Further Insight into the Bovine Serum Albumin Assay (The *in vitro* Anti-inflammatory Assay)

LAD Williams

ABSTRACT

The present article reports on the comparative cost of using the Bovine Serum Albumin as an assay for detecting natural products with anti-inflammatory activities relative to the use of animals. ***This is an addendum to the West Indian Medical Journal article; 2008; 57 (4); 327–31.***

Profundización en el Ensayo de Albúmina de Suero Bovino (El Ensayo Anti-inflamatorio *in vitro*)

LAD Williams

RESUMEN

El presente artículo consiste en un reporte comparativo del costo del uso de la albúmina de suero bovino en forma de ensayo para detectar productos naturales con actividad anti-inflamatoria en relación con el uso animal. ***El mismo constituye un apéndice al artículo de West Indian Medical Journal, 2008; 57 (4); 327-31.***

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INTRODUCTION

Williams *et al* (1) have proposed that stabilizing thermal immunogenic bovine serum albumin (BSA) by natural products is a feature that could be developed for selecting therapeutically interesting molecules without the use of animals. The above assay was developed from research done by Grant *et al* (2). Grant *et al* (2) stated that non-steroidal anti-inflammatory molecules will stabilize BSA when exposed to a few degree rise in temperatures. However, Williams *et al*, (1) revealed that a range of extracts and compounds with various biological properties can convey the protection (stability) to the protein (1). Thus, from the above it would appear that the assay could have a wider application than just detecting non-steroidal anti-inflammatory agents.

Stabilization of BSA imply that the protein is unable to loose its molecular confirmation and functions. Stabilizing the protein also means that it will be unable to express disease forming antigens. In a publication by Williams *et al*, (1) the cost for using animals and the BSA were not compared. The present article revealed the advantage of using the BSA assay relative to using animals for achieving the same outcome *ie* finding anti-inflammatory compounds.

Hypothetical comparative cost analyses for using the Bovine Serum Albumin (BSA) assay relative to the carageenan rat paw oedema assay to detect active anti-inflammatory compounds form a selected plant.

Step1.

Hexane, ethyl acetate and methanol extracts should be prepared from the dried powder plant material. A total of 20 rats (Sprague-Dawley rats) is required; 15 for the extracts (5 per extract) and five for the control. Animal cost = 20 x 3.75 = **75 US $**

(Assuming that the cost for one Sprague-Dawley rat is 3.75 US $ and the experimenter are working at one dose *eg* 50 mg/Kg body weight).

Step 2.

Assuming that from step 1 only the ethyl acetate fraction was positive for anti-inflammatory activity.

The ethyl acetate fraction can then be fractionated using a column, from this fractionation we obtained 50 fractions. These were then pooled using thin layer chromatography, 10 similar fractions were obtained. These

From: The Natural Products Unit, Scientific Research Council, P.O. Box 350, Hope Gardens, Kingston 6, Jamaica West Indies.

Correspondence: Dr LAD Williams, Scientific Research Council of Jamaica, PO Box 350, Hope Gardens, Kingston 6, Jamaica West Indies. E-mail: lawrencew@src-jamaica.org

fractions can then be tested for the anti-inflammatory active compounds. The animal cost for testing the 10 fractions would be 10 x 5 = 50, plus five animals for the control. Thus, the animal cost = 55 x 3.75 = **206.25 US $.**

Step 3.

Let us assume that from the assay two fractions were active for anti-inflammatory activity. These two fractions were then separated by high performance liquid chromatography (HPLC) revealing that both fractions contained 20 compounds. Thus, to find the active compounds from each fraction it would require 20 x 5 = 100 animals, plus the control of five animals *ie* 105 animals would be required at a cost of 393.75 US $. Thus, for the 40 compounds it would be 105 x 2 = 210 animals at a cost of 210 x 3.75 = **787.5 US** $.

CONCLUSION

Thus, from the above analyses the number of animals required would be 285 at a cost of **1068.75 US $** from the rat paw oedema assay, plus the cost for carageenan *etc* for finding the active compound (s). However, the cost of using the BSA assay would be **111.0 US $.** In addition, the experimenters would not need the problems associated with obtaining ethical approval since the BSA assay would not involve the use of living specimen.

REFERENCES

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