The Effects of cAMP Single Potencies and Mixed Potencies on Acid Phosphatase Activity

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Abstract

This study investigates whether the effects of cAMP Injeel® and cAMP Injeel® forte in a cell-free system are unique to these preparations or simply equal the additive effects of their single-potency components. When one of the single-potency components of cAMP Injeel® and Injeel® forte was selected as a base potency and the others were added in succession, recorded enzyme activity correlated with the number of potencies added. In all cases, the inhibiting effect of mixed potencies was greater than that of the single potencies, but in no case did the level of inhibition produced by a mixture equal the sum of the effects of its single-potency ingredients.

Introduction

Potency chords are mixed-potency preparations containing equal portions of three potencies of the same substance—a base potency and two additional levels.[1] These potency chords are available commercially under the trade name Injeels®. The "forte" variation includes four rather than three different potencies.[1]

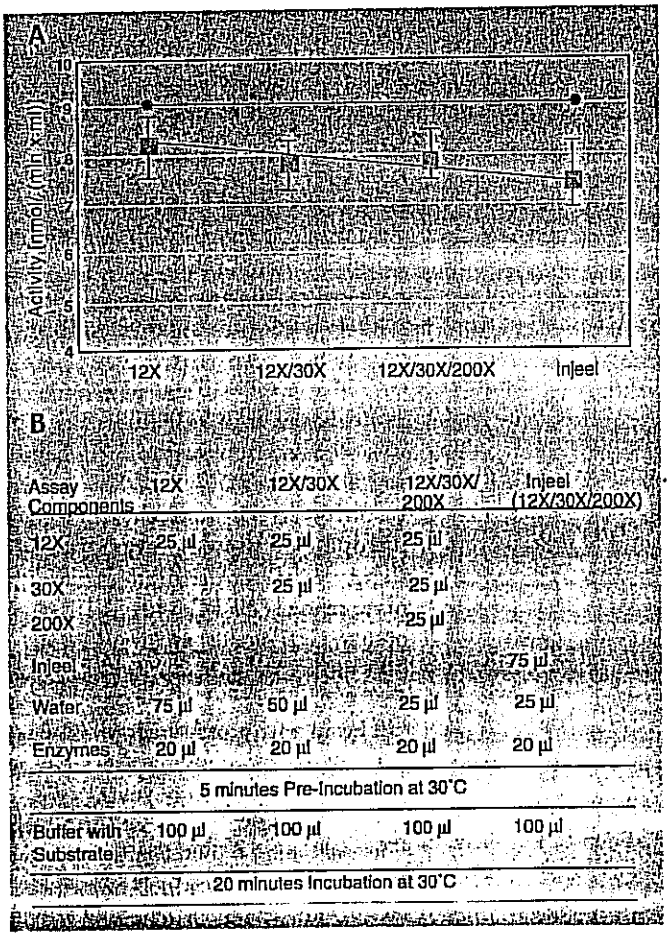


Fig.1: Activity of acid phosphatase (AP) in the presence of different cAMP preparations. The graph presents average values (± standard deviation). The line segment ending in circles represents the control. Statistics (n = 48): All experimental mixtures differed significantly from the control ($p < 0.01$), and 12X differed significantly from all mixtures ($p < 0.01$). To show the linear relationship among the values more clearly, the y axis begins at 4 instead of at 0, and a line has been drawn connecting the average values for 12X and the Injeel®. The table below the graph shows the composition of the experimental mixtures.

The authors confirm the results of the animal experiments reviewed in the article on potency chords in Volume 6/98 of *Biologische Medizin* (Frase W. [Efficacy of Homeopathic Dilutions In the Form of Potency Chords.] *Biol Med.* 1998;27(6):276-278). The work of the authors on the effects of single and mixed potencies of cAMP on acid phosphatase activity offers additional impressive proof (probability of error 1%, $p < 0.01$) that potency chords are superior to single potencies in efficacy.

Hartmut Heine, Ph.D.

The effects of Injeel® and Injeels® forte have already been studied in cell-free test systems. One such system involved xanthine oxidase, an enzyme catalyst in the transformation of xanthine into urate, one of the steps in purine breakdown.[2] Ubiquinon Injeel® and Ubiquinon Injeel® forte proved to have different effects on xanthine oxidase activity. The Injeel® clearly inhibited urate formation, while the Injeel® forte had a moderately stimulating effect.[3]

When we consider the effects of Injeel® and Injeel® forte preparations separately, the question arises, is the effect of the potency chord as such (either the three-potency Injeel® or the four-potency Injeel® forte) unique to that preparation, or does it simply reflect the sum of the effects of its single-potency components?

The first attempts to answer this question involved experiments designed to measure the catalytic activity of a specific enzyme system in the presence of two different single potencies, either used alone or combined in varying proportions.

These studies showed that the inhibiting effect of mixed potencies is greater than those of single potencies and that the degree of inhibition achieved by a mixture is not equal to the sum of the effects of the single potencies it contains.[4] These results suggest that the levels of efficacy of potency chords are new, unique, and different from those of their single-potency components.

The present study elaborates on these earlier experiments by investigating a cAMP Injeel® (12X, 30X, 200X) and a cAMP Injeel® forte (6X, 12X, 30X, 200X) that are not generally available in commerce. We began by selecting a base potency (6X or 12X). We then added the other single-potency components one at a time so that we could trace the change in effect and show how the effect related to that of the potency chord. This experimental design also enabled us to determine whether single potencies and potency chords had different effects.

Materials and Methods

Active Agents

The active substance cAMP was used in the following forms: 6X, 12X, 30X, 200X, cAMP Injeel® (12X/30X/200X), and cAMP Injeel® forte (6X/12X/ 30X/ 200X). All preparations were used in liquid form and were supplied in 5 ml ampules by Biologische Heilmittel Heel GmbH, Baden-Baden. (Please note that these cAMP Injeels® are not available commercially.) All cAMP potencies, as well as the Injeel® and its Injeel® forte variation, were produced according to homeopathic principles. The study utilized a blind test format; all preparations were coded prior to use but decoded prior to statistical analysis.

Chemicals

The synthetic enzyme substrate p-nitrophenyl phosphate was supplied by Serva of Heidelberg (Cat. # 30770). All other chemicals used were of the highest available degree of purity.

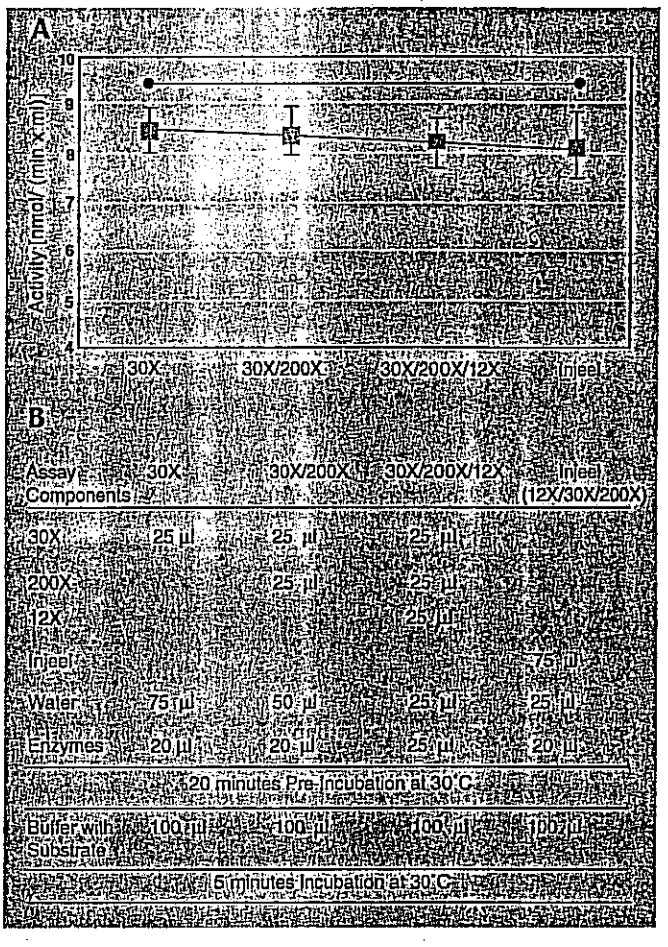


Fig.2: Activity of acid phosphatase (AP) in the presence of different cAMP preparations. The graph presents average values (± standard deviation). The line segment ending in circles represents the control. Statistics (n = 48): All experimental mixtures differed significantly from the control ($p < 0.01$), and 30X differed significantly from all mixtures ($p < 0.01$). To show the linear relationship among the values more clearly, the y axis begins at 4 instead of at 0, and a line has been drawn connecting the average values for 30X and the Injeel®. The table below the graph shows the composition of the experimental mixtures.

Enzyme Test System

The enzyme used was acid phosphatase (AP) derived from potatoes (Boehringer, Mannheim, Cat. # 108197). This enzyme model is biologically relevant because acid phosphatase occurs naturally in the lysosomes of human cells. For use in the experimental setups, the enzyme was diluted 1:200 with 10 mM of NaAc (pH 5.6).

Incubation Technique

The catalytic activity of AP was measured by determining the amount of p-nitrophenol formed in a microtiter plate assay. 20 µl of the enzyme suspension (out of a total volume of 120 µl) and the cAMP preparation being tested (or water, in the case of the control) were preincubated together at 30° C. Two series of assays were performed. In one, the preincubation period was 20 minutes, in the other, 40 minutes. After preincubation, each batch of assay components was mixed with 100 µl of the synthetic substrate (5.5 mM p-nitrophenyl phosphate in 0.1 M citrate buffer, pH 5.6) and reincubated at 30° C. After five minutes, the reaction was stopped by adding 100 µl of 1N NaOH, and the quantity of enzymatically formed p-nitrophenol was determined using a temperature-controlled microtiter plate reader (ATTC 340, SLT Instruments, Crailsheim) at a wavelength of 405 nm.

The quantity of p-nitrophenol was determined by applying the reference equation p-nitrophenol [nmol x ml -1] = 64.82 x OD 405 nm -3.373; correlation coefficient 0.998. P-nitrophenol in various concentrations served as the reference substance.

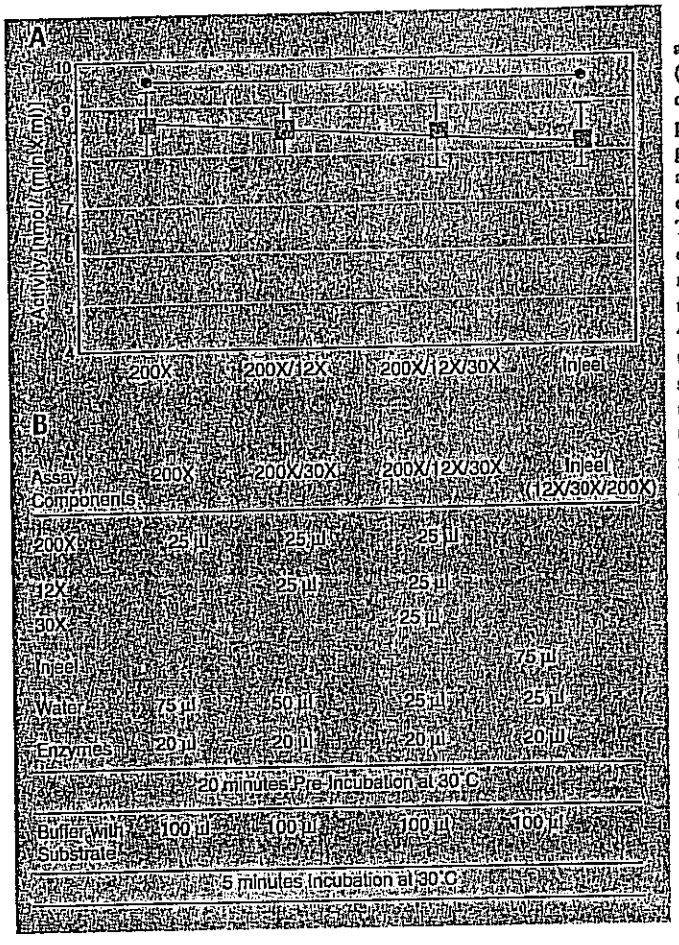


Fig.3: Activity of acid phosphatase (AP) in the presence of different cAMP preparations. The graph presents average values (± standard deviation). The line segment ending in circles represents the control. Statistics (n = 48): All experimental mixtures differed significantly from the control ($p < 0.01$), and 200X differed significantly from all mixtures ($p < 0.01$). To show the linear relationship among the values more clearly, the y axis begins at 4 instead of at 0, and a line has been drawn connecting the average values for 200X and the Injeel®. The table below the graph shows the composition of the experimental mixtures.

Statistics

The measured values obtained for all setups (activity per volume in nmol x min^{-1} x ml^{-1}) were subjected to single-factor variance analysis (ANOVA). Subsequently, the Fisher LSD test was performed to directly compare the effect of the control to that of each cAMP preparation or stage in the additive series of cAMP potencies. A probability of error of 1% ($p = 0.01$) was chosen as the limit of significance.

Results and Discussion

cAMP Injeel®

The cAMP Injeel® used in these experiments is a 1:1:1 mixture of the single potencies 12X, 30X, and 200X. In numerical terms, adding 75 µl of this mixture to a setup adds 25 µl each of 12X, 30X, and 200X. The design of our experiments took this fact into account, as exemplified by Series 1 (Figure 1):

Setup 1 (12X) contained 25 µl of cAMP 12X and 75 µl of water. Setup 2 (12X/30X) contained 25 µl each of 12X and 30X, plus 50 µl of water. Setup 3 (12X/30X/200X) contained 25 µl each of 12X, 30X, 200X, and water. Setup 4 (Injeel®) contained 75 µl of cAMP Injeel® and 25 µl of water. An additional setup serving as the control contained 100 µl of water. Each setup was mixed with 20 µl of AP suspension and then preincubated at 30° C. After 20 minutes, 100 µl of the substrate solution were added and incubation was continued for 5 more minutes. The reaction was then stopped and the quantity of enzymatically formed p-nitrophenol was determined through spectrophotometry.

As Figure 1 shows, all cAMP preparations inhibited the catalytic activity of AP. A linear decrease in enzyme activity is apparent in the series 12X, 12X/30X, 12X/30X/200X, Injeel®. A similar tendency was also observed both when 30X was chosen as the base potency and 200X and 12X were added one at a time (Figure 2) and when 200X was chosen as the base potency and 12X and 30X were added (Figure 3: Series 200X, 200X/12X, 200X/12X/30X, Injeel®).

A linear decrease in AP was also apparent when the preincubation period was increased from 20 to 40 minutes. Inhibition of AP was more moderate, however, and the recorded values were different (data not shown).

cAMP Injeel® forte

cAMP Injeel® forte is a mixed potency consisting of equal parts of the single potencies 6X, 12X, 30X, and 200X, so 100 µl of this mixture contains 25 µl each of 6X, 12X, 30X, and 200X. See the Table for the components and test sequence of the mixtures leading to the Injeel® forte.

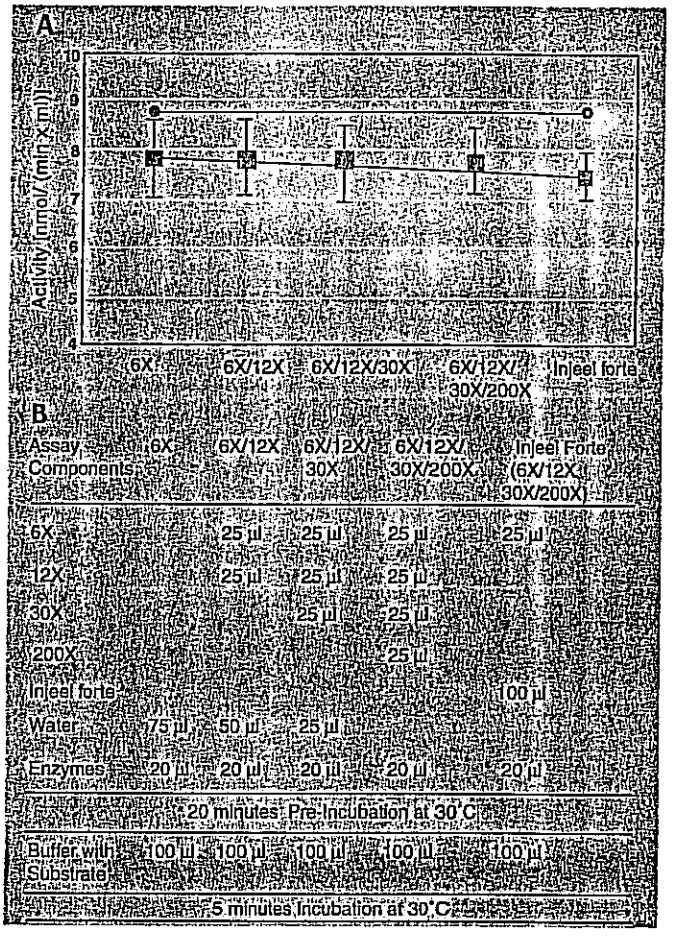
Setup 1	6X	25 µl cAMP and 75 µl Water
Setup 2	6X/12X	25 µl 6X, 25 µl 12X and 50 µl Water
Setup 3	6X/12X/30X	25 µl 6X, 25 µl 12X, 25 µl 30X and 25 µl Water
Setup 4	6X/12X/30X/200X	25 µl 6X, 25 µl 12X, 25 µl 200X and 25 µl 1000X
Setup 5	Injeel® forte	100 µl Injeel® forte
Setup 6	Control	100 µl Water

Tab.: Experimental sequence in analyzing the Injeel® forte variant

The experiments were conducted in the same way as the Injeel® experiments. As Figure 4 shows, all of these cAMP preparations inhibited AP catalytic activity. A linear decrease is again apparent in the series leading from 6X to the Injeel® forte. The same phenomenon is apparent when 12X, 30X, or 200X is chosen as the base potency (data not shown).

From these results, we can conclude that the effects of cAMP potency chords are not identical to the sum of the effects of the individual potencies they contain. As this example shows, potency chords such as Injeel® or Injeel® forte preparations seem to have qualitatively new and unique effects. Further investigation will be needed in order to demonstrate whether this conclusion also applies to Injeels® and Injeels® forte of other substances.

Fig.4: Activity of acid phosphatase (AP) in the presence of different cAMP preparations. The graph presents average values (± standard deviation). The line segment ending in circles represents the control. Statistics (n = 48): All experimental mixtures differed significantly from the control ($p < 0.01$), and 6X and 6X/12X differed significantly from Injeel® forte ($p < 0.01$). To show the linear relationship among the values more clearly, the y axis begins at 4 instead of at 0, and a line has been drawn connecting the average values for 6X and the Injeel®forte. The table below the graph shows the composition of the experimental mixtures.



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