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Quantification of Corticosteroid-Induced Skin Vasoconstriction: Visual Ranking, Chromameter Measurement or Digital Imaging Analysis

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Corticosteroids · Vasoconstriction · Bioequivalence · Visual ranking · Chromameter · Digital imaging

Abstract
Topical corticosteroid formulations have been evaluated by visual grading protocols for many years. Toward a more objective methodology, several instrumental methods have been evaluated for applicability in quantifying the vasoconstriction side-effect that follows corticosteroid application to the skin. Although the chromameter has been adopted by regulatory bodies throughout the world as the current standard for topical bioequivalence determinations, there is considerable criticism of this instrument from several quarters. A preliminary comparison reported here indicates that digital image analysis provides statistically significant results that are similar to those obtained by visual assessment techniques, and shows considerably greater precision than that obtained by the chromameter. Continued evaluation of objective assessment techniques, such as digital imaging, and continued modernisation of regulatory bioequivalence requirements will assist in protecting patients and optimising clinical results.

Introduction

Over the past 20 years it has become increasingly obvious that incorporating the same drug, at the same concentration, into different formulations of the same type (pharmaceutical equivalents), seldom produces formulations that are identical (or even similar) in drug delivery potential. These formulations may have markedly different clinical efficacies simply because of the differing potential of the vehicle to release the drug to the stratum corneum. Considerable effort has, therefore, been applied to the research of delivery vehicle optimisation and maximisation of the thermodynamic leaving potential of drugs in topical formulations [1]. Additionally, the (often marked) influence that the delivery vehicle itself may have on the skin barrier is seldom considered (or recognised) by researchers or clinicians [2]. An intricate part of this modern research effort has been the need to develop analytical systems capable of discriminating between the subtle drug delivery potentials of very similar formulations – especially important in topical bioequivalence testing.

After 40 years of topical corticosteroid delivery research and development, it is prudent to evaluate the status of the fundamental science that underlies the assay

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methodologies commonly employed. As yet we do not have answers to the following questions:

(1) What mass of a specific corticosteroid is required for a clinical effect? Theoretically, changing from one commercial product to a formulation judged to be equivalent should not affect the clinical outcome for the patient. In this respect, it is imperative to determine initially what degree of change in topical drug delivery is required to change the clinical response for the patient. Since much of the currently applied drug is not absorbed from the applied formulation (approximately 1% of the applied drug is absorbed), the 80–125% parameter for equivalence determination may not be appropriate for topical products.

(2) Do we have optimised vehicles to deliver the drug to the target site in the necessary concentrations? With the modern sophistication now available for the design of topical delivery vehicles (supersaturation, liposomes, penetration enhancers), it is possible to deliver the same mass of drug to the skin from much lower delivery formulation concentrations than those presently on the market. Almost all presently marketed preparations are gross overdoses [3, 4] of the drug in poorly designed delivery vehicles, exemplifying the status of the science 30 years ago.

(3) Are regulatory laws current with scientific technology? The emergence of the next generation of topical delivery vehicles may be hindered because of product registration regulations that are no longer applicable to today's technology.

The skin-blanching assay is unique in that the methodology utilises the localised vasoconstriction side-effect following topical corticosteroid application. The intensity of the induced vasoconstriction has been used for 50 years [5] to determine drug delivery to the skin, since the intensity of the blanching has been shown [6] to be proportional to the clinical efficacy of the formulation. McKenzie and Stoughton [7] developed the first documented, single-reading time, visual-assessment procedure for comparing corticosteroid performance. This methodology has been refined and improved over the decades [8–12] to the point where the multiple-reading, visual-assessment protocol, conducted by trained and experienced observers, is considered to be a highly sensitive and accurate tool in topical corticosteroid delivery vehicle research.

However, in the face of increasingly sophisticated analytical systems employed in other spheres of pharmaceutical research, regulators have become increasingly weary of the supposedly 'subjective' data generated by the visual blanching assay protocol, and the resultant inability to conduct inter-laboratory comparisons of results [13]. In recent years, regulatory agencies have advocated the Minolta chromameter [14] for the quantification of the intensity of the induced skin blanching. Although it was believed this instrument could produce more objective results than visual grading [15], the universal adoption of this instrument has not been without controversy. Lately, digital image analysis techniques have advanced dramatically in sophistication and complexity, and their application in clinical dermatology is now ubiquitous. It is clear that this technology presents a viable alternative to the subjective visual assessment protocol, and is superior in accuracy and the precision of colour quantification to the hand-held chromameter.

Visual Assessment

All variations of the vasoconstriction bioassay retain one essential cornerstone: the intensity of the drug-induced blanching side-effect must be quantified by some means or other. The methodology of the visual assessment assay, its sensitivity and reproducibility, have previously been documented at length [8, 10, 11, 16]. The visual methodology is routinely conducted for comparing two formulations containing the same corticosteroid in the same concentration [19], or for comparing different corticosteroids for clinical potency ranking [17]. This grading system has substantial theoretical merit: the human eye is an excellent discriminator of differences between very similar colours. The visual system simultaneously compares the skin colour of the application site with the surrounding (unmedicated) skin, and the global visual assessment subconsciously accounts for skin factors such as inherent skin pigmentation, hirsutism and mottling. These are fundamental facets of the assay that any instrumental/computational system would have to duplicate.

Refinements in visual assay methodology have markedly improved its credibility. Observations that the intensity of blanching produced by the same formulation depends on the forearm position to which it is applied [18], and varies between left and right arms, for example, has prompted the suggestion that each formulation to be compared should be applied to *multiple sites,* distributed over the *entire length* of *each arm.* Formulations initially judged to be equivalent by the single-point assessment procedures were not considered so by subsequent, multiple-point analyses [20].

Chromameter Assessment

The perceived subjectivity of the visual assessment procedure prompted the search for alternative corticosteroid test systems by the Food and Drug Administration (FDA) [15]. These efforts culminated in the release of a Guidance document [14] detailing the procedures to be followed for the determination of topical corticosteroid bioequivalence using the Minolta chromameter. This instrument analyses the light from a xenon source, incident onto the surface to be measured, quantifying the reflected light in terms of three colour indices: the a-scale (red-green), the b-scale (yellow-blue) and the L-scale (light-dark). These three values define a unique point on a three-dimensional colour map, absolutely defining the colour of the measured surface. The instrument performs accurately, precisely and reproducibly for solid, planar surfaces that are completely uniform in colour and topography. The Guidance [14] suggests the analysis of the a-scale index data, in a protocol of pilot and pivotal trials with corrections for baseline and unmedicated-site readings. The a-scale values appear to show the greatest sensitivity to change over the progression of the blanching response, followed by the L-scale and b-scale data, respectively.

Since its introduction in 1995, there have been several contentious publications in the literature concerning the use of the chromameter for skin vasoconstriction measurements [20–23]. For the purposes of determining a dose-response relationship, the Guidance protocol contends that different contact times – ranging from 15 min to several hours – between (an arbitrarily decided mass of) the reference formulation and the skin, parallels the dose of corticosteroid delivered to the stratum corneum. However, topical drug delivery is not a linear phenomenon; the vehicle typically changes markedly in composition after application to the skin because of the evaporation and penetration of constituents (i.e., the metamorphosis of the vehicle), and the vehicle may markedly change the biochemistry of the stratum corneum (both effects are time and dose dependent) [2]. There may, therefore, be profound changes in the thermodynamic activity of the drug in the metamorphosing matrix or in the barrier potential, with concomitant changes in the rate of drug delivery. Depletion of drug from the applied formulation film, extensive if the dose applied is small, may further confound the dose-response issue. The relationship between formulation-skin contact time and the mass of drug that penetrates the skin (reaching the dermal vasculature or forming a reservoir in the stratum corneum), has not been exhaustively evaluated. Recent developments in the field of skin-stripping methodology [24] (dermatopharmacokinetics) may be beneficial in helping to compare the mass of corticosteroid residing in the stratum corneum with the intensity of the induced vasoconstriction.

We have addressed the problems surrounding the correction of chromameter data for baseline and unmedicated site values [23]; irrespective of the manner in which these values are corrected (or not corrected at all), the shapes and variability of the blanching profiles remain essentially unchanged. However, potential problems of the double-correction procedure are the propagation of any errors from the time-zero baseline values throughout the entire data set, and the introduction of greater variability. This manipulation often generated negative and positive a-scale values for the measured response (negative values indicate greater blanching at the compared unmedicated skin sites than at medicated sites, even when vasoconstriction is clearly visible at the medicated sites). The problem that arises from this observation consists of selecting the appropriate method for the trapezoidal summation of the area under the effect curve (AUEC) for the dose duration: should one subtract positive values from negative values, calculate only the area under the abscissa, or assume that positive values are zero for the purpose of area computation? These different trapezoidal computation methods may lead to an appreciable variance in the calculated AUEC response value – an integral component of the response-modelling procedure used to estimate an effective dose for 50% response (ED_{50}) and dosing interval values corresponding to half ED_{50} and twice ED_{50} (D_1 and D_2). The dose so established for ED_{50} is used as the principal formulation contact time for the subsequent 40-subject pivotal trial, while the vasoconstriction responses resulting from the D_1 and D_2 doses are used as a screening tool in the pivotal trial to exclude subjects from the data pool in whom the ratio of these responses does not fall within a Guidance-specified range. Furthermore, one may also argue that it is inappropriate to model dose-response data when the exact dose of drug generating a specific vasoconstriction response cannot be determined.

Our experiences with the chromameter [20–24] have demonstrated that the results obtained from skin measurements (unlike measuring solid, homogeneous, planar surfaces) are relatively imprecise and are not totally objective. Measuring forearm skin poses several problems:

(1) The operator should strive to achieve negligible but intimate contact of the chromameter's measuring head with the skin. Although pressure is not a consideration when measuring solid surfaces, dynamic changes may take place in the microcirculation (and hence the colour)

of the skin due to vasocompaction in response to the superficial pressure from the measuring head.

(2) The angle at which the chromameter is presented to the skin by the investigator is critical to the magnitude of the masurement obtained. The measuring head should be positioned perpendicularly to the application site, hence the instrument analyses the reflected light from the measured surface. Although perpendicular positioning is not problematic when measuring a solid object, it becomes difficult when measuring a flexible, biological structure like the forearm.

(3) Positioning of the 8-mm-diameter measuring orifice of the hand-held probe is complicated by having to look through the surrounding Perspex shroud. If the vehicle application sites are small (typical of optimised protocols), then even a relatively small offset in placement of the head may result in a large discrepancy in the 'averaged' data obtained. Larger application sites may solve the positioning problem, but tend to induce vasoconstriction that is marginally less intense, more diffuse and less discriminating between similar formulations. It has been our experience that these placement parameters, alignment and pressure are more closely controlled if the subject carries out self-assessment with the chromameter probe than if this is carried out on the subject by an operator. However, this requires each subject to be fully trained in chromameter operation.

(4) The presence of hairs, moles and skin mottling on the arm can result in artefactual data since the darker colours of these structures would be averaged with the colour of the vasoconstricted skin.

(5) Anatomical and biological factors tend to confound the data further. There is a circadian variation in the intrinsic colour of the skin, which for the a-scale value on the forearm tends to generate the largest numbers at night. There is a definite gradient of skin colour between the wrist and the elbow, maximal at the mid-forearm. There is a difference in chromameter response along the inner and outer sides of the forearm. Protocols must, therefore, be designed in such a manner that all compared formulations are represented randomly at multiple sites across the entire forearm.

Other factors of concern, such as operator fatigue in protocols that require several hundred readings to be taken per hour in a topical availability trial involving a full panel of subjects, are less scientific, but are important in assessing the overall integrity of the data set. The inherent objectivity of the instrument is reduced by the investigator's subjective manipulation of the chromameter head, resulting in data that have a marked variability. The validity of the statistical modelling process, as discussed above, is dependent on the quality of the assessment data generated [20, 23]. The danger of such a data set is that one may assign a condition of equivalence to exist between compared formulations, when, in reality, the assessment methodology is not sufficiently precise to discriminate statistically between the performance of the dosage forms.

Digital Image Analysis

The technologies available for capturing, processing, and analysing digital images have improved greatly in recent years. Image analysis is already being applied to dermatological diagnostic and lesion-monitoring purposes; however, there does not seem to be widespread application in formulation research and development yet. Since the essential basis of the human skin-blanching assay is the quantification of normal and medicated skin colour, it seems logical that objective digital image analysis should yield more accurate and precise descriptors of these parameters than are currently possible with subjective visual assessment or the hand-held chromameter measurement. Analytical software algorithms can automatically identify the margins of colour regions in digital images (identifying vasoconstriction sites) and can provide colour quantification of these sites using the usual colour descriptors (R-G-B, L-a-b, etc.). On a less sophisticated level, this process may be accomplished manually by visual examination of the image and cursor delineation of the blanching areas and surrounding unmedicated skin. Advantageously, image analysis allows for exclusion of overt skin blemish colour (e.g. nevi) or hair colour in the overall analysis, a factor that may otherwise distort colour analysis if incorporated into the 'average colour' calculation (e.g. chromameter). The major advantage of digital image analysis is the size of the data set obtained, a 0.5-cm^2 skin site, even when captured at a relatively low resolution, typically comprises several thousand image pixels – each of which can be analysed for the composite colour descriptors. A drug application site showing vasoconstriction that is analysed in this manner may then be described by mean and SD values for the colour mode co-ordinates that are based on thousands of individual pixel measurements. The greater accuracy, precision and validity of these larger data sets appear to be highly advantageous when compared with the single visual grading or single chromameter values obtained for the entire incident measuring area.

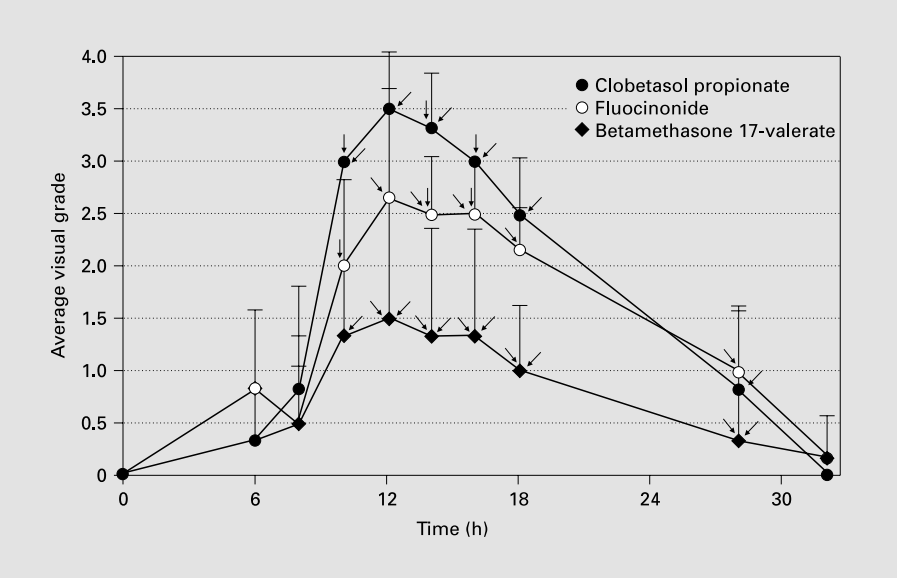

Fig. 1. Mean (± SD) visual response grade versus time profile for 3 corticosteroid formulations from different potency classifications (1 observer, n = 6 sites per formulation). Arrow pairs indicate statistically significant differences (p = 0.05).

Experimental Comparison

A comparison of the different quantitation techniques (visual, chromameter and digital image analysis) was performed to estimate the usefulness of the latter in defining the corticosteroid-induced skin-blanching response. The overall objective of this preliminary study was to assess the ability of the different techniques, when utilised in a standard human skin-blanching assay performed as described previously [8, 20], to make significant distinctions between the vasoconstriction responses induced by three formulations from different potency classes. One would expect to obtain three response versus time profiles that are significantly different from one another for the three formulations tested, i.e. betamethasone 17-valerate (B 17V), fluocinonide (F) and clobetasol propionate (CP). If a technique is to be used for bioequivalence evaluation purposes, then the methodology must be able to discriminate accurately and precisely between formulations that are known from clinical practice to be different from one another, in other words, the sensitivity of the technique must be proven. A quantification method that is not sensitive enough to differentiate statistically between formulations from different potency classes, cannot be applied to the bioequivalence evaluation of pharmaceutical equivalents.

A typical set of results are reported here for the evaluation study in which the visual assessment was carried out by an experienced, trained observer; the chromameter analysis was conducted according to the FDA Guidance suggestions [14], and digital images of each arm of each volunteer were captured at each observation time at a relatively low resolution of 60 pixels per cm. Adobe Photoshop (version 6.0) was used to analyse the digital images, with manual delineation of blanching sites and a comparison of the pixel a-scale values at medicated sites with those of the umedicated surrounding skin colour.

The non-parametric visual grades (0–4) recorded in this study were processed as numerical data for the purposes of this comparison, and are depicted in figure 1 as the average skin-blanching response grade versus time after formulation application to the skin. One standard deviation is shown to indicate the variance of the data, and pairs of arrows at each observation time indicate statistically significant differences between the mean data points (p = 0.05, n = 6 forearm sites per preparation). These results are similar to those obtained previously for these three formulations in our laboratories, and show the correct rank order for the formulations (the product that is most effective clinically induces the greatest vasoconstriction response and vice versa). Statistically, both CP and F are significant different from B 17V, and CP is significantly different from F at three observation times over the period of maximal vasoconstriction. Therefore, when the visual data are analysed in this form, there are significant distinctions between the potencies of the three formulations.

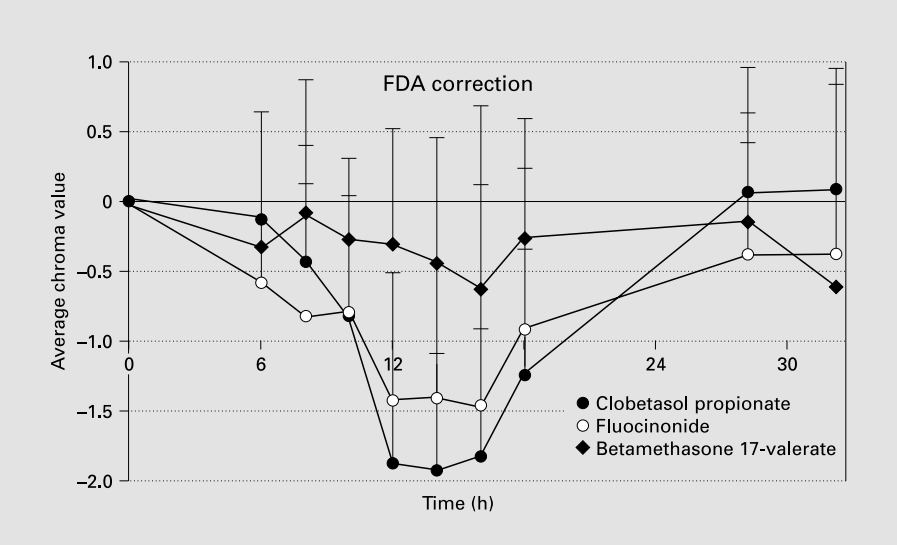

Fig. 2. Mean (± SD) chromameter a-scale response versus time profile for 3 corticosteroid formulations from different potency classifications (n = 6 sites per formulation). No statistically significant differences (p = 0.05) between the means at any observation time. Data have been corrected for time-zero skin colour values and for skin colour values at unmedicated sites at each observation time (FDA guidance methodology).

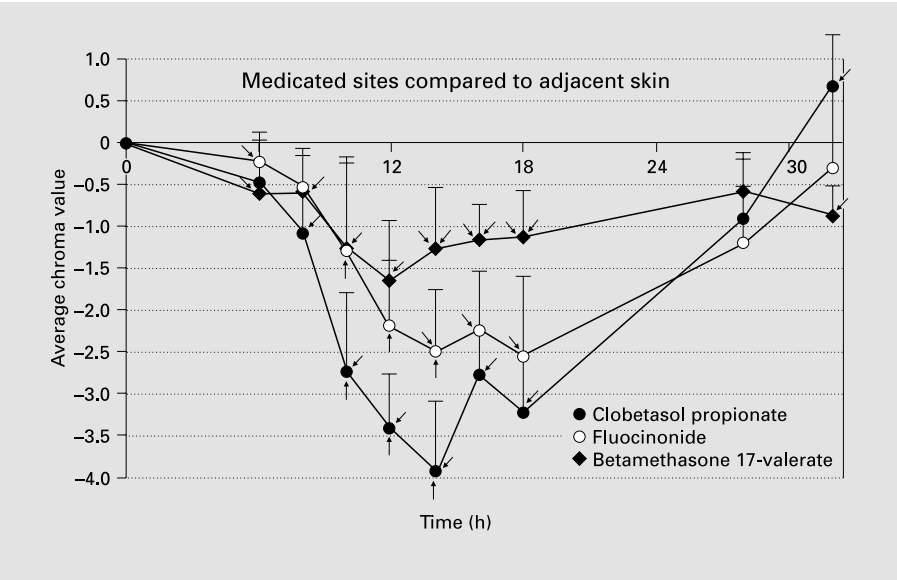

Fig. 3. Mean (± SD) digital imaging a-scale response values versus time profile for 3 corticosteroid formulations from different potency classifications (n = 6 sites per formulation). Arrow pairs indicate statistically significant differences (p = 0.05). Data have been corrected for skin colour values at surrounding unmedicated sites, at each observation.

The chromameter analysis of the skin-blanching response is depicted in figure 2 and clearly demonstrates the relatively poor precision of this instrumental data. Remarkably, there is *no* statistical distinction between the profiles of the different products at any observation time, although the rank order of the response profiles is as expected for the three products of different clinical potency. Therefore, here the chromameter is unable to differentiate statistically between the three formulations from different potency classes.

The digital image analysis data depicted in figure 3 is of clearly superior precision compared with the chromameter data and the visual data. The larger data set used for this quantitative analysis yields smaller standard deviations about the mean values, and there is a greater overall number of statistically significant differences between the data points of the three response profiles. The statistical differences here are similar to the visual data in that both CP and F are significantly different from B 17V, and CP is significantly different from F at three observation times over the period of maximal vasoconstriction. Therefore, when the digital image data are analysed in this form, there are significant distinctions between the potencies of the three formulations.

All three analytical methods (eye, chromameter and digital analysis of images) produced the anticipated potency rank order for the three formulations studied. However, quantitatively the precision of the data appears to decrease in the following order: digital-imaging analysis > eye > chromameter. There are clearly more instances of statistical significance in the digital imaging and visual data than there are in the chromameter data. The former methods would indicate significant differences between products that are known from clinical experience to be significantly different. Chromameter analysis alone would not have made a clear distinction between the products. It is presumed that the greater sensitivity of the digital image analysis is due to the much larger data set that is employed in the colour calculation of the medicated and unmedicated skin. It is also assumed that the delineation of the application site by cursor on the digital image is more accurate than the physical placement of the chromameter measuring head on the skin. Positioning and manipulation errors may also, therefore, be reduced.

Discussion

One may well ask if it is prudent to define topical equivalence determination protocols without knowing the answers to the fundamental questions posed in the introduction. Certainly, it is now known that pharmaceutical equivalence (the same concentration of the same drug in the same type of dosage form) is not required for pharmacodynamic and clinical equivalence, and that the majority of formulations on the market are not optimised in terms of drug delivery. In many cases, one may speculate that transdermal absorption occurs in spite of the delivery vehicle composition, not because of it. At best, we are currently attempting to define product comparison experiments that can show whether new products are 'equal' to products already on the market – a process that does not foster the incorporation of new delivery technology into developed formulations. This process is exacerbated by regulatory stipulations that generic equivalents should be pharmaceutical equivalents. A broader recognition and understanding of drug delivery may allow optimised vehicles with smaller drug concentrations to be classified as equivalents.

The visual assessment of corticosteroid-induces skin blanching is subjective by its very nature. Scientifically, it is highly preferable to have an objective method for the measurement of corticosteroid-induced skin blanching for bioequivalence evaluation purposes. However, the proven reproducibility and accuracy of the visual assay procedure provides the standard which objective techniques must equate or surpass. Previous studies, and the data presented here, indicate that digital image analysis may be a more robust method for the quantitative analysis of skin-blanching for bioequivalence testing purposes than visual assessment or, especially, chromameter assessment. Continued investigation, development and the improvement in sophistication of these test systems will, undoubtedly, produce reliable and robust protocols for topical corticosteroid equivalence evaluation in the future.

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