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(54) Title: METHYLENE BLUE DIAGNOSTIC AGENT AND DIAGNOSTIC METHODS FOR DETECTION OF EPITHELIAL CANCER

(57) Abstract: Methylene blue dye compositions and methods for detecting and/or delineating cancerous and precancerous epithelial tissue are presented.

METHYLENE BLUE DIAGNOSTIC AGENT AND DIAGNOSTIC METHODS FOR DETECTION OF EPITHELIAL CANCER

This invention relates to a novel diagnostic agent for detection of cancerous and precancerous epithelial tissue.

According to one aspect, the invention pertains to use of methylene blue for the manufacture of a dye stain composition for in vivo detection of premalignant epithelial lesions and carcinomas, wherein the dye stain composition is suitable for sequentially rinsing the epithelium such that the stain is selectively retained by cancerous and precancerous tissue and such that the unretained stain composition is capable of being removed by rinsing the epithelium with a rinse composition.

According to another aspect, the invention pertains to use of methylene blue for the manufacture of a dye stain composition for in vivo detection of premalignant epithelial lesions and carcinomas, wherein the dye stain composition is capable of being selectively retained by

cancerous and precancerous tissue and the unretained composition is capable of being removed by sequentially

rinsing the epithelium with the dye stain composition, and

rinsing the epithelium with a rinse composition for removing the unretained stain composition.

According to another aspect, the invention pertains to a biological stain composition for in vivo detection of cancerous and precancerous tissue, comprising

(a) methylene blue,

(b) a pharmaceutically acceptable aqueous solvent, and

(c) a pharmaceutically acceptable oxidizing agent for leuco methylene blue.

According to another aspect, the invention pertains to a dye stain composition which comprises methylene blue for use in a method for in vivo detection of premalignant epithelial lesions and carcinomas, including the steps of

sequentially rinsing the epithelium with dye stain composition which is selectively retained by cancerous and precancerous tissue, and

rinsing the epithelium with a rinse composition for removing unretained stain composition.

According to another aspect, the invention pertains to a dye stain composition which comprises methylene blue for use in a method for in vivo detection of premalignant epithelial lesions and carcinomas, including the steps of

sequentially

rinsing the epithelium with dye stain composition which is selectively retained by cancerous and precancerous tissue, and

rinsing the epithelium with a rinse composition for removing unretained stain composition.

According to one embodiment, the invention pertains to novel methods for detecting and/or delineating cancerous and/or precancerous tissue of the

epithelium.

In another embodiment the invention concerns to such diagnostic procedures and agents useful therein which are especially useful for in vivo screening of patients for possible oral cancer as part of routine dentist's or physician's examinations or procedures, such as periodic dental or physical examinations, dental cleaning, etc.

In yet another embodiment the invention relates to such procedures and compositions useful therein, which use dye stains that are more readily available and/or less expensive and less complicated to synthesize and/or purify than the dyes employed in prior art procedures.

In yet another embodiment, the invention concerns such in vivo procedures and compositions employing a dye which, despite prior art teachings otherwise, is sufficiently non-toxic that it can be employed by rinsing the entire oral cavity and/or gargling.

In-vivo diagnostic procedures for detecting premalignant epithelial lesions, such as oral lesions and oral carcinomas, employing dye compositions that are selectively retained by tissues rendered abnormal by dysplasia, hyperplasia, tumorigenesis and other active surface lesions, are known in the art. For example, procedures employing fluorescein or fluorescein derivatives are disclosed in Chenz, Chinese Journal of Stomatology (27: 44-47 (1992)) and Filurin (Stomatologiia (Russian) 72: 44-47 (1993)). These procedures involve application of the dye, followed by visual examination under ultraviolet light to detect cancerous/precancerous tissue, which is selectively fluorescent.

Another prior art procedure involves in vivo application by rinsing with toluidine blue 0, followed by normal visual examination to detect any selectively stained tissue. Such procedures are disclosed, for

example, in U.S. Patent 5,372,801 to Tucci, et al. and in U.S. Patent 4,321,251 to Mashberg. Toluidine blue has been used for decades as a histopathological stain for in-vitro use. Through this use it has become known as a metachromatic dye, staining nuclei rich in DNA and RNA a purple to pink color. The inherent deep blue color of toluidine blue O is changed to purple or pink when the dye is bound to nucleic acid or other acidic cellular macromolecules. Of course, this type of staining is dependent on the dye gaining access to internal subcellular structures such as the nucleus. Such access is readily obtained only by "fixing" a tissue sample with formaldehyde or other reagent that disrupts the cellular membrane without destroying general cellular structure.

In contrast to the mechanism involved in in-vitro use, the staining of oral tissue in vivo by toluidine blue O is due to its ability to penetrate the cell walls and attach to the mitochondria, which retains the dye longer than components of the extracellular matrix.

The Mashberg procedure involves application of the toluidine blue O solution as a rinse of the entire oral cavity, with gargling, followed by rinses with water and

acetic acid to remove dye that is not retained by the cancerous or precancerous tissue. The preliminary diagnosis by the Mashberg procedure is then confirmed by direct application of the toluidine blue O composition to the suspect site 10-14 days later. The Tucci '801 patent discloses an improved toluidine blue O composition for use according to the general procedure taught by Mashberg.

An in vivo procedure involving use of Lugol's solution (iodine) and toluidine blue O was proposed for detecting esophageal cancer synchronous with upper aerodigestive tract cancers in Papazian, Gastroenterologic Clinique et Biologique 9:16-22 (1985).

More recently, Pomerantz U.S. Patent 5,882,627 disclosed a structurally defined class of oxazine and thiazine dyes that are useful in general accordance with the Mashberg diagnostic protocol. However, the Pomerantz '627 patent expressly excludes methylene blue from this defined class, asserting that it is too toxic for application by oral rinse and/or gargling procedures.

Contrary, however, to the disclosure of the

Pomerantz '627 patent, methylene blue and its ionic derivatives, in the concentrations and quantities employed for in vivo tissue staining, is safely employed as a topically applied selective stain for detecting and/or delineating epithelial cancerous and precancerous tissue.

Suitable compositions of methylene blue for application of the dye to epithelial tissue are prepared by mixing the dye with a suitable pharmaceutically acceptable solvent. Preferably the pH of the methylene blue solution is adjusted with a suitable pharmaceutically acceptable buffer system to yield a final solution that is substantially isotonic and has a pH in the range of approximately 2.5 to 7.0, preferably 4.0-5.0. This can be accomplished by an acetic acid-sodium acetate buffer system. Other suitable buffer systems include citric acid-sodium citrate or mixed acid salt systems such as citric acid-sodium phosphate and the like.

The solvent used to provide the liquid methylene blue dye compositions of the invention is an aqueous solvent. According to the presently preferred embodiment

of the invention, the solvent included a pharmaceutically acceptable, i.e., non-toxic, non-reactive alcohol, e.g., ethyl alcohol. Such solvents do not appreciably interfere with the staining mechanism and do not themselves contribute to the reduction of chromo forms of the dye to leuco forms.

Flavoring, stable to the other components of the dye composition, may be added to improve the palatability of the composition if it is to be used as an oral "rinse."

The amount of methylene blue dye in the liquid composition is preferably adjusted to yield a concentration of approximately 1% by weight of the final composition, although higher concentrations can be employed and lower concentrations are at least partially effective. At present, I prefer to employ dye compositions containing from about 0.5 to about 3.5% by weight of the methylene blue component.

The invention also contemplates compositions for use in accordance with the methods of the invention, in which any leuco form of the dye present in the composition is oxidized to the chromo form, by inclusion of a

pharmaceutically acceptable oxidizing agent, in the manner analogous to that disclosed in the Tucci '801 patent.

EXAMPLES

The following examples are presented in order to illustrate practice of the invention to those skilled in the art and not by way of limitation of the scope thereof, which is defined only by the appended claims.

Example 1

Preparation of Diagnostic Composition

A diagnostic composition is prepared by mixing each of the indicated components in the following proportions (% by weight):

Purified Water U.S.P.	83.85
Glacial Acetic Acid U.S.P.	4.61
Sodium Acetate Trihydrate U.S.P.	2.45
SD18 Ethyl Alcohol	7.48
Hydrogen Peroxide 30%, U.S.P.	0.41
IFF Raspberry IC563457	0.20
Methylene Blue	1.00

Example 2

Preparation of Rinse Solution

A rinse solution is prepared by mixing the following components in the indicated proportions (weight %):

Purified Water U.S.P.	98.70
Glacial Acetic Acid U.S.P.	1.00
Sodium Benzoate U.S.P.	0.10
IFF Raspberry IC563457	0.20

Example 3

Clinical Effectiveness

The clinical effectiveness of the compositions of Examples 1 and 2, is compared to toluidine blue O

diagnostic control compositions prepared in accordance with the Tucci '801 patent, using the diagnostic protocol disclosed in the Mashberg '251 patent.

Patients are first screened for oral pathology employing the TBO control composition. After identifying potential cancerous or precancerous pathology, all traces of the TBO are removed by repeatedly rinsing the suspect sites with water and the acetic acid rinse of Example 2.

Those patients exhibiting oral pathology are then used as test subjects for the methylene blue diagnostic composition of Example 1. 2-3 cc of the methylene blue composition is applied by painting the pathologic mucosal surface, followed by rinsing with the rinse mixture and water to remove excess methylene blue composition.

Histological examination of tissue from the areas stained by the methylene blue diagnostic composition of Example 1 confirms that the methylene blue composition is at least as effective as toluidine blue O in identifying and delineating cancerous and precancerous epithelial tissue.

Example 4

The procedures of Example 3 are repeated, except that the test and control compositions are applied to the oral mucosa by rinsing, with gargling, instead of by direct application to the locus of the suspect sites. Equivalent results are obtained.

Having disclosed my invention in such terms as to enable those skilled in the art to understand and practice it, and having disclosed the presently preferred embodiment, I CLAIM:

CLAIMS

1. Use of methylene blue for the manufacture of a dye stain composition for in vivo detection of premalignant epithelial lesions and carcinomas, wherein the dye stain composition is suitable for sequentially rinsing the epithelium such that the stain is selectively retained by cancerous and precancerous tissue and such that the unretained stain composition is capable of being removed by rinsing the epithelium with a rinse composition.

2. Use of methylene blue for the manufacture of a dye stain composition for in vivo detection of premalignant epithelial lesions and carcinomas, wherein the dye stain composition is capable of being selectively retained by cancerous and precancerous tissue and the unretained composition is capable of being removed by sequentially

rinsing the epithelium with the dye stain composition, and

rinsing the epithelium with a rinse composition for removing the unretained stain composition.

3. A biological stain composition for in vivo detection of cancerous and precancerous tissue, comprising

(a) methylene blue,

(b) a pharmaceutically acceptable aqueous solvent, and

(c) a pharmaceutically acceptable oxidizing agent for leuco methylene blue.

4. A dye stain composition which comprises methylene blue for use in a method for in vivo detection of premalignant epithelial lesions and carcinomas, including the steps of

sequentially rinsing the epithelium with dye stain composition which is selectively retained by cancerous and precancerous tissue, and

rinsing the epithelium with a rinse composition for removing unretained stain composition.

5. A dye stain composition which comprises methylene blue for use in a method for in vivo detection of premalignant epithelial lesions and carcinomas, including the steps of sequentially

rinsing the epithelium with dye stain composition which is selectively retained by cancerous and precancerous tissue, and

rinsing the epithelium with a rinse composition for removing unretained stain composition.