	60	0.62 ± 0.04a_2b_3	1.42 ± 0.04
Rocuronium	48	80	0.75 ± 0.02a_3b_3	1.46 ± 0.06
Rocuronium	48	100	0.82 ± 0.06a_2b_3	1.46 ± 0.05

Data are expressed as the mean values (±S.E) obtained from four donors; $n = 4$

a significant from negative control, *b* significant from positive control

a_1b_1 $p \leq 0.05$, a_2b_2 $p \leq 0.01$, a_3b_3 $p \leq 0.001$

Table 4 Proliferation index (PI) and mitotic index (MI) in human peripheral blood lymphocytes treated with rocuronium for 24- and 48-h

Test substance	Treatment		M1	M2	M3	PI ± SE	MI ± SE
	Time (h)	Concentration (μg/mL)					
Neg. Control	–	–	62	116	222	2.40 ± 0.12	4.06 ± 0.53
MMC	24	0.25	152	116	132	1.95 ± 0.14	2.70 ± 0.35
Rocuronium	24	60	106	115	179	2.18 ± 0.21	4.60 ± 1.08
Rocuronium	24	80	80	114	206	2.31 ± 0.15	4.32 ± 1.25
Rocuronium	24	100	103	104	193	2.22 ± 0.26	4.33 ± 0.95
MMC	48	0.25	217	110	73	1.64 ± 0.03	2.11 ± 0.27
Rocuronium	48	60	50	113	237	2.46 ± 0.07b_3	5.03 ± 0.97b_1
Rocuronium	48	80	55	112	233	2.44 ± 0.12b_2	4.94 ± 0.50b_1
Rocuronium	48	100	66	104	230	2.41 ± 0.09b_2	4.69 ± 0.59b_1

Data are expressed as the mean values (±S.E) obtained from four donors; $n = 4$

a significant from negative control, *b* significant from positive control

M1 first mitosis, *M2* mitosis, *M3* third mitosis

a_1b_1 $p \leq 0.05$, a_2b_2 $p \leq 0.01$, a_3b_3 $p \leq 0.001$

mL, where a clear cytotoxicity was gained at concentrations >0.00236 mg/mL. Based on these two findings, it may be proposed that RB may interfere with the normal cell cycle of human lymphocytes, or may have a threshold-like cytotoxicity which could have been detected if higher concentrations were employed.

The top concentration used in this study did not lead to a cytotoxic manifestation. It has been pointed out that the chromosome-damaging properties of various compounds are often associated with high toxicity (Müller and Sofuni 2000; Galloway 2000). Thus, cytotoxicity of RB should not be responsible for the genotoxic effects detected in this study.

There is no precise information on the genotoxic features of RB, however, the present study indicates that RB is capable of causing genotoxicity via clastogenic effects at concentrations at which a significant cytotoxic effect does not occur. Further analysis need to be performed to clarify whether this agent has a threshold-like cytotoxicity in vitro and in vivo.

Acknowledgments This study was supported by the grant from Cukurova University research fund (FEF2006YL28).

References

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice R, Waters MD, Aitio A (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res 463:111–172

Baird M, Futter M (1996) Anaphylaxis to mivacurium. Anaesth Intensive Care 42:486–488

Brambilla G, Martelli A (2009) Update on genotoxicity and carcinogenicity testing of 472 marketed pharmaceuticals. Mutat Res 681:209–229

Buckton KE, Evans HJ (eds) (1973) Methods for the Analysis of Human Chromosome Aberrations. World Health Organisation, Geneva

Clendenen S, Harper J, Wharen R, Guarderas J (1997) Anaphylactic reaction after cisatracurium. Anesthesiology 87:690–692

Evans HJ (1970) Population cytogenetics and environmental factors. In: Jacobs PA, Price WH, Law P (eds) Pfizer Medical Monographs, vol 5. Edinburgh University Press, Edinburgh, pp 192–216

Evans HJ (1982) Chromosomal mutations in human populations. Cytogenet Cell Genet 33:48–56

Evans HJ (1984) Human Peripheral Blood Lymphocytes for the Analysis of Chromosome Aberrations in Mutagen Tests. In: Kilbey BJ, Legator M, Nichols W, Ramel C (eds) Handbook of mutagenicity test procedures, 2nd ed. Elsevier Science Publishers BV, Amsterdam, pp 405–427

Evans HJ, Buckton KE, Hamilton GE, Carothers A (1979) Radiation-induced chromosome aberrations in nuclear-dockyard workers. Nature 277:531–534

Farrell A, Gowland G, McDowell J, Simpson K, Watkins J (1988) Anaphylactoid reaction to vecuronium followed by systemic reaction to skin testing. Anaesthesia 43:207–209

Fenech M (2000) The in vitro micronucleus technique. Mutat Res 455:81–95

Galloway SM (2000) Cytotoxicity and chromosome aberrations in vitro: Experience in industry and the case for an upper limit on toxicity in the aberration assay. Environ Mol Mutagen 35:191–201

Heier T, Guttormsen AB (2000) Anaphylactic reactions during induction of anaesthesia using rocuronium for muscle relaxation: A report including 3 cases. Acta Anaesthesiol Scand 44:775–781

Huong TT, Szentesi I, Czeizel A (1988) Lower prevalence of chromosome aberrations and SCEs in self-poisoned pregnant women. Mutat Res 198:255–259

Husum B, Wulf HC, Niebuhr E, Rasmussen JA (1985) SCE in lymphocytes of patients treated with single, large doses of diazepam. Mutat Res 155:71–73

Ishii Y, Bender MA (1980) Effects of inhibitors of DNA synthesis on spontaneous and ultraviolet light-induced sister chromatid exchanges in Chinese hamster cells. Mutat Res 79:19–32

Karabulut AK, Reisli R, Uysal II, Çelik JB, Ziylan T (2004) An investigation of non-depolarizing muscle relaxants on embryonic development in cultured rat embryos. Eur J Anaesthesiol 21:715–724

Karahalil B, Yağar S, Bahadır G, Durak P, Sardaş S (2005) Diazepam and propofol used as anesthetics during open-heart surgery do not cause chromosomal aberrations in peripheral blood lymphocytes. Mutat Res 581:181–186

Kirsch-Volders M, Sofuni T, Aardemac M, Albertini S, Eastmond D, Fenech M, Ishidate M Jr, Kirchner S, Lorge E, Morita T, Norppa H, Surralles J, Vanhauwaert A, Wakata A (2003) Report from the in vitro micronucleus assay working group. Mutat Res 540:153–163

Klimova EM, Paskevich IF (1984) Cytogenetic effect of barbiturate anesthetizing substances in a human peripheral blood leukocyte culture. Tsitol Genet 18:13–17

Kumar A, Thys J, Van Aken H, Stevens E, Grul J (1993) Severe anaphylactic shock after atracurium. Anesth Analg 76:423–425

Laake JH, Røttingen JA (2001) Rocuronium and anaphylaxis–a statistical challenge. Acta Anaesthesiol Scand 45:1196–1203

Leal Garza CH, Valenciano Cedillo GG, Rojas Alvarado MA, Cortes Gutierrez EI (1998) Mutagenic activity of diazepam evaluated by in vivo cytogenetic tests. Arch Med Res 29:285–289

Lynch A, Harvey J, Aylott M, Nicholas E, Burman M, Siddiqui A, Walker S, Rees R (2003) Investigations into the concept of a threshold for topoisomerase inhibitor-induced clastogenicity. Mutagenesis 18(4):345–353

Mace ML Jr, Daskal Y, Wray W (1978) Scanning electron microscopy of chromosome aberrations. Mutat Res 52: 199–206

Mishima S, Yamamura T (1984) Anaphylactoid reaction to pancuronium. Anesth Analg 63:865–866

Müller L, Sofuni T (2000) Appropriate levels of cytotoxicity for genotoxicity tests using mammalian cells in vitro. Environ Mol Mutagen 35:202–205

Natarajan AT (2002) Chromosome aberrations: past, present and future. Mutat Res 504:3–16

O'Callaghan A, Scadding G, Watkins J (1986) Bronchospasm following the use of vecuronium. Anaesthesia 41:940–942

Olivier M, Bautista S, Vallès H, Theillet C (1998) Relaxed cell-cycle arrests and propagation of unrepaired chromosomal damage in cancer cell lines with wild-type p53. Mol Carcinog 23:1–12

Paz-y-Miño C, Bustamante G, Sánchez ME, Leone PE (2002) Cytogenetic monitoring in a population occupationally exposed to pesticide in Ecuador. Environ Health Perspective 110:1077–1080

Perry PE, Thomson EJ (1984) The methodology of sister chromatid exchanges. In: Kilbey BJ, Legator M, Nichols W, Ramel C (eds) Handbook of Mutagenicity Test Procedures, 2nd ed. Elsevier, Amsterdam, pp 459–529

Rothfuss A, Schütz P, Bochum S, Volm T, Eberhardt E, Kreirenberg R, Vogel W, Speit G (2000) Induced micronucleus frequencies in peripheral lymphocytes as a screening test for carriers of a BRCA1 mutation in breast cancer families. Cancer Res 60:390–394

Speit G, Haupter S (1985) On the mechanisms of differential giemsa staining of bromodeoxyuridine-substituted chromosomes. II. Differences between the demonstration of sister chromatid differentiation and replication patterns. Human Genet 70:126–129

Synder RD (2009) An Update on the Genotoxicity and Carcinogenicity of Marketed Pharmaceuticals with Reference to In Silico Predictivity. Environ Mol Mutagen 50:435–450

Synder RD, Green JW (2001) A review of the genotoxicity of marketed pharmaceuticals. Mutat Res 488:151–169

Synder RD, Pearl GS, Mandakas G, Choy WN, Goodsaid F, Rosenblum IY (2004) Assessment of the Sensitivity of the Computational Programs DEREK, TOPKAT, and MCASE in the Prediction of the Genotoxicity of Pharmaceutical Molecules. Environ Mol Mutagen 43:143–158

Toh K, Deacock S, Fawcett W (1999) Severe anaphylactic reaction to cisatracurium. Anesth Analg 88:462–464

Topaktaş M, Rencüzoğulları E (1995) Cytogenetics. Cukurova University, Faculty of Science and Letters, Department of Biology. p. 182

Treuren B, Buckley D (1990) Anaphylactoid reaction to vecuronium. Br J Anaesth 64:125–126