Use of Supporting Laboratory or Clinical Data in Caries Clinical Trials

W. H. BOWEN

University of Rochester, Department of Dental Research, 601 Elmwood Avenue, Rochester, New York 14642

J Dent Res 63(Spec Iss):757-759, May, 1984

In the course of development of chemotherapeutic or preventive agents, it is usual to assess their potential effectiveness before reaching the obvious end point of a clinical study. For example, in a clinical trial to determine the therapeutic effect of an antimicrobial agent, it is not uncommon to monitor levels of the test material in appropriate body fluids. In addition, various specimens are obtained to determine whether the infectious agent is being eliminated or brought under control. Similarly, in the development of vaccines, it is usual to ascertain antibody response to either the whole cells or a defined antigen, and eventually attempt to correlate antibody response to protection. Thus, it is frequently possible to predict the outcome of a clinical trial on the basis of laboratory tests long before the conclusion of the investigation.

However, in the conduct of clinical trials in dentistry to determine the effect of a cariostatic agent, we have been content to assess effectiveness, for the most part, on the basis of the number of new caries lesions in control and test children. In rare instances, biochemical (Birkeland *et al.,* 1971) and microbiological studies have been conducted concurrently, and these will be referred to below.

Because so few laboratory tests have been carried out in conjunction with clinical trials, we find that little information is available on fluoride levels in plaque and saliva from subjects who participate in fluoride mouthrinsing trials and dentifrice studies. The net result is that we now find ourselves conducting a series of *ad hoc* clinical trials to determine the effect, for example, of various fluoride regimes continuing for at least two years, costing several hundred thousand dollars. Because of declining caries increment rates, difficulty in obtaining suitable populations, and increasing expense, it is imperative that appropriate laboratory tests be used to ensure that clinical trials do not continue longer than is essential. Monitoring of biochemical changes can in many instances be used to test whether subjects are using the test preparations appropriately; in some clinical trials, perhaps we are measuring compliance with a regimen and not therapeutic efficacy. Use of laboratory tests will often help to elucidate the mechanism of action of the test substance. Satisfactory use of laboratory tests and, indeed, perhaps trials in animals could eventually obviate the need for the presently required two clinical trials necessary for FDA approval.

In assessing the potential effect of any substance for prevention of dental caries, we can attempt to examine its effect on the tooth and/or plaque or saliva. Any proposed test must not only be simple to carry out but also must clearly not require complicated procedures in the field. Sources of material upon which to carry out a laboratory test are limited. Clinical information is usually limited to presence or absence of caries or tooth staining. Routine enamel biopsies of teeth, even if they are informative, probably are not practical to carry out in clinical trials (Mellberg *et al.,* 1973). Saliva and dental plaque are the only materials readily available to us on which to conduct laboratory tests. Because dental plaque is without a doubt related to the pathogenesis of dental caries, we are fortunate that we can carry out direct biochemical and bacteriological examinations. Saliva usually provides more indirect information, largely because we do not know biochemical values of salivary constituents associated with susceptibility, onset, and progress of dental caries (Mandel, 1978). However, in contrast, there is a growing amount of microbiological information which appears to show that there is a good correlation in populations between numbers of *Strep. mutans* and *Lactobacilli* in saliva and the prevalence and incidence of dental caries. Estimations of populations of these micro-organisms are routinely used in the cariology clinic in the University of Göteberg to identify highly-caries-susceptible subjects and to monitor the effect of cariostatic procedures (Zickert *et al.,* 1982). I believe that the use of salivary counts of these "indicator" organisms could lead to selection of more homogeneous study populations, and I suspect that by including only those who appear to be most susceptible, the number of subjects required in a clinical trial could be reduced. The procedures required are simple, relatively non-time-consuming, and are readily available in most microbiological laboratories.

Dental plaque, the other easily accessible material, has, under certain well-defined circumstances, biochemical properties which are apparently associated with lack of pathogenicity. Plaque from persons or sub-human primates which receive their entire diet by gastric intubation lacks the ability to lower the pH of sugar solutions (Bowen, 1974; Littleton *et al.,* 1967). Plaque from persons or primates with restricted sugar intake has less acid-producing ability than that from other subjects. Indeed, the acidogenic potential of plaque probably provides the explanation of difference in caries scores in two populations in Colombia, S.A. Sugar solutions prepared in water from the low-caries town lowered the pH of plaque little, compared with sugar solutions in water from the high-caries town (Bowen *et al.,* 1977).

Other chemical and biochemical procedures can provide useful information. Several epidemiological studies have shown that there is a strong inverse relationship between concentration of fluoride in dental plaque and the prevalence of dental caries (Dawes *et al.,* 1965; Stiles *et al.,* 1979). Although the determination of fluoride in dental plaque is not as easy to carry out as are microbiological counts in saliva, nevertheless, the procedure is not difficult. There is also some epidemiological evidence which suggests that the concentrations of calcium and phosphate in dental plaque are inversely related to the prevalence of caries (Ashley and Wilson, 1977; Ashley, 1975). Although this relationship appears to be somewhat dependent on the sites in the mouth from which plaque is removed, the association is nevertheless clear.

From the foregoing, it is apparent that the potential, at least, exists to use several laboratory tests during the course of a clinical trial. These might be helpful in predicting the outcome and provide supporting data for clinical observations which frequently are subjective. Furthermore, they might reduce the costs associated with having large populations.

The laboratory tests selected must, for the most part, be determined by the predicted mode of action of the therapeutic agent. The value of the laboratory tests will clearly be enhanced if they have been used in forming the study

groups. For example, if populations have been selected on the basis of the number of *Strep. mutans* or *Lactobacilli* that they harbor, then treatment effects reflected in changes of these populations will be readily detected.

Overall, disappointingly few clinical trials have used laboratory data to support their clinical observations or to predict the outcome of studies. There are, however, a few studies available which serve to illustrate the manner in which laboratory tests may be used and also their potential value in clinical studies. One of the best examples can be found in studies related to the introduction of calcium glycerophosphate as a cariostatic agent (Bowen, 1969 and 1972). Although this material may not be the best cariostatic agent available, its manner of introduction serves as a useful model.

Several years ago, we observed that the inclusion of calcium glycerophosphate in a sugar solution prevented pH fall in monkey plaque. Based on that observation, we included calcium glycerophosphate in sugar fed between meals to a small group of primates. We observed a significant reduction in caries over a three-year period. Perhaps just as interesting as the effect detected on caries were the differences in the volume and biochemical composition of plaque that we saw in the primates. We noted that the calcium content of plaque was enhanced.

Subsequently, investigators attempted to raise the concentration of calcium and phosphate in human plaque by having small groups of humans allow tablets or lozenges containing calcium glycerophosphate to dissolve slowly in their mouths (Brook *et al.*, 1975). Elevated levels of calcium and phosphate were detected in plaque. A clinical trial (Naylor and Glass, 1979) was conducted in which the effects of a fluoride dentifrice containing calcium glycerophosphate were compared with those of a fluoride dentifrice containing calcium carbonate. In a subset of the study population, differences in the composition of plaque were detected (Duke *et al.*, 1979). Two examiners showed that the calcium glycerophosphate-containing dentifrice gave consistently higher protection than did the control, even though the difference was not statistically significant.

The combination of laboratory tests (Forward *et al.*, 1979) with the clinical observation leads one to be confident that calcium glycerophosphate is having a protective effect. Furthermore, based on observations that there are elevated calcium and phosphate levels in plaque from animals (Bowen, 1974) that receive their entire diet by gastric intubation, it could be predicted that any agent that results in enhanced levels of these elements in plaque would be cariostatic.

There is unequivocal evidence that the concentrations of fluoride in dental plaque and saliva are related to the presence of fluoride in drinking water, and furthermore that fluoride in dental plaque contributes to the levels of fluoride in enamel (Jenkins and Edgar, 1977; Klimek *et al.*, 1982; Charlton *et al.*, 1974). Nevertheless, it has been difficult to find studies in which the levels of fluoride in plaque and saliva have been monitored in the course of a clinical trial to determine the effect of a topical application of fluoride preparation (Birkeland *et al.*, 1971). It appears inconceivable that serum levels of a drug undergoing a clinical trial to determine its effect on, for example, a systemic infection would not be monitored. Nevertheless, in caries research, for the most part we appear satisfied to determine the final clinical result without determining the level of the therapeutic agent in oral fluids (Poulsen *et al.*, 1981). Indeed, the plethora of clinical trials comparing the effects of various topical fluoride regimens might well have been circumvented if such studies had been conducted in the past.

The evidence calling for systematic studies is overwhelming. Significantly higher levels of fluoride were found in plaque (Dawes *et al.*, 1965) from subjects residing in an area where the water contained 2 ppm fluoride compared with that found in subjects from a low fluoride area. Stiles *et al.* (1979) have also observed high levels of fluoride in plaque from subjects residing in areas where water contained up to 4 ppm fluoride. We have shown that monkeys (Bowen, 1973) given 2 ppm fluoride in their drinking water for five years had three to four times more fluoride in their plaque than did control animals.

All the available evidence appears to suggest that the level of protection against caries from fluoride is for the most part dependent on the ambient levels of fluoride in the oral environment (Birkeland and Lokken, 1972). Studies conducted in rodents by Larson *et al.* (1976) and by Mirth *et al.* (1982) using fluoride-releasing devices are consistent with this hypothesis. A clinical trial conducted by Stephen and Campbell (1978) – who achieved over 80% protection against caries using fluoride tablets – is also consistent with this concept. Indeed, McCall *et al.* (1981) showed that by allowing unflavored tablets to dissolve slowly in the mouth (as opposed to sucking or chewing them), protracted elevation of the fluoride levels could be achieved, and they attributed the high level of protection to this phenomenon in saliva. Unfortunately, fluoride levels in plaque were not determined.

A fine example of the use of laboratory data can also be found in the Turku sugar studies (Makinen and Scheinin, 1974). In this investigation, a group of humans had most sucrose in their diet substituted by xylitol. A range of biochemical and microbiological tests on oral fluids was conducted, all of which were aimed at both determining the effects of xylitol on the physiology of the mouth and correlating any observed phenomena with reduction in the prevalence of caries.

Several notable effects were observed long before any effect on caries could be recorded. There was a marked decline in the acidogenic flora of the saliva. In addition, in a comparatively short period, ingestion of xylitol was followed by an increase in the concentration of salivary lactoperoxidase. This enzyme is believed to contribute to the antimicrobial properties of saliva. Proteinases and transaminases increased in saliva together with the concentration of amino acids. The ratio of protein to carbohydrate in plaque increased during the consumption of xylitol. Thus, it is apparent that in the Turku studies the ingestion of xylitol resulted in increased nitrogen metabolism in plaque and whole saliva, a phenomenon clearly associated with cariostasis. Subsequently, it was observed that other sugar alcohols have comparable effects.

Although we have not yet reached the stage of clinically testing a caries vaccine, it is clear, nevertheless, that before we conduct a full-scale trial, the antibodies reactive with cariogenic organisms will have to be monitored in saliva in small groups of subjects. Furthermore, if a full clinical trial is conducted, the antibody response will be determined in the study population. The appropriate route of administration will have to be determined before trials are initiated. We will never have the luxury of determining in a full clinical study whether an immunogen is more effective given orally than when given intramuscularly or by a combination of routes.

There is a growing interest in the development of safe and effective antimicrobial agents for oral use (Luoma *et al.*,

1978). There are many agents which have low minimal effective doses, and which are bactericidal to a wide spectrum of oral micro-organisms. For example, several quaternary ammonium compounds are more effective *in vitro* than is chlorhexidine, yet, paradoxically, chlorhexidine is much more effective clinically than are the many other compounds. Laboratory tests reveal that antimicrobial levels of chlorhexidine can be detected in saliva up to eight hours after a single rinse (Bonesvoll *et al.*, 1974; Gjermo *et al.*, 1975). Quaternary ammonium compounds, in contrast, are rapidly eliminated or inactivated in the mouth. This observation has helped to identify one of the most important properties that an effective oral antiseptic must possess and indicates that agents not retained in the mouth for reasonable periods are likely to be ineffective. Furthermore, it provided strong evidence for the molecular structure of agents that might be retained in the mouth sufficiently long to exercise their maximum therapeutic potential.

In conclusion, I believe it is in the best interests of industry, the clinical investigator, and, most importantly, the patient that appropriate laboratory tests be carried out in all clinical trials. In the future, I hope that those who sponsor clinical trials will insist that supporting laboratory data be sought in all studies. Clinical trials are far too important to be left to clinicians.

REFERENCES

ASHLEY, F.P.: Calcium and Phosphorus Concentrations of Dental Plaque Related to Dental Caries in 11- to 14- year-old Male Subjects, *Caries Res* 9:351-362, 1975.

ASHLEY, F.P. and WILSON, R.F.: Dental Plaque and Caries. A 3-year Longitudinal Study in Children, *Br Dent J* 142:85-91, 1977.

BIRKELAND, J.M.; JORKJEND, L.; and von der FEHR, F.R.: The Influence of Fluoride Rinses on the Fluoride Content of Dental Plaque in Children, *Caries Res* 5:169-179, 1971.

BIRKELAND, J.M. and LOKKEN, P.: The Pharmacokinetics of Fluoride in Mouth Rinses as Indicated by a Reference Substance (^{51}Cr-EDTA), *Caries Res* 6:325-333, 1972.

BONESVOLL, P.; LOKKEN, P.; RØLLA, G.; and PAUS, P.N.: Retention of Chlorhexidine in the Human Oral Cavity after Mouth Rinses, *Arch Oral Biol* 19:209-212, 1974.

BOWEN, W.H.: Effect of Restricting Oral Intake to Invert Sugar or Casein on the Microbiology of Plaque in *Macaca fascicularis* (Irus), *Arch Oral Biol* 19:231-239, 1974.

BOWEN, W.H.: The Cariostatic Effect of Calcium Glycerophosphate in Monkeys, *Caries Res* 6:43-51, 1972.

BOWEN, W.H.: The Effect of Fluoride and Molybdate on Caries Activity in Monkeys (*Macaca fascicularis*), *Br Dent J* 135:489-493, 1973.

BOWEN, W.H.; VELEZ, H.; AGUILA, M.; VELASQUEZ, H.; SIERRA, L.I.; and GILLESPIE, G.: The Microbiology and Biochemistry of Plaque, Saliva and Drinking Water from Two Communities with Contrasting Levels of Caries in Colombia, S.A., *J Dent Res* 56(C):33-39, 1977.

BOWEN, W.H.: The Monitoring of Acid Production in Dental Plaque in Monkeys, *Br Dent J* 126:506-508, 1969.

BROOK, A.H.; GAWTHORPE, J.; and WINTER, G.B.: Calcium Glycerophosphate and Dental Plaque, *Caries Res* 9:156-162, 1975.

CHARLTON, G.; BLAINEY, B.; and SCHAMSCHULA, R.G.: Associations between Dental Plaque and Fluoride in Human Surface Enamel, *Arch Oral Biol* 19:139-143, 1974.

DAWES, C.; JENKINS, G.N.; HARDWICK, J.L.; and LEACH, S.A.: The Relation between the Fluoride Concentrations in the Dental Plaque and in Drinking Water, *Br Dent J* 119:164-167, 1965.

DUKE, S.A.; REES, D.A.; and FORWARD, G.C.: Increased Plaque Calcium and Phosphorus Concentrations after Using a Calcium Carbonate Toothpaste Containing Calcium Glycerophosphate and Sodium Monofluorophosphate, *Caries Res* 13:57-59, 1979.

FORWARD, G.C.; BELL, M.A.; and DUKE, S.A.: Improving the Performance of Sodium Monofluorophosphate to Protect Enamel against Acid, *Caries Res* 13:61-67, 1979.

GJERMO, P.; BONESVOLL, P.; HJELJORD, L.G.; and RØLLA, G.: Influence of Variation of pH of Chlorhexidine Mouth Rinses on Oral Retention and Plaque-inhibiting Effect, *Caries Res* 9: 74-82, 1975.

INGRAM, G.S. and NASH, P.F.: A Mechanism for the Anticaries Action of Fluoride, *Caries Res* 14:298-303, 1980.

JENKINS, G.N. and EDGAR, W.M.: Distribution and Forms of F in Saliva and Plaque, *Caries Res* 11(Suppl. 1):226-242, 1977.

KLIMEK, J.; HELLWIG, E.; and AHRENS, G.: Fluoride Taken up by Plaque, by the Underlying Enamel and by Clean Enamel from Three Fluoride Compounds *in vitro*, *Caries Res* 16:156-161, 1982.

LARSON, R.H.; MELLBERG, J.R.; ENGLANDER, H.R.; and SENNING, R.: Caries Inhibition in the Rat by Water-borne and Enamel-bound Fluoride, *Caries Res* 10:321-331, 1976.

LARSON, R.H.; MELLBERG, J.R.; ENGLANDER, H.R.; and SENNING, R.S.: Relation of Ambient and Enamel Fluoride (F) to Rat Caries, IADR *Progr & Abst* 53:No. 864, 1974.

LITTLETON, N.W.; McCABE, R.M.; and CARTER, G.H.: Studies of Oral Health in Persons Nourished by Stomach Tube. II. Acidogenic Properties and Selected Bacterial Components of Plaque Material, *Arch Oral Biol* 12:601-609, 1967.

LUOMA, H.; MURTOMAA, H.; NUUJA, T.; NYMAN, A.; NUMMIKOSKI, P.; AINAMO, J.; and LUOMA, A.-R.: A Simultaneous Reduction of Caries and Gingivitis in a Group of Schoolchildren Receiving Chlorhexidine-Fluoride Applications. Results after 2 Years, *Caries Res* 12:290-298, 1978.

MAKINEN, K. and SCHEININ, A.: Turku Sugar Studies. II. Preliminary Biochemical and General Findings, *Acta Odontol Scand* 32:413-421, 1974.

MANDEL, I.D.: Salivary Factors in Caries Prediction. In: **Methods of Caries Prediction**, Bibby, B.G. and Shern, R.J., Eds., Washington and London: Information Retrieval, Inc., 1978, pp. 147-158.

McCALL, D.; STEPHEN, K.W.; and McNEE, S.G.: Fluoride Tablets and Salivary Fluoride Levels, *Caries Res* 15:98-102, 1981.

MELLBERG, J.R.; NICHOLSON, C.R.; and TRUBMAN, A.: The Acquisition of Fluoride by Tooth Enamel *in vivo* from Self-applied APF Gel and Prophylaxis Paste, *Caries Res* 7:173-178, 1973.

MIRTH, D.B.; SHERN, R.J.; EMILSON, C.; ADDERLY, D.; LI, SHOU-HUA; GOMEZ, I.; and BOWEN, W.: Clinical Evaluation of an Intraoral Device for the Controlled Release of Fluoride, *JADA* 105:791-797, 1982.

NAYLOR, M.N. and GLASS, R.L.: A 3-year Clinical Trial of Calcium Carbonate Dentifrice Containing Calcium Glycerophosphate and Sodium Monofluorophosphate, *Caries Res* 13:39-46, 1979.

POULSEN, S.; GADEGAARD, E.; and MORTENSEN, B.: Cariostatic Effect of Daily Use of a Fluoride-containing Lozenge Compared to Fortnightly Rinses with 0.2% Sodium Fluoride, *Caries Res* 15:236-242, 1981.

STEPHEN, K.W. and CAMPBELL, D.: Caries Reduction and Cost Benefit after 3 Years of Sucking Fluoride Tablets Daily at School. A Double-blind Trial, *Br Dent J* 144:202-206, 1978.

STILES, H.M.; BOWEN, W.H.; WITTIG, A.B.; and BRUNELLE, J.: Plaque Fluoride Concentrations and Caries Prevalence in 12-18-year-old Children, IADR *Progr & Abst* 58:No. 1343, 1979.

ZICKERT, I.; EMILSON, C.; and KRASSE, B.: *Streptococcus mutans, Lactobacilli* and Dental Health in 13-14-year-old Swedish Children, *Community Dent Oral Epidemiol* 10:77-81, 1982.