Exacerbation of Airway Hyperreactivity by ($\pm$)Salbutamol in Sensitized Guinea Pigs

Ken-ichiro Hoshiko^{#,*} and John Morley^{##}

Preclinical Research, Sandoz Pharma, Ltd., CH-4002, Basel, Switzerland

Received February 22, 1993 Accepted July 1, 1993

ABSTRACT—In guinea pigs passively sensitized to ovalbumin, sustained (6 days) subcutaneous infusion of ($\pm$)salbutamol (1 mg/kg/day) induced significant airway obstruction and heightened responsivity to airway spasmogens. Of these animals, a substantial proportion (78/235) were too responsive to injected spasmogens to permit infusion of ovalbumin or died following infusion of ovalbumin; yet there were few deaths (2/166) amongst the sensitized animals not exposed to ($\pm$)salbutamol. In comparison to the animals not exposed to ($\pm$)salbutamol, infusion of ovalbumin led to exaggerated responsivity of the airways to leukotriene C₄, leukotriene E₄, histamine, serotonin and acetylcholine, but not to prostaglandin F_{2 α} or bradykinin. The capacity of sustained exposure to high doses of ($\pm$)salbutamol to induce airway hyperreactivity to allergic mediators may account for an association between asthma death and regular, excessive use of sympathomimetics.

Keywords: Salbutamol, Hyperreactivity (airway)

For many years, it has been known that repeated exposure of guinea pigs to sympathomimetics leads to an increased susceptibility to airway spasmogens. Thus, regular injection of epinephrine (1) increased the lethality of inhaled antigen; and regular injection of ($\pm$)isoprenaline, ($\pm$)salbutamol or ($\pm$)terbutaline (2) increased the susceptibility of guinea pigs to the lethal effects of inhaled histamine. No mechanism was established to account for these phenomena and the possibility of increased airway obstruction does not appear to have been considered, even though overt airway obstruction in reaction to ($\pm$)isoprenaline was known from clinical (3–5) and animal (6) studies.

More recently, it has been established that acute intravenous infusion of ($\pm$)isoprenaline induces airway hyperreactivity in guinea pigs (7), and this effect has been shown to extend to β_2 -selective sympathomimetics such as ($\pm$)salbutamol (8). Acutely, this property of ($\pm$)salbuta-

mol is masked by the bronchodilator action of (–)salbutamol, but following sustained infusion of ($\pm$)salbutamol over several days, increased sensitivity of the airways can be demonstrated to antigen (9) and to airway spasmogens (10). Such findings are consistent with clinical reports of exacerbation of airway hyperreactivity following regular administration of ($\pm$)fenoterol (11), ($\pm$)terbutaline (12–14) and ($\pm$)salbutamol (15); loss of protection against constrictor stimuli despite persisting bronchodilator responses, as reported for ($\pm$)salbutamol (16) and ($\pm$)salmeterol (17); and intensification of late-onset reactions to inhaled allergen, as following inhalation of ($\pm$)rimiterol (18).

The present study has defined the capacity of sustained infusion of ($\pm$)salbutamol to cause airway obstruction in passively sensitized animals, before and after expression of allergic reactions, and has defined airway hyperreactivity in these circumstances using seven airway spasmogens.

MATERIALS AND METHODS

Animals

Male Dunkin Hartley guinea pigs (450–650 g; WIGA,

[#]Present address: Sandoz Tsukuba Research Institute, 8, Ohkubo, Tsukuba, Ibaraki 300–33, Japan

^{*}To whom reprint requests should be addressed.

^{##}Present address: Mumed, Ltd., Building 15, The Paddocks, Frith Lane, Mill Hill, NW7 1PS, London, UK

Charles River, Sulzfeld, Germany) were used throughout. Animals, which were housed in groups (4–6), received food and water ad libitum. All experimental procedures were approved by the "Kantonale Tierversuchs-Kommission von Basel-Stadt und Baselland."

Materials

Al(OH)₃ (Merck, Darmstadt, Germany); ACh iodide, histamine dihydrochloride and OA (Fluka, Buchs, Switzerland); *Bordetella pertussis* vaccine (IMPF, Bern, Switzerland); PGF_{2α} and 5-HT creatinine sulfate (Sigma, Buchs, Switzerland); BK (Bachem, Budendorf, Switzerland); gallamine triethiodide (Davis-Geck, New York, NY, USA); sodium phenobarbitone, sodium pentobarbitone and ether (Siegfried, Zofingen, Switzerland) were commercial preparations. LTC₄ (purity >95%), LTE₄ (purity >90%) and (±)salbutamol were synthesized.

Measurement of lung function

Guinea pigs were anesthetized by intraperitoneal injection of sodium phenobarbitone (100 mg/kg) supplemented with sodium pentobarbitone (30 mg/kg) and paralyzed by intramuscular injection of gallamine (10 mg/kg). Animals were ventilated (8 ml/kg, 1 Hz) with a mixture of air and oxygen (40 : 60, v/v) via a tracheal cannula. Ventilation was monitored at the trachea by a pneumotachograph (Fleisch type 0000; Zabona, Basel, Switzerland) connected to a differential pressure transducer (MP 4514871; Validyne, North Reading, MA, USA). Coincident pressure changes within the thorax were measured via an intrathoracic cannula, using a differential pressure transducer (MP 4524, Validyne), so that the pressure difference between the trachea and thorax could be measured and displayed. To monitor the physiological status of the animal during experimental investigations, blood pressure and heart rate were recorded from the carotid artery by a pressure transducer (Isotec; Hugo Sachs, Freiburg, Germany), and a cannula was introduced into the right jugular vein to allow intravenous infusion of OA or vehicle. The left jugular vein was cannulated for administration of spasmogens, which were injected intravenously in a fixed volume (0.2 ml), i.e., within the dead space in the cannula. This material was infused at a constant rate (1.0 ml/min) by delivery of saline (0.5 ml over 30 sec) from an infusion pump (Perfusor IV; Bender & Hobein, Zürich, Switzerland). From measurements of air-flow and transpulmonary pressure, both R_L and C_{dyn} were calculated after each respiratory cycle by a digital electronic pulmonary monitoring system (PMS; Mumed, London, UK) which displayed blood pressure, intrathoracic pressure and airflow and computed R_L and C_{dyn} in real time for display on a visual display unit (Premium II 486/33; AST, Irvine, CA, USA). Experimental data

was stored continuously and, on termination of an experiment, experimental traces or processed data were plotted on a laser jet printer (Laser Jet Series II; Hewlett Packard, Palo Alto, CA, USA).

Sensitization procedure

OA (10 µg) was added to Al(OH)₃ (10 mg) in sterile physiological saline (0.75 ml) and mixed in a blender. This mixture, together with 0.25 ml of *Bordetella pertussis* vaccine, was injected intraperitoneally into recipients; and after 14 days, the procedure was repeated. After a further 7 days, blood was collected by cardiac puncture under anesthesia with sodium pentobarbitone (30–50 mg/kg, i.v.). Blood was allowed to clot and stored (4°C) overnight. Following clot retraction, decanted serum was filtered prior to centrifugation (200 × g for 10 min). Aliquots (10 ml) were stored at –20°C. For passive sensitization, anti-OA guinea pig serum (1 ml/animal, i.v.) was injected 7 days before an intravenous infusion of OA (32 µg/kg) over one hr, a procedure which consistently induced hyperreactivity of the airways. Seven days after intradermal injection of antiserum, PCA reactions (mm diameter) (n=5) were: 15, 12, 6, 0 and 0 when the serum had been heated (56°C for 4 hr), as compared with >30, >30, 25, 22 and 20 when the serum was not heated.

Experimental protocol

Anti-OA guinea pig serum (1 ml/animal) was injected intravenously 7 days prior to study. To provide sustained infusion of (±)salbutamol (1 mg/kg/day), osmotic minipumps (model 2001; Alza, Palo Alto, CA, USA) containing a solution of (±)salbutamol (41.3 mg/kg/ml) were implanted subcutaneously in the nuchal region under ether anesthesia, 6 days prior to the study on pulmonary function. Intravenous injections of either 5-HT (1.0, 1.8, and 3.2 µg/kg), ACh (5.6, 10, and 18 µg/kg), histamine (0.56, 1.0, and 1.8 µg/kg), LTC₄ (100, 180, and 320 ng/kg), LTE₄ (180, 320, and 560 ng/kg), or PGF_{2α} (32, 56, and 100 µg/kg) were made at 10-min intervals or for BK (0.32, 1.0, and 3.2 µg/kg) at 30-min intervals to define sensitivity of the airways. These procedures were repeated 10 min after termination of intravenous infusion of OA (32 µg/kg) over 1 hr (3 ml/hr).

Statistics

Data are presented as means ± S.E.M., and Student's *t*-test was used for comparison between treatments. When multiple doses of spasmogen were used, airway responsiveness to the smooth muscle spasmogen was defined as the dose-effect slope for R_L (cmH₂O/l/sec) over a log₁₀ dose-meter, using RS1 (BBN Software Product Corporation, Cambridge, MA, USA). For purposes of comparison, altered sensitivity has been defined as a ratio between

Table 1. R_L and C_{dyn} following intravenous infusion of vehicle (saline) ($n=80$) or OA ($n=80$), without, or with, sustained (6 days) infusion of ($\pm$)salbutamol (1 mg/kg/day) ($n=97$) into passively sensitized animals

		Vehicle	OA	OA after ($\pm$)salbutamol
R_L (cmH ₂ O/l/sec)	pre	91.1 $\pm$ 1.7	94.6 $\pm$ 2.0	86.8 $\pm$ 1.4 ⁺⁺⁺
	post	93.6 $\pm$ 1.8	102.8 $\pm$ 2.8 ^{**}	128.2 $\pm$ 6.6 ⁺⁺⁺⁺
	difference	2.5 $\pm$ 1.1	8.1 $\pm$ 2.2 [*]	41.4 $\pm$ 6.2 ⁺⁺⁺⁺⁺
C_{dyn} (ml/cmH ₂ O)	pre	0.93 $\pm$ 0.02	0.97 $\pm$ 0.03	0.91 $\pm$ 0.02
	post	0.88 $\pm$ 0.02	0.96 $\pm$ 0.03 [*]	0.60 $\pm$ 0.03 ⁺⁺⁺⁺⁺
	difference	-0.05 $\pm$ 0.01	-0.01 $\pm$ 0.03	-0.31 $\pm$ 0.03 ⁺⁺⁺⁺⁺

Significant difference from the vehicle, * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). Significant difference from OA, ++ ($P < 0.01$), +++ ($P < 0.001$).

the dose-effect slope after infusion of OA (or vehicle) and the corresponding dose-effect slope before infusion.

RESULTS

Intravenous infusion of OA (32 μ g/kg) over one hr caused slight airway obstruction (Table 1). Infusion of this dose of OA into passively sensitized animals that had been previously exposed to a sustained (6 days) infusion of ($\pm$)salbutamol (1 mg/kg/day) led to significantly ($P < 0.001$) greater airway obstruction (Table 1). Of the animals exposed to ($\pm$)salbutamol, 38 were so responsive to spasmogen as to preclude infusion of OA; of the remaining 197, 40 died during or following the infusion of antigen. In animals not exposed to ($\pm$)salbutamol, none were so responsive as to preclude infusion of OA, and only 2 of 166 died subsequent to this infusion.

Airway responsivity to injected spasmogens has been defined in naive and in passively sensitized animals, using a range of spasmogens. Following sustained infusion of ($\pm$)salbutamol, responsivity to test spasmogens in passive-

ly sensitized animals was greater for LTC₄, LTE₄ and BK, but less for ACh, histamine, 5-HT and PGF_{2 α} than in naive animals or sensitized animals not exposed to ($\pm$)salbutamol (Table 2). Animals that had been exposed to ($\pm$)salbutamol and which received (and survived) an infusion of OA (32 μ g/kg) and subsequent reinjection of spasmogen exhibited exaggerated responsivity to LTC₄, LTE₄, histamine, 5-HT and ACh, but not to PGF_{2 α} and BK. Such increased responsivity has been contrasted with the corresponding changes in sensitized animals not exposed to ($\pm$)salbutamol (Table 3). Since approximately 30% of the animals exposed to ($\pm$)salbutamol either died or were discarded, the observed differences are not representative and may underestimate the difference between the two populations.

Table 2. Slopes (log₁₀ metameter) of dose-effect relationships for spasmogens in naive animals, in sensitized animals and in sensitized animals after exposure for 6 days to ($\pm$)salbutamol (1 mg/kg/day) ($n=10$ for each group).

Spasmogen	Naive	Sensitized	Sensitized and exposed to ($\pm$)salbutamol
Bradykinin	120	141	352
PGF _{2α}	148	186	76
LTE ₄	150	182	327
Histamine	163	144	91
LTC ₄	209	137	626
Acetylcholine	524	326	272
5-HT	561	445	211

Table 3. Slope ratios of increased airway resistance to LTC₄, LTE₄, histamine, 5-HT, acetylcholine, PGF_{2 α} , and bradykinin following infusion of vehicle (saline) or OA in the absence, or presence of, sustained infusion of ($\pm$)salbutamol into passively sensitized animals

Spasmogen	Slope ratio (% increase over vehicle)		
	Saline	OA with ($\pm$)salbutamol	OA [†]
LTC ₄	0.19	9.60 (+4953)	5.48 (+2748)
LTE ₄	0.96	5.03 (+424)	2.51 (+161)
Histamine	1.50	6.32 (+321)	3.16 (+111)
5-HT	1.23	3.91 (+218)	1.31 (+7)
Acetylcholine	1.23	2.31 (+88)	1.13 (-8)
PGF _{2α}	1.78	2.35 (+32)	3.11 (+75)
Bradykinin	1.63	1.69 (+4)	2.70 (+66)

To calculate slope ratios, in animals exposed to ($\pm$)salbutamol, only responses to lowest and intermediate doses of spasmogen were used, since responses to the intermediate dose were near maximal. [†]Data from reference 19.

DISCUSSION

The present observations demonstrate that sustained exposure to ($\pm$)salbutamol exacerbates acute allergic bronchospasm in the anesthetized guinea pig, and this effect can be sufficient to convert a response from barely detectable airway obstruction into a reaction that is lethal in about a third of the animals. This finding is consistent with earlier reports of increased lethality following exposure to inhaled histamine, in animals which had received repeated injections of β_2 -selective sympathomimetics (2).

There are three reasons why this effect of ($\pm$)salbutamol cannot be accounted for by adrenoceptor occupancy. First, activation of β_2 -adrenoceptors on mast cells or basophils is known to reduce the release of allergic mediators (20); secondly, activation of β_2 -adrenoceptors on airway smooth muscle is spasmolytic (21); thirdly, acute inhalation of ($\pm$)isoprenaline can abolish allergic bronchospasm in animals previously exposed to protracted infusion of ($\pm$)salbutamol (8), so that adrenoceptor tachyphylaxis cannot account for this form of airway hyperreactivity. It is known that ($\pm$)isoprenaline produces airway hyperreactivity in the guinea pig by a mechanism independent of adrenoceptor occupancy (7), and corresponding studies with ($\pm$)salbutamol have revealed a similar profile. Thus, in contrast to effects that depend upon interaction with adrenoceptors, development of airway hyperreactivity following exposure to ($\pm$)salbutamol is not prevented by ($\pm$)propranolol, is prevented by bilateral section of the vagus nerves and is evidenced by (+)salbutamol, the enantiomer which does not interact significantly with adrenoceptors (22). Abrogation by vagal section implies an action in neural tissue; however, it is not easy to ascertain the locus of such an effect since atropine did not inhibit the corresponding action of ($\pm$)isoprenaline (5) and since use of hexamethonium is precluded owing to an intrinsic capacity of this compound to induce airway hyperreactivity.

Following sustained infusion of ($\pm$)salbutamol into sensitized animals, responsivity to LTC₄, LTE₄ and BK were increased, whereas responsivity to histamine, ACh, PGF_{2 α} and 5-HT were diminished. This dichotomy has been reported previously, when exaggerated airway obstruction due to intravenous or intratracheal antigen was accompanied by proportionately less increased responsivity to histamine in actively sensitized animals (9). This difference presumably reflects a greater capacity of (–)salbutamol to suppress constrictor responses to histamine than those to spasmogens such as LTC₄ and LTE₄ whose formation accounts in large part for acute allergic bronchospasm in this species (23). A possible counterpart in clinical asthma is the sustained efficacy of ($\pm$)salbutamol

or ($\pm$)salmeterol as bronchodilators, despite loss of protection from bronchoconstrictor stimuli (16, 17). The rank order of changed responsivity differs when the effects of antigen are contrasted with the effect of antigen in animals pretreated with ($\pm$)salbutamol. It may be presumed therefore that the mechanism underlying the exaggeration of airway hyperreactivity by prolonged infusion of ($\pm$)salbutamol is distinct from that invoked by the acute allergic reaction. However, in neither instance is it possible to propose a molecular basis for the observed phenomena.

Increased morbidity and mortality in asthma have been associated with regular and excessive use of ($\pm$)isoprenaline (24) and, more recently, β_2 -selective sympathomimetics (25). Previous attempts to establish a causal basis for such association led to recognition that repeated exposure to relatively high dose levels of sympathomimetics made guinea pigs susceptible to lethal effects of the airway spasmogen histamine (2), in confirmation of effects upon antigen in earlier studies (1). The present investigation has confirmed the occurrence of this phenomenon in the guinea pig and has revealed that deaths in animals pretreated with ($\pm$)salbutamol result from increased reactivity of the airways, particularly to peptidoleukotrienes. Whether this conclusion extends to allergic bronchospasm in asthma patients has yet to be investigated. It is already known that regular use of existing β_2 -selective sympathomimetics can induce modest exacerbation of reactivity of asthma patients to inhaled methacholine, but the effect of regular administration of sympathomimetics upon allergic hyperreactivity has yet to be defined for any spasmogen. Use of methacholine may be misleading, since it has been reported that the intensity and duration of allergic hyperreactivity to inhaled bradykinin exceeds, by an order of magnitude, concomitant responsivity to inhaled methacholine (24). Studies of the effect of racemic mixtures of β_2 -sympathomimetics upon allergic hyperreactivity could be confounded by persistent bronchodilator effects of (–)enantiomers. It is of practical importance, therefore, that development of airway hyperreactivity is a feature of exposure to (+)salbutamol, (+)terbutaline and (+)isoprenaline (22), since use of such enantiomers in clinical studies will circumvent this limitation.

REFERENCES

- 1 Izard, S.R., Henson, E.C., Collins, A.D. and Brunson, J.G.: Increased sensitivity to anaphylactic shock in guinea pigs induced by prolonged treatment with epinephrine prior to challenge. *J. Allergy* **47**, 309–314 (1971)
- 2 Conolly, M.E., Davies, D.S., Dollery, C.T. and George, C.F.: Resistance to β -adrenoceptor stimulants (a possible explanation for the rise in asthma deaths). *Br. J. Pharmacol.* **43**, 389–402 (1971)

- 3 Reisman, R.E.: Asthma induced by adrenergic aerosols. *J. Allergy* **46**, 162–177 (1970)
- 4 Trautlein, J., Allegra, J., Field, J. and Gillin, M.: Paradoxical bronchospasm after inhalation of isoproterenol. *Chest* **70**, 711–714 (1976)
- 5 Paterson, J.W., Evans, R.J.C. and Prime, F.J.: Selectivity of bronchodilator action of salbutamol in asthmatic patients. *Br. J. Dis. Chest* **65**, 21–38 (1971)
- 6 Cho, Y.W., Aviado, D.M. and Lish, P.M.: Efficacy of a new bronchodilator, soterenol, on experimental locked-lung syndrome in dogs. *J. Allergy Clin. Immunol.* **42**, 36–48 (1968)
- 7 Sanjar, S., Kristersson, A., Mazzoni, L., Morley, J. and Schaeublin, E.: Increased airway reactivity in the guinea pig follows exposure to intravenous isoprenaline. *J. Physiol. (Lond.)* **425**, 43–54 (1990)
- 8 Morley, J., Chapman, I.D., Foster, A., Hoshiko, K. and Mazzoni, L.: Effect of (+) and racemic salbutamol on airway responses in the guinea-pig. *Br. J. Pharmacol.* **104**, 295P (1991)
- 9 Chapman, I.D., Mazzoni, L. and Morley, J.: An anomalous effect of salbutamol in sensitized guinea pigs. *Br. J. Pharmacol.* **99**, 66P (1990)
- 10 Morley, J., Hoshiko, K., Chapman, I.D. and Mazzoni, L.: The selective nature of airway hyperreactivity. *J. Physiol. (Lond.)* **446**, 117P (1992)
- 11 Sears, M.R., Taylor, D.R., Print, C.G., Lake, D.C., Li, Q., Flannery, E.M., Yates, D.M., Lucas, M.K. and Herbison, G.P.: Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* **336**, 1391–1396 (1990)
- 12 Kraan, J., Koeter, G.H., Mark, Th.W., Sluiter, H.J. and de Vries, K.: Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J. Allergy Clin. Immunol.* **76**, 628–636 (1985)
- 13 Kerrebijn, K.F., Van Essen-Zandvliet, E.E.M. and Neijens, H.J.: Effect of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. *J. Allergy Clin. Immunol.* **79**, 653–659 (1987)
- 14 Vathenen, A.S., Knox, A.J., Higgins, B.G., Britton, J.R. and Tattersfield, A.E.: Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* **ii**, 554–557 (1988)
- 15 van Schayck, C.P., Graafma, S.J., Visch, M.B., Dompeling, E., van Weel, C. and van Herwaarden, C.L.A.: Increased bronchial hyperresponsiveness after inhaling salbutamol during 1 year is not caused by subsensitization to salbutamol. *J. Allergy Clin. Immunol.* **86**, 793–800 (1990)
- 16 Jenne, J.W. and Ahrens, R.C.: Pharmacokinetics of beta-adrenergic compounds. In *Drug Therapy for Asthma: Research and Clinical Practice*, Edited by Jenne, J.W. and Murphy, S., pp. 213–258, Marcel Dekker, New York (1987)
- 17 Cheung, D., Timmers, M.C., Zwinderman, A.H., Bel, E.H., Dijkman, J.H. and Sterk, P.J.: Long-term effects of a long-acting β_2 -adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N. Engl. J. Med.* **327**, 1198–1203 (1992)
- 18 Lai, C.K.W., Twentyman, O.P. and Holgate, S.T.: The effect of an increase in inhaled allergen dose after rimeterol hydrobromide on the occurrence and magnitude of the late asthmatic response and the associated change in nonspecific bronchial responsiveness. *Am. Rev. Respir. Dis.* **140**, 917–923 (1989)
- 19 Hoshiko, K. and Morley, J.: Allergic bronchospasm and airway hyperreactivity in the guinea pig. *Japan. J. Pharmacol.* **63**, 151–157 (1993)
- 20 Butchers, P.R., Fullarton, J.R., Skidmore, I.F., Thompson, L.E., Vardey, C.J. and Wheeldon, A.: A comparison of the anti-anaphylactic activities of salbutamol and disodium cromoglycate in the rat, the rat mast cell and in human lung tissue. *Br. J. Pharmacol.* **67**, 23–32 (1979)
- 21 Paterson, J.W., Woolcock, A.J. and Shenfield, G.M.: Bronchodilator drugs. *Am. Rev. Respir. Dis.* **120**, 1149–1187 (1979)
- 22 Morley, J.: Adverse reactions to sympathomimetics in laboratory animals. In *Beta Agonists in the Treatment of Asthma*, Edited by Costello, J.F. and Mann, R.D., pp. 57–68, The Parthenon Publishing Group, Camforth (UK) (1992)
- 23 Undem, B.J., Pickett, W.C. and Adams, G.K.: Antigen-induced sulfidopeptide leukotriene release from the guinea pig superfused trachea. *Eur. J. Pharmacol.* **142**, 31–37 (1987)
- 24 Djukanovic, R.J.: Is airway hyperreactivity selective or non-selective? *Agents Actions* **43**, Supp. 231–239 (1993)
- 25 Spitzer, W.O., Suissa, S., Ernst, P., Horwitz, R.I., Habbick, B., Cockcroft, D., Boivin, J.-F., McNutt, M., Buisst, A.S. and Rebuck, A.S.: The use of β -agonists and the risk of death and near death from asthma. *N. Engl. J. Med.* **326**, 501–506 (1992)