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ABSORPTION AND DISTRIBUTION OF CYTOPLASMIC ORGAN LYSATES (CONJUNCTISAN A EYEDROPS) FOLLOWING INTRACONJUNCTIVAL APPLICATION

J. Seifert, R. Ganser, A. Pfleiderer, W. Brendel

(Institute of Surgical Research at Grosshadern Clinic, University of Munich; Director: Prof. Dr. h. c. W. Brendel)

Summary

The absorption and transport pathways of Conjunctisan A were determined, and its mode of action clarified, by means of radioactive labelling. A dose of 0.5 ml of this drug, labelled with I^{131}, was applied to the conjunctival sacs of rats and rabbits. After six hours there was highly significant enrichment in the ocular tissues. The highest degree of enrichment was found in the uvea, i.e. the ciliary body, choroid and retina. However there was also moderate enrichment in the vitreous body. It was proved immunologically that the radioactivity was identical with the original Conjunctisan A molecule. In particular it was demonstrated that the enriched Conjunctisan A in the aqueous humour precipitates with the specific antibody.

Introduction

The therapeutic efficacy of some substances has been proven, while their mechanisms of absorption, transportation and action remain undefined. One such substance is Conjunctisan A. J. Fuchs (Med. Mschr. 29 (1975) 224) has reported very good results with this preparation in the treatment of senile cataract, providing proof of his observation in a large patient study. No studies have however investigated whether Conjuctisan A is absorbed or transported after it has been applied to the conjunctiva in drop form, and whether any of its constituents actually penetrate the eye. It is feasible that Conjunctisan A exerts an indirect therapeutic effect, by influencing hormone, immune or enzyme systems.

Studies were carried out in rabbits and rats with the aim of throwing more light on the mechanism of activity of Conjunctisan A, by ascertaining whether the preparation can be absorbed, how it is transported, and whether its constituents can be detected in the structure of the eye following absorption from the conjunctival sac. It was necessary to attach a radioactive label to Conjuntisan A eyedrops in order to solve this question. The main constituents of Conjunctisan A are proteins and amino acids from the lens, vitreous body, retina, cornea and optic nerve. Since it is relatively easy to label proteins and certain amino acids with iodine-131, their passage through the system can be followed very well using radioisotope detection methods.

Material and method

A concentration of 0. 3 mg Conjunctisan A (vitOrgan Arzneimittelfabrik, Dr. Theurer, Stuttgart, Germany) per ml was labelled with iodine-131 (Amersham-Buchler, Brunswick, Germany). Low-molecular substances were then removed from the preparation by column chromatography on Sephadex G25. The resulting protein concentration was 0.15 mg/ml.

Inbred rats (strain BD 9) and non-pedigree rabbits of both sexes were used in the study. Prior to the study the animals had been kept under normal conditions, with free acces to water, and a diet of Altromin animal feed. The radioactivity was measured in cps/g of organ tissue in a multi-channel gammaspectrophotometer (Packard, USA), and results were given in % of the dose applied.

The mean and mean error of the mean were calculated, and where necessary Student's t-test was applied to examine the significance of differences between two groups.

Results and Discussion

In order to ascertain whether Conjunctisan A is absorbed at all following drop application to the conjunctival sac, rats were given doses of 0. 5 ml into the eye. As figure 1 shows, the eyes of the treated animals were removed after 2 or 6 hours, and the radioactivity levels were determined.

Skin from the same animals was used as a comparison. Only 2 hours after administration of radioactive Conjunctisan, the eye contains significantly more radioactivity than the skin. After 6 hours, the concentration in the eye is 5 times higher, while in the comparative tissue, the skin, there is no change. This accumulation of radioactivity in the eye over 6 hours indicates that radioactive Conjunctisan A is selectively absorbed into structures of the eye.

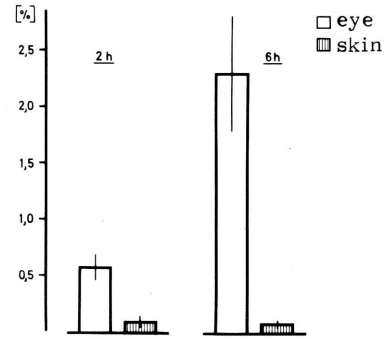

Figure 1: Percent of the dose found in the eye and comparative tissue of rats following administration of 0. 5 ml of Conjunctisan A Eyedrops labelled with ^{131}I.

Since the absorption of a preparation and its accumulation in a particular type of tissue are two different phenomena, the next step was to ascertain the time in which the absorption of radioactive Conjunctisan A takes place. In addition we wished to establish whether the preparation also accumulates in the eye if it is given orally. In the next part of the study, radioactive Conjunctisan was given orally. As figure 2 shows, the radioactivity was first measured per ml of blood for the 6-hour observation period, and recorded at hourly intervals. The graph shows two things very clearly: it is seen that Conjunctisan A is also absorbed from the gastro-intestinal tract, and secondly, absorption is complete within one hour. The concentrations fall gradually from hour 1 to hour 6. The best explanation for this is specific accumulation in a particular type of tissue - the eye - as well as elimination via the liver and kidneys.

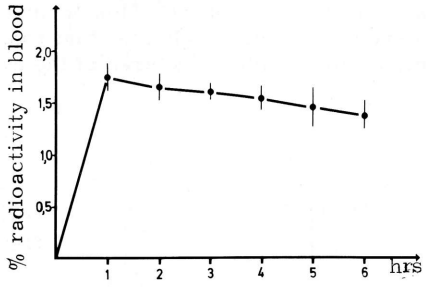

Figure 2: Radioactivity in 1 ml of blood in % of the oral dose of ^{131}I-Conjunctisan A in rats.

Figure 3 shows that Conjunctisan A also accumulates in the eye after oral administration. Here again we observe a significant increase in the level after 6 hours, although the level is not as high as after administration of eyedrops into the conjunctival sac. This is probably due to enzyme activity in the gastro-intestinal tract, breaking down a certain proportion of the dose administered. As figure 2 shows, absorption is complete after 1 hour, and it is only after this that specific accumulation in the structures of the eye begins.

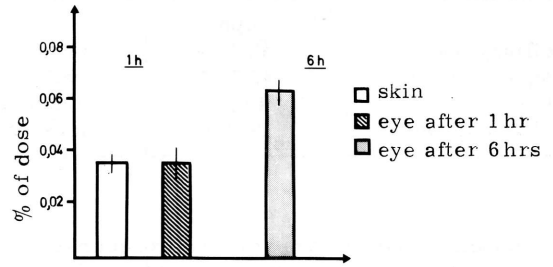

Figure 3: Accumulation of ^{131}I-labelled Conjunctisan A in the eyes of rabbits following oral administration.

If the mechanism of action of Conjunctisan A is to be ascertained, it is very important to know in which structures of the eye it accumulates. The rat is not a suitable model for the investigation of this question, so only rabbits were used. Conjunctisan A was again dropped into the conjunctival sac of the animals. After a 6-hour absorption, distribution and accumulation period, the eyes were removed, the various structures (lens, vitreous body, ciliary body, aqueous humour, cornea, retina and bulbus oculi) were dissected, and their radioactive content measured individually. Table 1 shows the radioactivity per gram of tissue for each of these. It is clear that those parts of the eye which are well-perfused with blood contain more radioactive Conjunctisan A, i. e. the retina and the ciliary body. The aqueous humour, lens and vitreous body have moderate concentrations, and the cornea has markedly less. The lowest level is found in the reference tissue, muscle. This distribution of radioactivity in the structures of the eye is a result of transport through the bloodstream, and perhaps also the lymph, but only to a very small extent through the cornea, of radioactive Conjunctisan A absorbed from the conjunctival sac.

We have so far spoken only of accumulation of radioactivity. It is important to prove that the radioactivity in the eye is in fact identical with the Conjunctisan organ lysate initially applied to the

Table 1: Distribution of radioactivity in the eyes of rabbits 6 hours after drop administration of 5 ml of ^{131}I-labelled Conjunctisan A.

	cpm/g		cpm/g
ciliary body	95 ± 34	cornea	24 ± 2.5
aqueous humour	45 ± 17	retina	114 ± 54.0
lens	45 ± 14	bulbus oculi	38 ± 32.0
vitreous body	38 ± 15	muscle	10 ± 7.4

conjunctival sac. Of the various procedures available for demonstrating this, we chose the immunological method. This assumes that the preparation Conjunctisan A Eyedrops has antigenic properties. An antibody is raised, and is then used to demonstrate the antigen in Conjunctisan A following the absorption and accumulation in the eye.

The immunisation of rabbits with Conjunctisan A proved to be very difficult, and considerable time and material were expended. After a three-month treatment period with very high doses combined with an adjuvant, we finally succeeded in raising antibodies which precipitate with Conjunctisan A.

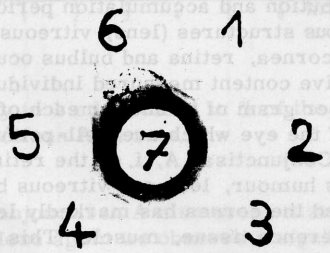

Figure 4: An anti-Conjunctisan antibody (7) precipitates with the aqueous humour of rabbits (1 - 5) which had had Conjunctisan A Eyedrops administered into the conjunctival sac.

A double diffusion test on agar gel was run with these precipitating antibodies and aqueous humour from animals which had had Conjunctisan A dropped into their eyes. Figure 4 shows how Conjunctisan A absorbed from the conjunctival sac and concentrated in the aqueous humour of the rabbit precipitates with the antibody. This means that the Conjunctisan in the aqueous humour still has its original molecular structure. This animal study thus proves that Conjunctisan A can be absorbed, and that it accumulates specifically in the tissue of the eye after absorption. As a result of this study, the therapeutic efficacy of this preparation in the treatment of senile cataract is more readily explained.

Address for correspondence: Prof. Dr. J. Seifert, Institut für Chirurgische Forschung, Klinikum Grosshadern, Marchioninistrasse 15, 8000 Munich 70, Germany.