Effects of bronchodilator particle size in asthmatic patients using monodisperse aerosols

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Usmani, Omar S., Martyn F. Biddiscombe, Julia A. Nightingale, S. Richard Underwood, and Peter J. Barnes. Effects of bronchodilator particle size in asthmatic patients using monodisperse aerosols. J Appl Physiol 95: 2106–2112, 2003. First published August 1, 2003; 10.1152/japplphysiol.00525.2003.—Aerosol particle size influences inhaled drug deposition. Current inhaler devices are inefficient, delivering a heterodisperse distribution of drug particle sizes, whereas only 10–20% of the drug dose reaches the lungs. Monodisperse aerosols are the appropriate research tools to investigate basic aerosol science concepts within the human airways. We hypothesized that engineering such aerosols of albuterol would identify the ideal bronchodilator particle size, thereby optimizing inhaled therapeutic drug delivery. Eighteen stable mildly to moderately asthmatic patients [mean forced expiratory volume in 1 s (FEV1) 74.3% of predicted] participated in a randomized, double-blind, crossover study design. A spinning-top aerosol generator was used to produce monodisperse albuterol aerosols that were 1.5, 3, and 6 μm in size, and also a placebo, which were inhaled at cumulative doses of 10, 20, 40, and 100 μg. Lung function changes and tolerability effects were determined. The larger particles, 6 and 3 μm, were significantly more potent bronchodilators than the 1.5-μm and placebo aerosols for FEV1 and the forced expiratory flow between exhalation of 25 and 75% of forced vital capacity. A 20-μg dose of the 6- and 3-μm aerosols produced FEV1 bronchodilation comparable to that produced by 200 μg from a metered-dose inhaler. No adverse effects were observed in heart rate and plasma potassium. The data suggest that in mildly to moderately asthmatic patients there is more than one optimal β2-agonist bronchodilator particle size and that these are larger particles in the higher part of the respirable range. Aerosols delivered in monodisperse form can enable large reductions of the inhaled dose without loss of clinical efficacy.

albuterol; drug delivery; human lungs; spinning-top aerosol generator

THE CLINICAL EFFICACY OF INHALED therapy is dependent on the ability to deliver sufficient drug to the lower respiratory tract, yet current inhaler devices are very inefficient because only 10–20% of the drug dose reaches the lungs (24). The majority impacts in the oropharynx, which is wasted, and although devices compensate with higher drug doses to achieve an adequate clinical response, there is equal potential for systemic adverse effects to occur. Drug particle size is the major aerosol characteristic determining the extent, distribution, and site of inhaled drug deposition within the airways. Experimental predictive models suggest that submicrometer particles generally deposit in the alveoli or are exhaled, whereas those larger than 8 μm characteristically undergo inertial impaction within the oropharynx (13, 15). Therefore, to reach the lower respiratory tract, the majority of the aerosol mass should be within the 2- to 6-μm respirable range of particle size diameters (28).

β2-Agonists are the most widely prescribed inhaled drugs for the treatment of airway disease. Several investigators have explored the relationship between bronchodilator aerosol particle size and the clinical response in asthmatic patients, yet they have reached different conclusions for the optimal size (8, 9, 17–19, 25, 29). These studies employed heterodisperse aerosols where the drug mass is distributed across a wide range of particle size diameters, and the broad and overlapping aerosol distributions may have confounded the results attributable to one particular particle size (23). Differences in aerosol generation technique, airway disease severity, the inhalation maneuver, and clinical efficacy end points used may also have contributed to the different interpretations.

Monodisperse pharmacological aerosols are highly relevant to clinical practice because they allow us to undertake translational aerosol research, accurately exploring fundamental in vitro concepts of basic aerosol science within the human airways in vivo. In contrast to heterodisperse aerosols, they are composed of uniform-sized particles where the majority of the drug mass is within a narrow size distribution and therefore have greater discriminative power to explore differences due to aerosol particle size. Two studies have used monodisperse β2-agonist aerosols to investigate particle size and bronchodilator response (27, 35). Patel et al. (27) found that 2.8-μm particles of isoproterenol achieved better improvement in lung function indexes than 5.5-μm particles, albeit in a non-placebo-controlled study involving eight mildly asthmatic

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patients. Zanen et al. (35) showed greater potency of 2.8-μm particles of albuterol over 1.5- and 5-μm particles in eight mildly to moderately asthmatic patients; however, tolerability effects were not concurrently monitored.

We undertook to improve on these earlier attempts, to address and clarify the uncertainty of the optimal particle size for β2-agonists. Carefully controlled, validated, and consistent methods were used to generate and deliver monodisperse albuterol aerosols (6) to 18 well-characterized asthmatic patients in whom airway function and systemic tolerability were simultaneously monitored within a randomized, double-blind, placebo-controlled study design. We hypothesized that this modification of aerosol delivery would allow us to identify the ideal bronchodilator particle size and would potentially optimize therapeutic inhaled drug delivery.

METHODS

Subjects. Eighteen stable patients who fulfilled the American Thoracic Society diagnostic criteria for mild to moderate asthma were studied (Table 1): (2). All were nonsmokers, took inhaled albuterol for symptomatic relief, and were main-
tained on ≤500 μg inhaled beclomethasone dipropionate daily, but none was receiving long-acting β2-agonists or oral antiasthmatic medication. Each showed ≮15% improvement in their screening forced expiratory volume in 1 s (FEV1) with 200 μg of albuterol inhaled from a metered-dose inhaler (MDI) and spacer. Two actuations of 100 μg were separately delivered, and after each administration, patients inhaled slowly from functional residual capacity (FRC), followed by a breath-hold pause of 10 s and then a slow exhalation. The study was approved by the Ethics Committee of the Royal Brompton and Harefield Hospital National Health Service Trust, and patients gave their written, informed consent.

Study design. The study was randomized, crossover, and placebo controlled. During four visits, monodisperse aerosols of albuterol sulfate, 1.5-, 3-, and 6-μm mass median aerodynamic diameter (MMAD) in size, were administered as the active treatments, whereas the carrier formulation (ethanol-water) for albuterol was used as placebo. At each visit, the monodisperse aerosols were delivered in a cumulative dosing schedule of 10, 20, 40, and 100 μg at 0, 30, 60, and 90 min, respectively. Patients attended at the same time of day with a minimum washout period of 48 h between visits, and bronchodilator medication and caffeinated beverages were withheld for at least the previous 12 h.

Aerosol generation and delivery. Monodisperse aerosols (geometric SD <1.22) were generated by an air-driven spinning-top aerosol generator (STAG) (Mark II, Research Engi-
neers, London, UK), as previously described (6). Briefly, a solution of albuterol sulfate was supplied to the center of a spinning disk, and by altering the disk speed and drug concentration, different-sized aerosols were generated. Aerosol particle size, stability, and delivered drug dose were validated as previously reported (6). In particular, the drug concentration measured by an aerodynamic particle sizer (APS), was validated with filters at the inhalation port of the STAG.

Patients inhaled 1-liter single breaths of aerosol from FRC, via the inhalation port leading from the STAG chamber, at an inspiratory flow rate between 30 and 60 l/min guided by a visual indicator, followed by a 10-s breath-hold pause. Patients practiced the inhalation maneuver by using room air at the beginning of each visit. The volume of air inhaled and the breathing pattern were controlled by using a pneumotachograph attached to an electronic control circuit. The number of 1-liters required to achieve each dose were predetermined. Patients consecutively inhaled one breath for the first (10 μg) and second (10 μg) doses, two breaths for the third (20 μg), and four breaths for the fourth (60 μg) dose. There was no variation in the number of breaths for each dose, between different particle size treatments, or between patients.

The number of particles within a given aerosol dose decreases by a factor of 8 on doubling the size of the particles, and each individual 6-μm particle carries 64 times the drug mass of an individual 1.5-μm particle (23). However, for our study, the number of particles within each aerosol distribution was not important. Rather, the mass of the drug delivered at a given MMAD was the significant and relevant common denominator, and this was constant for the three different-sized aerosols delivered at the mouth. Essentially, the study was investigating how asthmatic airways handle bronchodilator aerosols of different-sized MMAD. From the experimental data of Fuchs (12), and correcting for particle density, we estimated the expected settling velocity of the 1.5-, 3-, and 6-μm particles to be 0.068, 0.137, and 0.82 cm/s, respectively. All three particle sizes will have a higher deposition probability because of the increased airway residence time afforded by the 10-s breath-hold pause. During this time, most of the 3- and 6-μm particles will deposit in the airways, whereas the 1.5-μm particles will have a higher deposition probability distally, where the airway diameter is less than the distance sedimented in 10 s (34).

Clinical measurements and protocol. A medical practitioner who was unaware of the aerosol administered undertook all physiological measurements. Baseline spirometry was performed following a standardized protocol (3). FEV1, forced vital capacity (FVC), forced expiratory flow between exhalation of 25 and 75% of FVC (FEF25-75), and peak expiratory flow (PEF) were measured by using a calibrated portable electronic spirometer (Vitalograph, Buckingham, UK). Patients were rescheduled if their baseline FEV1 was not within 15% of the screening FEV1 value. Blood samples (2.5 ml) were obtained via an indwelling intravenous forearm line and metered at the inhalation port of the STAG. Details of the protocol and measurements were previously described (9).

Table 1. Patient demographic data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
</tr>
<tr>
<td>Age, yr</td>
<td>33.0 ± 8.0</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.70 ± 0.08</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67.0 ± 11.0</td>
</tr>
<tr>
<td>Screening spirometry</td>
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<tr>
<td>Predicted FEV1, liters</td>
<td>3.47 ± 0.63</td>
</tr>
<tr>
<td>FEV1, liters</td>
<td>2.59 ± 0.66</td>
</tr>
<tr>
<td>FEV1, %predicted</td>
<td>74.3 ± 13.8</td>
</tr>
<tr>
<td>FEF25-75, liters</td>
<td>1.67 ± 0.86</td>
</tr>
<tr>
<td>Postbronchodilator reversibility</td>
<td></td>
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<tr>
<td>FEV1, ml</td>
<td>608 ± 186</td>
</tr>
<tr>
<td>FEV1, %predicted</td>
<td>24.5 ± 8.6</td>
</tr>
</tbody>
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n, No. of subjects; FEV1, forced expiratory volume in 1 s; FEF25–75, forced expiratory flow between exhalation of 25 and 75% of forced vital capacity.
PEF, FVC) and heart rate measurements were taken at 10-min intervals, and plasma potassium measurement was taken after 20 min. Additional measurements were undertaken at 10-min intervals (spirometry and heart rate) and 20 min (plasma potassium) after further aerosol doses at 30 min (10-μg dose), 60 min (20-μg dose), and 90 min (60-μg dose).

Final measurements were taken at 120 and 150 min. The total cumulative dose schedule resulted in 10, 20, 40, and 100 μg of albuterol being delivered.

**Statistical analysis.** An intention to treat approach was adopted in the analysis of efficacy and tolerability. For each treatment, physiological response-time curves were obtained by plotting the mean change of each measured variable from baseline values against time. The baseline (time 0) of all outcome variables was the value taken 5 min before dosing. The weighted area under the curve (wAUC) was used in the statistical comparison of treatment effects. The area under the curve (AUC) is commonly used to summarize the information from a series of physiological measurements from an individual (1). AUC was calculated by summing the area under the curve between each pair of consecutive measurements for the entire response-time curve by using the trapezium rule. The resulting value was standardized for time of follow-up, dividing by the number of minutes the patient had measurements performed, to give the wAUC.

The primary end point was wAUC FEV1 (AUC of FEV1 mean change from baseline, standardized for time). Secondary end points were AUC for FEF25–75, PEF, FVC, potassium concentration and heart rate. The study was analyzed by using analysis of covariance, including factors for treatment, baseline value, subject, period, and randomization sequence using analysis of covariance, including factors for treatment, subject, period, and randomization sequence. The area under the curve (AUC) is commonly used to summarize the information from a series of physiological measurements from an individual (1). AUC was calculated by summing the area under the curve between each pair of consecutive measurements for the entire response-time curve by using the trapezium rule. The resulting value was standardized for time of follow-up, dividing by the number of minutes the patient had measurements performed, to give the wAUC.

The primary end point was wAUC FEV1 (AUC of FEV1 mean change from baseline, standardized for time). Secondary end points were AUC for FEF25–75, PEF, FVC, potassium concentration and heart rate. The study was analyzed by using analysis of covariance, including factors for treatment, baseline value, subject, period, and randomization sequence in the model. Because there was only one primary end point, no adjustment for multiple comparisons was required. With 18 patients, there was 80% power to detect a clinically relevant difference of 0.25 liter in FEV1 between the treatments, assuming a significance level of 0.05 and an estimate of variability of 0.25 liter.

**RESULTS**

All 18 patients completed the study without any noticeable adverse effects. Fifteen were steroid-naive asthmatic patients. The mean percent difference between the baseline and screening FEV1 values was 0.31 ± 6.39% (SD), indicating that the with-in-patient FEV1 variability was minimal. The mean baseline FEV1 values at the start of the study visits were similar between the four treatment groups: placebo = 2.52 ± 0.65 (SD), 1.5 μm = 2.58 ± 0.58, 3 μm = 2.60 ± 0.60, and 6 μm = 2.55 ± 0.56 liters (P > 0.05).

**Particle size effects on airway function.** A steep increase in FEV1 was observed with inhalation of each particle size, beginning within 10 min after dosing, and it progressively rose to reach plateau of the dose-response relationship over the study time period (Fig. 1). This suggests that the monodisperse aerosol doses were on the linear part of the dose-response curve. The larger particles produced the greatest clinical response in the order 6 μm > 3 μm > 1.5 μm > placebo. Significant differences were observed between the 6- and 1.5-μm aerosols (P < 0.01), between the 3- and 1.5-μm aerosols (P = 0.01), and between all particle sizes and the placebo (P < 0.001) (see Fig. 3). Although the 6-μm particles were clinically more efficacious than those 3 μm in size, this difference was not significant (P = 0.85) (Fig. 1).

The results for FEF25–75 closely reflected those obtained for FEV1 (Fig. 1). The 6-μm particles achieved a greater therapeutic response compared with the 3-μm aerosol (P = 0.08) and 1.5-μm aerosol (P < 0.01) particles. No significant difference was observed between the 3- and 1.5-μm aerosols (P = 0.09). Each particle size produced a highly significant improvement compared with placebo (P < 0.001) (see Fig. 3).

**Dose effects on particle size.** Successive doses of albuterol produced incremental increases in FEV1 and FEF25–75 such that higher doses achieved a greater degree of bronchodilation for all particle sizes (Fig. 2). At each dose, the 6-μm aerosols were the most potent for these lung function parameters after adjustment for baseline values. After the 20-μg cumulative dose, 6- and 3-μm aerosols achieved 79 ± 9.1% (SD) and 76 ± 9.9% of their maximal monodisperse FEV1 response, respectively.

**Comparison to MDI.** For the 3- and 6-μm aerosols, a cumulative 20-μg monodisperse albuterol aerosol was as efficacious as the 200-μg MDI screening FEV1 response (Fig. 2). At plateau, a cumulative 100-μg dose for the 6- and 3-μm aerosols achieved a greater increase in FEV1 than the screening MDI 200-μg dose [746 ± 272 (SD), 713 ± 319, and 608 ± 186 ml, respectively]; however,
these differences were not significant, whereas the 1.5-μm aerosols (569 ± 315 ml) were unable to match the MDI response. The polydisperse size distribution of the MDI was 2.7 (mean MMAD) and 1.5 (mean geometric SD) (n = 5 experiments).

**Other lung function variables.** All particle sizes produced significantly higher values than placebo (P < 0.01) for FVC and PEF; however, there was no statistical difference observed among the particle sizes (Fig. 3). The trend seen with PEF, though, was consistent with a greater effect of the 6-μm aerosols.

**Tolerability variables.** There were no clinically relevant or significant differences observed in the heart rate and plasma potassium concentrations when each particle size was compared with placebo (Fig. 4).

**DISCUSSION**

We used monodisperse albuterol aerosols of 1.5-, 3- and 6-μm MMAD to investigate the effects of bronchodilator particle size in asthmatic patients. Our results demonstrate that the larger particles were more potent bronchodilators achieving the greatest improvement in FEV₁ and FEF₂₅₋₇₅ and that they enabled a reduction in the delivered drug dose without compromising the clinical response, such that a 20-μg dose of the 6- and 3-μm aerosols was as efficacious as 200 μg from a MDI (Fig. 2). All aerosols were shown to be safe because no adverse effects were observed. We believe the observed differences result from the preferential innate physical deposition properties of the particle sizes chosen, in that the larger particles were better matched to their target site of action within the airways.

β₂-Agonists achieve bronchodilation by stimulating β₂-adrenoreceptors to relax airway smooth muscle. Although β₂-receptor density is greatest within the alve-
be targeted (4, 10). We hypothesize the importance of delivering the 6- and 3-\(\mu\)m aerosols were able to selectively deposit to a greater extent within the conducting region, and evidence from predictive experimental models on airway particle deposition supports this (13, 15). Heyder et al. (15) showed greater total deposition of 6-\(\mu\)m particles in the tracheobronchial (conducting) region compared with 3-\(\mu\)m particles, whereas all the 1.0-\(\mu\)m particles deposited within the alveolar compartment. These deposition characteristics could explain the better bronchodilator response observed with the 6-\(\mu\)m aerosol over the poorer effect of the 1.5-\(\mu\)m aerosol. Although greater oropharyngeal deposition would be anticipated with the 6-\(\mu\)m aerosol, Ruffin et al. (30) found \(\beta_2\)-agonist delivered to the buccal cavity did not contribute toward bronchodilation. The regional airway mucosal drug concentration also determines the therapeutic response to inhaled drug, and, as a result of the exponential increase in airway surface area, topical drug concentration within the conducting airways will be greater than in the lung periphery (7). Hence, higher conducting airway concentrations, and therefore greater bronchodilator potential, will be achieved with the 6- and 3-\(\mu\)m aerosols than with the 1.5-\(\mu\)m aerosols delivered to the alveoli (13). Our data reinforce the importance of delivering \(\beta_2\)-agonists to the conducting region for bronchodilator efficacy, and it may be to our advantage to target regional airways as a function of aerosol particle size.

Conversely, the deposition characteristics of the 1.5- and 6-\(\mu\)m aerosols would suggest a greater likelihood for adverse events as a result of systemic absorption of deposited drug from the alveolar and oropharyngeal regions, respectively. We, however, administered very small doses, which may predictably explain the absence of clinically significant differences within the tolerability variables. Indeed, Bennett et al. (5) failed to observe a significant change in heart rate or plasma potassium in asthmatic patients after 100 \(\mu\)g of albuterol optimally delivered from a MDI. Inhaler devices overcome their inefficiency in lung deposition, by compensating with higher drug doses delivered at the mouth. The effective dose range of MDI albuterol in asthmatic patients has been reported as between 100 and 4,000 \(\mu\)g, although conventional doses used in everyday clinical practice (200–400 \(\mu\)g) achieve a good therapeutic response (21). Although we did not undertake a cumulative dose response with the MDI, our findings agree with earlier observations that lower doses of monodisperse aerosol may be used to achieve similar degrees of bronchodilation (36).

In contrast to previous findings, our data show that the 6-\(\mu\)m particles were as efficacious as 3-\(\mu\)m particles and that both significantly achieved a better clinical response than the 1.5-\(\mu\)m particles (27, 35). Differences in aerosol delivery, patient characteristics, and methodology between studies may have contributed to the different results. Fast inspiratory flow rates from a MDI have been shown to achieve less lung deposition (11, 26). By inhaling the actuated MDI drug through a spacer, the dispersed aerosol cloud slows down, and there is less need for coordination by the patient. Both these modifications minimize the high oropharyngeal impaction component of the dose. In our system, the STAG chamber acts like a holding reservoir analogous to a MDI and spacer, and we postulate our slower inhalation maneuver allowed longer retention of the monodisperse 6-\(\mu\)m particles within the air stream, thereby minimizing oropharyngeal impaction and consequently greater aerosol delivery to the conducting airways. This may partly account why our larger particles were more efficacious than those in previous studies (27, 35). Indeed, Svartengren et al. (32) found that 6-\(\mu\)m particles deposited in more distant airways with slow deep breathing.

We employed a constant inhaled volume and number of breaths to achieve the required dose between and within our patients to minimize intrapulmonary variations in drug dose and distribution. Previous reports, however, varied the inhaled volume delivered to achieve the target dose, so there may not have been any aerosol delivery or consistency in the depth and distribution of the airways and receptor sites reached, and this may have affected the observed clinical responses (35). Also, in these earlier studies, all patients were steroid treated, which may have masked the full potential of their bronchodilator response, whereas 15 of our 18 asthmatic patients were steroid naive. As we
have described elsewhere (6), our methodology was robust, well validated, and consistent throughout the study. Importantly, unlike in previous studies (27, 35), we had validated the drug dose administered at the inhalation port of the STAG, as determined by an APS, against an established chemical analysis method (6).

In addition, we believe that we controlled patient factors that would appreciably influence drug deposition such that the data presented are a reflection of the intrinsic behavior of the physical property of interest, i.e., aerodynamic particle size. The inherent asthmatic airways variability of our patients was controlled by ensuring that their FEV1 at the start of each visit was within 15% of their screening value. The degree of airway narrowing in our patients, though, did not influence the clinical response because no correlation was observed between baseline FEV1 and maximal bronchodilation achieved with each particle size (data not shown). It is conceivable, however, that there was better matching, particularly of the larger aerosols to their target site of action, because airway narrowing favors more proximal aerosol deposition (20). Conversely, progressive bronchodilation was observed with our cumulative-dose regimen, which may have allowed successive doses to reach more peripheral airways (9). Clinical factors beyond our control that may have influenced our data include hygroscopic growth and blood flow redistribution. Particle growth within the humid lung environment is a complex process dependent on its chemical composition, airway residence time and aerodynamic size; however, sparse data support such in vivo deposition behavior with pharmacological agents (31). In fact, growth may be a misnomer, because bronchodilator aerodynamic particle size may actually decrease with water vapor absorption as a result of lowering of the overall droplet density (22). There are also limited data in vivo to support the bronchial circulation as a local redistribution system to other lung regions after inhaled drug treatment, and venous admixture would significantly dilute any absorbed drug effect (33). We observed early and sustained separation of particle size effects, more marked at plateau, which suggests blood redistribution may not be that important. Urinary salbutamol detection may have indicated different rates of absorption for the different-sized albuterol aerosols, and we would hypothesize greater systemic absorption with the smaller particles, implying more peripheral lung deposition (16).

We cannot directly infer that our observations favor regional airway over total lung deposition, because tests of forced expiration are unable to accurately distinguish changes in small-airway function from more proximal airways. Although FEV1 is considered more of a proximal airway marker than FEF25–75, there is considerable overlap, because changes in the conducting airways contribute toward both indexes. Indeed, the similarity of responses observed with FEV1 and FEF25–75 would support this. The trend seen with PEF, a large-airway index, was consistent with a greater effect of the 6-µm aerosol as a result of relative proximal airway deposition. However, because there is greater population variability and effort dependence with PEF, this may account for the magnitude of the response observed with the 1.5-µm aerosol.

In conclusion, our results demonstrate that, for β2-agonists, there may be a range of optimal bronchodilator particle sizes that deliver greatest clinical efficacy, rather than a single size per se. Notably, we have shown these to be larger 3- and 6-µm particles, in the higher part of the respirable range, rather than small 1.5-µm particles. Monodisperse aerosols enable significant reductions of the inhaled dose without compromising efficacy and thus have potential for improving therapeutic drug delivery to the lungs. With intense interest in the evolution of new inhaler technologies (14), monodisperse pharmacological aerosols are able to address essential scientific concepts of inhalational aerosol research in vivo. Further studies investigating the importance of regional airway targeting may guide the future development of more efficient and purpose-specific inhalers.

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DISCLOSURES

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REFERENCES


