

Trapidil, an Inhibitor for Phosphodiesterase and Platelet-Derived-Growth Factor, Ameliorates Corrosive Esophageal Burn in Rats

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— Corrosive esophageal burn is a common health problem in the pediatric age group and causes serious esophageal injuries. The medical treatment in acute phase of corrosive esophageal injury is of particular importance for prevention of esophageal stricture. We therefore aimed to investigate the possible beneficial effect of trapidil (triazolopyrimidine), an inhibitor for phosphodiesterase and platelet-derived-growth-factor, during acute phase of esophageal corrosive injury. Wistar albino rats were randomly allocated to untreated, treated, and sham-operated groups ($n = 10$ for each group). Corrosive esophageal burn was generated with 10% NaOH solution. The rats were left untreated (untreated group) or treated with trapidil as a single dose of 40 mg/kg intraperitoneally after one hour of the injury (treated group). Abdominal esophageal segment was isolated and tied in sham-control group. The studied esophageal segment was removed from each animal after 24 hours. Malondialdehyde (MDA) and nitric oxide (NO) levels were measured in the esophageal tissues. The ulcer depth was graded by histopathologic examination. MDA and NO levels were significantly higher in the untreated group than in the treated group. Namely, trapidil treatment significantly decreased MDA and NO levels in the injured tissues, the levels of which are similar to those in the tissues of control animals. The grades of ulcer depth were significantly improved in the treated group. These results indicate that the reactive oxygen radicals increase in the early phase of corrosive esophagitis and cause tissue damage. We suggest that trapidil treatment may be useful in acute phase of corrosive esophageal injury.

— corrosive esophagitis; free oxygen radicals; trapidil; triazolopyrimidine

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Infants and young children often accidentally ingest corrosive substances. Ingestion of corrosive substance is a common health problem in the pediatric age group. Accidental ingestion of cor-

rosive substances leads to serious upper aerodigestive and esophageal injuries. Corrosive esophageal burns are the most common cause of esophageal stricture development (Haller et al.

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1971). Corrosive esophageal burns are caused by alkali in 90% of cases and by acid substances in 10%. Esophageal injury is observed in 24% of children who have ingested alkali substances, and serious esophageal stricture can be seen in 12% to 35% of these children (Bautista et al. 1996).

Development of esophageal stricture is related with the severity of the initial injury. Thus, initial treatment plays an important role in corrosive esophagitis (Capella et al. 1992). Acute necrotic phase is seen at the injury region in the first 1-4 days (Rothstein et al. 1986). It has been known that post-ischemic tissue damage appears to be caused by formation of reactive oxygen radicals (Miller et al. 1988). Reactive oxygen radicals have been found to be increased in early phase of corrosive esophagitis in a rat model (Gunel et al. 1999).

Although several drugs and enzymes have been used for treatment of corrosive esophagitis and prevention of esophageal strictures in experimental studies, trapidil has not been used previously. Trapidil (5-methyl-7-diethylamino-s-triazolopyrimidine), an inhibitor for phosphodiesterase and platelet-derived-growth-factor, was originally developed as an antianginal drug. Trapidil has effects of nitroglycerin like vasodilatation, inhibition of platelet aggregation via blockage of thromboxane A₂ (TXA₂) and reduction of lipid peroxidation, interleukin 6 and 12 (IL-6 and IL-12) and procoagulant activity by inhibiting the CD 40 pathway in monocytes (Heinroth-Hoffman et al. 1987; Neider et al. 1995; Block et al. 1998; Zhon et al. 1999).

We investigated the beneficial effects of trapidil and explored the involvement of free oxygen radicals in the acute phase of corrosive esophageal injury in a rat model.

MATERIALS AND METHODS

Wistar albino rats (body weights 190-210 g) were used. The animals were housed in a temperature and light controlled room with ad libitum access to water and rat chow. All animals received humane care in compliance with the European Convention on Animal Care. The study was approved by the Kırıkkale University School of Medicine Ethical Committee. All surgical pro-

cedures were performed under general anesthesia by 40 mg/kg Ketamine HCl (Ketalar, Eczacıbaşı, Istanbul, Turkey) intramuscularly. Animals were divided into 3 groups, each containing 10 rats. Standard esophageal corrosive burn was generated in the untreated and treated groups, as described by Gehanno and Guedon (1981). Median laparotomy incision was performed, and a 1.5-cm abdominal esophageal segment was isolated and tied both upper and lower point with 2-0 chromic catgut suture in all groups. One ml of 10% NaOH solution was instilled through the isolated segment via 24 F intravenous cannule for 3 min, then esophagus was rinsed for 1 min with distilled water in the untreated and treated groups.

In the treated group ($n = 10$), corrosive esophageal burn was produced and then trapidil (Rocarnal, Rentschler-UCB GmbH, Kerpen, Germany) was administered in a single dose of 40 mg/kg intraperitoneally after one hour, as described previously (Gocer et al. 2001). In sham control group ($n = 10$), median laparotomy incision was performed and 1.5-cm abdominal esophagus segment was isolated and tied both upper and lower point with 2-0 chromic catgut suture.

All animals were harvested after 24 hours surgical procedure and the studied 1.5 cm abdominal esophageal segment was removed from each animal in all groups and then specimens were prepared for biochemical analysis and histopathologic study. All animals could be evaluated at the end of the experiment.

Biochemical analysis

Esophageal tissue samples were stored at -70°C for biochemical study. The frozen tissue samples of esophagus were homogenized (Art-Micra D-8, Menheim, Germany) in 2 ml 0.02 M EDTA. Samples were kept in an ice bath during the study and centrifuged (5,000 g for 10 min) after homogenization. The supernatant was used for all the analysis. The protein content of the supernatant was measured by using urinary/csf protein kit (Olympus, Tokyo) in Olympus AU 800 autoanalyzer. Shimadzu UV-1601 spectrophotometer (Auburn, Austria) was used for measurement.

Malondialdehyde assay

Malondialdehyde (MDA) levels were measured as an indicator of free radical generation. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA (Yagi et al. 1976). Results were calcu-

lated as nanomoles per gram (nmol/g) protein.

Nitric oxide assay

Nitrate and nitrite were measured as oxidized stable end products of nitric oxide (NO). Total nitrite level in the sample was determined by Griess reaction after nitrate was reduced to nitrite by Vanadium Cl_3 (VCl_3) (Miranda et al. 2001). Results were calculated as μmoles per gram ($\mu\text{mol/g}$) protein.

Histopathologic study

Tissue samples were inflated and fixed with 10% formalin. Then tissue samples were embedded in paraffin. Tissues were sectioned in $4\text{ }\mu\text{m}$ pieces and stained with routine hematoxyline-eosine stain. The specimens were examined by same pathologist who was blind to the study. Depth of the ulcer and polymorphonuclear leukocyte (pmn) infiltration were evaluated. The injury was graded according to the depth of the ulcer as grade 0 (no ulcer); grade I (ulcer involving only the epithelium); grade II (ulcer involving epithelium and muscularis mucosa); grade III (ulcer proceeding to submucosa); and grade IV (ulcer proceeding to inner circular muscular layer and full thickness ulcer development) (Ozel et al. 2004).

Statistical study

Kruskal-Wallis test was used to detect differentiation among groups. Mann Whitney's U-test was used to detect differentiation between two groups. A p value of less than 0.05 was considered as statistically significant. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, version 12.0, Chicago, IL, USA).

RESULTS

MDA levels (nmol/g protein) and NO levels ($\mu\text{mol/g}$ protein) in esophagus tissues of the trapidil-treated and untreated rats are summarized together with the levels in sham control esophagus tissues (Table 1). The MDA level in the untreated group was significantly higher than in the treated and sham control groups ($p < 0.001$ and $p < 0.05$, respectively). There was no significant difference in MDA levels between the treated and sham control groups ($p > 0.05$). Likewise, the NO level in untreated group was significantly higher than treated and sham control groups ($p < 0.05$). Again, no significant difference was found in NO levels between the treated and sham control groups ($p > 0.05$).

Histopathologic examinations revealed that grade of ulcer increase in the injured esophagus in untreated group (Fig. 1) and decrease by trapidil in treated group (Fig. 2). Histopathologic examinations revealed the increased PMN infiltration in injured esophagus of untreated group (Fig. 3) and the decreased PMN infiltration by trapidil in treated group.

The results of histopathologic examination are summarized in Table 2. The grades of ulcer depth were significantly higher in the untreated group than the treated group ($p < 0.05$). PMN infiltration was 60% (6/10) in the untreated group and 20% (2/10) in the treated group.

TABLE 1. The levels of MDA and NO in the untreated, treated and sham control groups (Means $\pm$ S.E.M.)

Groups	MDA (nmol/g protein) (Means $\pm$ S.E.M.)	NO ($\mu\text{mol/g}$ protein) (Means $\pm$ S.E.M.)
Untreated group	2.32 $\pm$ 0.05*	5.52 $\pm$ 0.18*
Treated group	1.50 $\pm$ 0.15**	3.32 $\pm$ 0.47**
Sham control group	1.78 $\pm$ 0.18#	3.10 $\pm$ 0.58#

/ $p < 0.001$ for MDA.

*/# $p < 0.05$ for MDA.

/ and */# $p < 0.05$ for NO.

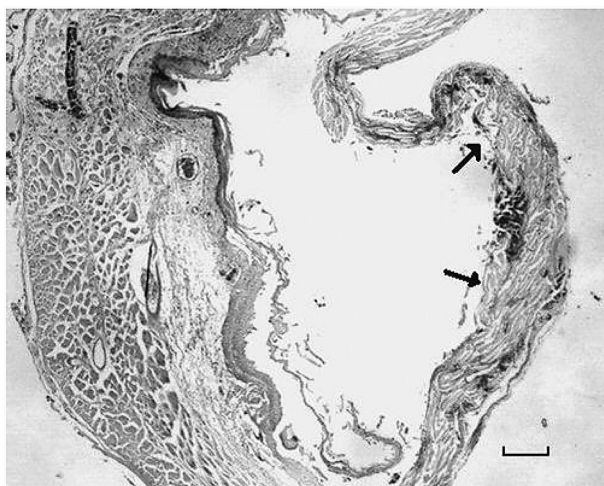


Fig. 1. The view of deep ulceration in untreated group in histopathologic examination. Arrows shows deep ulceration (HE $\times 40$). Scale bar is 10 μm .

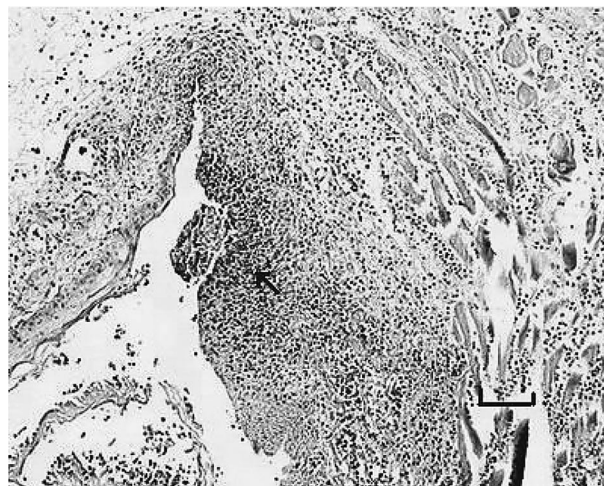


Fig. 3. The view of intensive PMN infiltration in injured esophagus in the untreated group. Arrow shows intensive PMN infiltration (HE $\times 100$). Scale bar is 100 μm .

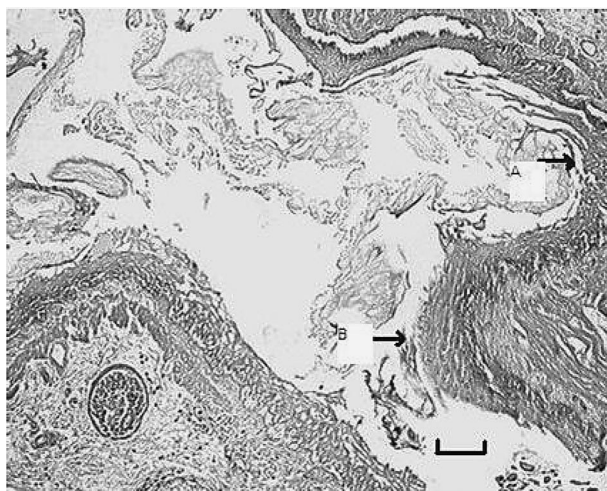


Fig. 2. The view of superficial ulceration and only epithelial necrosis in the treated group in histopathologic examination. Arrow A shows superficial ulceration. Arrow B shows only epithelial necrosis (HE $\times 100$). Scale bar is 10 μm .

TABLE 2. *The grades of ulcer depth in the untreated, treated and sham control groups*

Groups	Ulcer depth (Means $\pm$ S.E.M.)
Untreated group	2.50 $\pm$ 0.27*
Treated group	0.40 $\pm$ 0.15**
Sham control group	No ulcer

* / ** $p < 0.05$ for grades of ulcer depth.

DISCUSSION

Chemical substance ingestion may result in corrosive esophagitis. Corrosive esophageal injury depends on concentration and nature of the substance, the amount of the swallowed substance and the contact time (Day 1989). Since alkali substances penetrate deeply into the muscle layers

by liquefaction necrosis, they are highly destructive (Aschcraft and Padula 1974). Acute necrotic phase is seen over the first 1 to 4 days (Rothstein 1986) and is characterized by decreasing the perfusion of the tissue and increasing the breakdown of cellular membranes by lipid peroxidation and hydrolysis at the injury site (Cadranet et al. 1993). Hydroxyl radicals directly react with polyunsaturated fatty acids and lead to lipid peroxidation with the cell membranes. Reactive oxygen radicals indirectly trigger the accumulation of neutrophils within the affected tissue initiating an inflammatory process and lead to severe mucosal damage (Günel et al. 1999).

Experimental studies on corrosive esophagitis are generally performed to investigate the late-

term stricture development. Corticosteroids and pentoxifylline were previously used in experimental and clinical studies to show the decrease in scar formation during the phase of collagen synthesis. However, enough preventive effects were not found (Cakmak et al. 1997; Ulman and Mutaf 1998). It was also reported in experimental model that estradiol and progesteron, ketotifen, Caffeicacid Phenyl Ester (CAPE), Epidermoid Growth Factor (EGF) inhibited collagen synthesis, but, these have not been used in clinic (Demirbilek et al. 1994; Koltuksuz et al. 2001; Yukselen et al. 2004).

Very few studies were found to investigate the acute phase of corrosive esophagitis (Gunel et al. 1999; Ozel et al. 2004). Gunel et al. (1999) showed that tissue oxygen radicals were significantly increased within first 24 hour in corrosive esophageal injury. The release of reactive oxygen radicals has been detected in thermal burns and has extended the degree of tissue damage (Till et al. 1989). Mutaf et al. (1996) have shown that reactive oxygen radicals are responsible for the early phase of corrosive esophagitis and increasing tissue damage. Mutaf et al. (1996) have shown that the prognosis of corrosive esophageal burns and esophageal stricture are determined at the time of corrosive substance ingestion and the severity of the initial lesion also.

Various studies were done to understand the effect of NO in different tissues previously (Whittle 1994). NO is a highly reactive free radical with a multitude of organ specific regulatory functions. NO plays a major role in many organ systems and deranged NO synthesis causes a number of pathological states (Ferguson and Granton 2000). NO can cause mucosal hyperemia with vasodilatation and leucocytic translocation to the extra vascular area and increase tissue edema (Whittle 1994). Ozel et al. (2004) suggested that leucocytes, vascular endothelium, free oxygen radicals, NO and endothelin may be responsible for the early effects on the corrosive injury of esophagus.

Trapidil has effects of nitroglycerine-like vasodilatation, inhibition of platelet aggregation, facilitation of the biosynthesis of prostacycline,

reduction of phosphodiesterase, reduction of production of interleukin 6 and 12 (IL6 and 12) (Heinroth-Hoffman et al. 1987; Neider et al. 1995; Block et al. 1998; Zhon et al. 1999). Previous studies have shown that trapidil significantly reduces cellular damage and edema in the injury site. This finding is explained by the membrane stabilizing effect of trapidil by inhibiting of TXA₂ synthesis (Gocer et al. 2001). It was reported that trapidil has a marked protection against ischemia-reperfusion injury in peripheral nerves (Bagdatoglu et al. 2002). It was reported that trapidil has positive effect on anastomotic healing due to its preventive effects on inflammatory response and lipid peroxidation (Colak et al. 2003). Therefore, we aimed to determine the tissue levels of reactive oxygen radicals and to investigate the beneficial effects of trapidil therapy in corrosive esophagitis.

Gunel et al. (1999) found that MDA levels increase in the early phase (first 24, 48 and 72 hours) of corrosive esophagitis. Ozden et al. (2004) used MDA as an indicator of free radical generation in their study. We found that MDA level significantly increased in early phase of corrosive esophagitis (first 24 hour) and significantly decreased by trapidil treatment. These findings suggest that trapidil reduces lipid peroxidation and free oxygen radicals in the injured tissue. Reactive oxygen radicals, NO, endothelin were found to increase in the early phase of corrosive esophagitis in an experimental study by Ozel et al. (2004). We found that NO levels significantly increase in the untreated group and significantly decrease by trapidil in the treated group. These findings suggest that free oxygen radicals and NO may be responsible for the early phase of corrosive esophagitis. In addition, the present study has shown that trapidil decreases PMN infiltration in acute phase of corrosive injury and reduces cellular damage, ulceration and edema in injured tissue.

In conclusion, leucocytic infiltration, free oxygen radicals and NO may be responsible for the early phase of the corrosive injury. Trapidil usage at early phase of corrosive esophagitis may ameliorate corrosive esophageal injury and reduce

the stricture formation in the healing of corrosive esophageal burns.

References

- Aschcraft, K.W. & Padula, R.T. (1974) The effect of dilute corrosives on the esophagus. *Pediatrics*, **53**, 226-232.
- Bagdatoglu, C., Saray, A., Surucu, H.S., Ozturk, H. & Tamer, L. (2002) Effect of trapidil in ischemia- reperfusion injury of peripheral nerves. *Neurosurgery*, **51**, 212-220.
- Bautista, A., Varela, R., Villanueva, A., Estevez, E., Tojo, R. & Cadranet, S. (1996) Effects of prednisolone and dexamethasone in children with alkali burns of esophagus. *Eur. J. Pediatr. Surg.*, **6**, 198-203.
- Block, H.U., Dunemann, A., Niebrisch, M. & Mest, H.J. (1998) Influence of the antianginal drug trapidil and its derivate AR 12463 on arachidonic acid liberation and thromboxane formation in thrombin stimulated platelets. *Biomed. Biochem. Acta.*, **47**, 141-144.
- Cadranet, S., Scaillon, M., Goyens, P. & Rodesch, P. (1993) Treatment of esophageal caustic injuries: Experience with high-dose dexamethasone. *Pediatr. Surg. Int.*, **8**, 97-102.
- Cakmak, M., Nayci, A., Renda, N., Ereku, S., Gokcora, H. & Yucesan, S. (1997) The effect of corticosteroids and pentoxifylline in caustic esophageal burns. *Int. Surg.*, **82**, 371-375.
- Capella, M., Goldberg, P., Quaresma, E., Araujo, E. & Pereira, M. (1992) Persistence of corrosive esophageal stricture due to gastro esophageal reflux in children. *Pediatr. Surg. Int.*, **7**, 180-182.
- Colak, T., Nayci, A., Polat, G., Polat, A., Comelekoglu, U., Kanik, A., Turkmenoglu, O. & Aydin, S. (2003) Effect of trapidil on the healing of colonic anastomosis in an experimental rat model. *ANZ J. Surg.*, **73**, 916-921.
- Day, D.W. (1989) Infective, physical and chemical oesophagitis. In: *Gastrointestinal and oesophageal pathology*. edited by R. Whitehead, Churchill Livingstone, London, pp. 377-383.
- Demirbilek, S., Bernay, F., Rizalar, R., Baris, S. & Gurses, N. (1994) Effects of estradiol and progesterone on the synthesis of collagen in corrosive esophageal burns in rats. *J. Ped. Surg.*, **29**, 1425-1428.
- Ferguson, N.D. & Granton, J.T. (2000) Inhaled nitric oxide for hypoxemic respiratory failure: passing bad gas? *C. M. A.J.*, **162**, 85.
- Gehanno, P. & Guedon, C. (1981) Inhibition of experimental esophageal lye strictures by penicillamine. *Arch. Otolaryngol.*, **107**, 145-147.
- Gocer, A.I., Ildan, F., Tuna, M., Polat, S., Tamer, L., Erman, T. & Kaya, M. (2001) Effects of trapidil on ATPase, lipid peroxidation with ultra structure in experimental spinal cord injury. *Neurosurg Res.* **24**, 136-142.
- Gunel, E., Caglayan, F., Caglayan, O. & Akilloglu, I. (1999) Reactive oxygen radical levels in caustic esophageal burns. *J. Ped. Surg.*, **34**, 405-407.
- Haller, J.A., Andrews, H.G., White, J.J., Tamer, M.A. & Cleceland, W.W. (1971) Pathophysiology and management of acute corrosive burns of esophagus. Results of treatments in 285 children. *J. Ped. Surg.*, **6**, 578-584.
- Heinroth-Hoffman, I., Hauser, A. & Mest, H.J. (1987) Inhibition of thromboxane A₂ production by trapidil and trapidil derivatives in the arachidonic acid injected rat. *Prostaglandins. Leukot. Med.*, **30**, 87-92.
- Koltuksuz, U., Mutus, M., Kutlu, R., Ozyurt, H., Cetin, S., Karaman, A., Gurbuz, N., Akyol, O. & Aydin, E. (2001) Effects of caffeic acid phenyl ester and epidermoid growth factor on the development of caustic esophageal stricture in rats. *J. Ped. Surg.*, **36**, 1504-1509.
- Miller, M.J., Mc Neill, H., Mullane, K.M., Caravella, S.J. & Clark, D.A. (1988) SOD prevents damage and attenuates eicosanoid release in a rabbit model of necrotizing enterocolitis. *Am. J. Physiol.*, **255**, G556.
- Miranda, K.M., Espey, M.G. & Wink, D.A. (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, **5**, 62-71.
- Mutaf, O., Genc, A., Herek, O., Demircan, M., Ozcan, C. & Arkan, A. (1996) Gastro esophageal reflux: a determinant in the outcome of caustic esophageal burns. *J. Pediatr. Surg.*, **31**, 1494-1495.
- Neider, J., Claus, P. & Auguntin, W. (1995) Effects of trapidil on the PGI₂ and TxA₂ synthesis in human umbilical veins perused in vitro. *Prostaglandins*, **49**, 311-318.
- Ozden, T.A., Uzun, H., Bohloli, M., Toklu, A.S., Paksoy, M., Simsek, G., Durak, H., Issever, H. & Ipek, T. (2004) The effects of hyperbaric oxygen treatment on oxidant and antioxidants levels during liver regeneration in rats. *Tohoku J. Exp. Med.*, **203**, 253-265.
- Ozel, K., Dagli, T., Yuksel, M., Kiyani, G. & Kotiloglu, E. (2004) The roles of free oxygen radicals, nitric oxide, and endothelin in caustic injury of rat esophagus. *J. Ped. Surg.*, **39**, 1381-1385.
- Rothstein, F.C. (1986) Caustic injuries to the esophagus in children. *Pediatr. Clin. North Am.*, **33**, 665-674.
- Till, G.O., Guilds, L.S., Mahrougui, M., Friedl, H.P., Trentz, O. & Ward, P.A. (1989) Role of xanthine oxidase in thermal injury of skin. *Am. J. Pathol.*, **135**, 195-202.
- Ulman, I. & Mutaf, O. (1998) A critique of systemic steroids in the management of caustic esophageal burns in children. *Eur. J. Pediatr. Surg.*, **8**, 71-74.
- Whittle, B.T.J. (1994) Nitric oxide in gastrointestinal physiology and pathology. In: *Physiology of the gastrointestinal tract*. edited by L.R. Johnson, New York, NY, Raven Press, pp. 267-294.
- Yagi, K. (1976) A simple fluorimetric assay for lipoperoxide in blood plasma. *Biochem. Med.*, **15**, 212-216.
- Yukselen, V., Karaoglu, A.O., Ozutemiz, O., Yenisey, C. & Tuncyurek, M. (2004) Ketotifen ameliorates development of fibrosis in alkali burns of the esophagus. *Pediatr. Surg. Int.*, **20**, 429-433.
- Zhon, L., Ismaili, J., Stordeur, P., Thielemans, K., Goldman, M. & Pradier, O. (1999) Inhibition of the CD40 pathway of monocyte activation by triazolopyrimidine. *Clin. Immunol.*, **93**, 232-238.